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CASE STUDY ARTICLE

Competitor Avian Species and their Impact on Sparrows - A Case Study of Saran Division

Deepshikha[1]* and Reeta Kumari[2]

[1]Research Scholar, Department of Zoology, Jai Prakash University, Chapra, Bihar, India.
[2]Professor and HOD, Department of Zoology, Jai Prakash University, Chapra, Bihar, India.

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***Address for Correspondence**
Deepshikha
Research Scholar,
Department of Zoology,
Jai Prakash University, Chapra, Bihar, India.
E.Mail: sanu1994deepshikha786@gmail.com

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ABSTRACT

The house sparrow (*Passer domesticus*), which used to be found nearly everywhere, is now endangered in urban and rural India due to a number of challenges faced in the ecosystem. Interspecific competition from both resident and migratory avian species is one understudied but more important factor. The emphasis of this study lies on the competition dynamics faced by house sparrows in Saran Division of Bihar which includes Chapra, Siwan and Gopalganj districts. Direct field observations, citizen science data and structured interviews were the methods used to show how the increase of avian diversity, urban expansion, food scarcity and nesting sites competition combined together resulted in the spatial redistribution and decline of sparrow populations. Common Myna (*Acridotheres tristis)*,Rose ringed Parakeet (*Psittacula krameri*), Rock Pigeon and migratory Warblers and Buntings are some aggressive competitor avian species that affect local niche occupancy patterns. The paper underscores the importance of localized preservation measures and strategies that take into account interspecific dynamics besides anthropogenic factors.

Keywords: Interspecific competition, *Passer domesticus*, Conservation, Gangetic Plains, Bihar, Synanthropic birds.

INTRODUCTION

Since the dawn of human civilization, the house sparrow (*Passer domesticus*), a tiny passerine bird native to Eurasia has cohabited with humans. But sadly, this bird has seen a declining trend in its population over a past few decades in many parts of India and Bihar is not exception to that (Sharma and Mishra, 2021; Bhattacharya *et al*.,2011). The bird has suffered catastrophic global declines (60-80% in urban centres since 1990) with India reporting particularly severe losses (Anderson, 2006; Singh *et al*., 2020). Amongst the obvious reasons of this decrease like food shortage, habitat

loss, and pollution, interspecific competition has emerged as a significant but unexplored factor contributing to their loss(Choudhary *et al*; 2015). Competition from aggressive avian species remains critically understudied in South Asia (Mohanty *et al*.,2018). The districts of Chapra, Siwan and Gopalganj collectively under Saran Division feature combination of rural agriculture based zones and urbanizing towns. Such a unique ecological mix is regularly visited by a variety of migratory avian fauna. The proximity of the area to wetlands and riverine system encourage frequent visits of the birds causing seasonal influx of avian competitors (Ali and Ripley,1983; Wetlands and International,2020). Several research studies report that dependence on human habitation makes house sparrows susceptible to alterations in nesting areas and food resources (Summer-Smith, 2023). Some avian species like Common Myna, House Crow (*Corvus splendens*) are more aggressive and competent in securing nesting sites and food materials than house sparrows (Goyal and Mehta, 2010). While Parakeets and Warblers cause small insects and seeds less available to sparrows thus indirectly affecting sparrows by reducing food sources (Kumar and Singh, 2017). As far as the Indian environment is concerned, it is observed that as urbanisation increases sparrow abundance decreases demonstration inverse relation between the two variables (Bhattacharya *et al*.,2011). According to Rao and Murali (2012) increasing dominance of more adaptable and aggressive avian species is one important amongst the culprits of sparrow decline Delhi and Bengaluru. Regional factors should also be understood as they may be important factors behind the loss as suggested by studies. In north parts of Bihar, the migratory influx of birds alters local avian food chains especially during the months of winter affecting residential birds like sparrows (Mukherjee *et al*.,2020). The mechanism of competition in avian ecology operates through exploitative competition that leads to resource depletion in the form of food and nesting sites (Dhondt,2011); interference competition causing direct aggression or eviction (Shochat *et al*.,2010) and apparent competition that involves shared predators(Holdt,1977) causing granivorous and cavity nesting birds like sparrows face disproportionate pressure (Macarthur,1958). In the Indian context, key competitor species of birds like Common Myna employs nest eviction type of competition as evident in 74% nest failure in Delhi (Sandhu, 2004); Rose ringed Parakeet showing cavity domination competition strategy and occupying 89% of urban nest sites (Menon, 2020); Migratory Buntings competing with local birds by depleting winter food cause 50% grain resource reduction (Datta, 2022) and Eurasian Tree Sparrow by niche overlapping cause rural displacement (Zhang,2013). Competition gets intensified in urban environments where habitats often homogenize ,in spite of the fact coexistence demands niche differentiation according to Mac Arthur's (1918) Niche Theory (Mac Leod *et al*.,2006).

This is evident from the example of Europe where due to reduced nesting cavities and fragmented green spaces competition heightened amongst sparrows in cities (Summer-Smith,1988). In the same way due to niche overlap with aggressive finches and starlings in North America, House Sparrows decline (Cabe ,1993). Grain eating birds give direct competition to sparrows for seeds and grains like House Finches (*Haemorhous mexicanus*) and Eurasian Collared Doves (*Streptopelia decaocto*). With larger body size House Finches aggressively displaced House Sparrows at feeders as reported by Anderson (2006). For feeding on cereal crops sparrows and buntings compete causing resource depletion especially in winters driving sparrow mortality (Robinson *et al*., 2005). Opposite to this, a behavioural adaptation is seen when sparrows alter foraging strategies when competitors are present by feeding in human generated wastes, thus decreasing competitive pressure although not eliminating (Gonzalez-Solis *et al*.,2011). Sparrows nest sites are aggressively usurped by cavity – nesting species such as European Starlings (*Sturnus vulgaris*) and House Martins (*Delichon urbicum*). By monopolizing crevices and eaves in buildings starlings reduce House Sparrow reproductive success (Jones *et al*.,1997). A similar example of Asia where invasive common Myna (*Acridotheres tristis*) evict sparrows from nest boxes as documented by Sandhu and Sandhu (2004). As noticed by Moller (1994), Sparrows are forced into suboptimal sites by Barn Swallows (*Hirundo rustics*), a noncavity nester, that compete for sheltered anthropogenic structures. Displacement of sparrows from urban nest sites by key competitor species can be seen by case studies of North America in 1890s when starlings introduced into the area become major competitor of sparrows (Kessel, 1957). Koening (2003) attributed 30% of sparrows nest failure in New York to starling interference. Hill (1993) documented that House Finches, native to Western North America, expanded eastwards in the 20th century threatening sparrow survival by overlapping with them in both diet and habitat. Also localized sparrow declines have been connected to the dominance of House Finches in feeder interaction (Belhoff and Gauthreaux, 1991). Interspecific competition between Tree Sparrows and House Sparrows in Asia restricts House

Sparrows expansion into rural regions to some extent (Zhang *et al*.,2013). Resources get concentrated by urbanization that in turn amplifies interspecific competition among birds. In Europe increased density of starlings and reduction in green space can be attributed to about 60% sparrow decline between 1980s and 2000 (Chamberlain *et al*.,2009). In agricultural setting, sparrows are forced to compete more aggressively with Rock Pigeon (*Columba livia*) for scattered grains when traditional grain storage is replaced by sealed silos (Da Laet and Summers-Smith, 2007). Bihar's Saran Division represents a unique agro-urban mosaic in Gangetic plain, with Chapra an urban center having more building density; Siwan , an intensive agricultural region and Gopalganj, a peri urban transitional zone. According to Kumar (2021), the region of Bihar witnessed a 47% decline in sparrow population since 2010, yet interspecific avifaunal interaction remain unexplored. Amongst 45 migratory winter visitor bird species (Shamshad, Kumari, 2023), which ones are out competing House Sparrows are still unquantified. This study addresses spatial variation in competitor density across habitats and relative impact of residential and migratory bird species and provides conservation solutions.

MATERIALS AND METHODS

The research was done across key urban, semi urban, and rural zones of the districts of Chapra, Siwan and Gopalganj. We employed three simple field techniques over 60 sites (20 per district chosen as residential colonies, marketplaces, agricultural fields, river banks, railway stations where sparrows were historically abundant. The observation was carried out over a 12 months period covering both migratory and non-migratory seasons. Early morning (6-9 AM) surveys were conducted to record bird activity. The methods of point count (bird counting surveys), nest monitoring and resource competition experiments were used in the study. For mapping bird neighbourhoods we recorded all birds seen or heard within 40m by standing quietly at fixed points for 15 minutes in the morning and late afternoon (Bibby *et al*., 2000). Except the time of monsoon, monthly surveys from June 2023 to June 2024 were done for 12 months. The tools and devices used were binoculars, field notebook, G.P.S enabled phone application, density maps of sparrows and competitors. Active nests were located in buildings, trees, crevices etc. After every 3 days targeted places were visited to record egg or chicks present, competitor interactions and final outcome in the form of success and failure. Mirror poles were used for locating high nests. To assess resource competition, seed trays were placed in markets, farmlands, residential colonies, and other chosen places. The visitor species and their aggression are recorded as well as leftover seeds were weighed hourly. The nest box trials were done by installing 30 boxes, of them 15 were having small entrances (4 cm: Myna- proof) and 15 with large entrances (6cm standard). Analysis of the data collected were done using free software tools like Me recorder and QGIS. The correlation tests were employed to know whether competitor number predicts sparrow number; chi-square tests used to quantify nest failures linked to competition and GIS used to know the factors that best explain sparrow distribution.

RESULT AND DISCUSSION

In urban areas of Chapra town centre and Siwan Sader, House Sparrows were found to coexist with 18-22 other avian species and this species richness increases in peri urban zones. The species of common Myna, Feral Pigeon (*Columba Livia*), Rose ringed Parakeet and Brown Rock Chat (*Oenanthe fusca*) are more competitive towards House Sparrows. Sparrow nests are displaced by pigeons and mynas in marketplaces. The hollow cracks and crevices that were historically occupied by sparrows were occupied by Parakeets. The migratory influx during winter season especially by Winter Buntings cause overlapping diet patterns for insects and grains. But this doesn't cause significant harm to sparrows. Though during peak migratory season during November to February, the sightings of sparrows become less in areas of high migratory birds density in wetlands (Suraila Chaur, Mirzapur Bahiyara in Bhagwanpur Hatt).(Table:1) The impact of other bird species can be seen as 46% of sparrows nests failed due to bird competitors and 68% of these are due to mynas. Also on arriving mynas, sparrows leave 70% of seed trays. Myna-proof boxes show 80% occupancy by sparrows as compared to 29% in standard boxes. When competitor bird density increased 12 birds per kilometer square, sparrow density fell. When winter buntings visit early winter in October as opposed to

their routine visit in November, underweight chicks were found in rural areas. The findings of the research study suggests that any form of interspecific competition, whether direct or indirect, impacts the distribution of sparrows in Saran Division . In these areas sparrows face a complex web of ecological pressure due to invasive nesting of mynas and pigeons, dietary overlap with warblers ,babblers, parakeets and competition posed by migratory birds during winter. When competition combines with anthropogenic disturbances in line with Newton (1988) and Dhondt (2012). The unique environmental context of Saran Division having a mix of urban and agrarian landscapes provides challenges and unique insights into avian community dynamics. The inclusion of interspecific competition as a significant factor in devising conservation strategies turn out to be better conservation measures. In Saran House Sparrows distribution is mainly constrained by residential mynas particularly in Chapra regions where sparrows are restricted to less than 10% of green spaces by myna dominance. In winter rice fields, buntings reduce sparrow feeding efficiency by 40%. A significant 3.5 times more sparrows are seen in riverine villages where traditional grain storage is still used. Conservation must address both species interactions and landscapes connectivity to reverse declines.

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Table 1. Bird Density per Km²			
Species	**Chapra (Urban)**	**Siwan (Farmland)**	**Gopalganj (Mixed)**
Common Myna	17.1 ± 2.9	7.2 ± 1.4	12.2 ± 1.9
House Sparrow	4.2 ± 0.7	15.2 ± 2.2	6.9 ± 1.3
Rose-ringed Parakeet	3.8 ± 0.8	11.7 ± 1.9	8.1 ± 1.1
Winter Buntings	0.7 ± 0.3	23.5 ± 3.5	9.3 ± 1.5

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RESEARCH ARTICLE

Medicinal Flora Inventory of Mallem Konda - An Unexplored, Forgotten Sacred Grove of Eastern Ghats, Andhra Pradesh, India

K. Manipal[1], M. Ramakrishna[2], S. Kameswaran[2]* and B. Swapna[2]

[1]Research Scholar, Department of Botany, Sri Venkateswara University, Tirupati, Andhra Pradesh, India.
[2]Assistant Professor, Department of Botany, V.S.U. College, Kavali, (Affiliated to Vikrama Simhapuri University, Nellore), Andhra Pradesh, India.

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***Address for Correspondence**
S. Kameswaran
Assistant Professor,
Department of Botany,
V.S.U. College, Kavali,
(Affiliated to Vikrama Simhapuri University, Nellore),
Andhra Pradesh, India.
E.Mail: kambharath@gmail.com

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ABSTRACT

The present work is an inventorization of valuable medicinal plants of 80 species that belongs to 37 families wildly grown in Mallem konda – an unexplored, forgotten sacred grove, located at the border of Nellore and Kadapa districts in Eastern Ghats of Andhra Pradesh. The inventorization was carried out after the successive field trips in different seasons during the years 2019 to 2021. Sacred groves are the exemplary examples of climax communities in forest ecosystem which exhibits species richness of trees, shrubs, herbs along with climbers, lianas and other Sciophytes. Many ethnobotanical investigations across the country, revealed the usage of medicinal plants besides utility of medicinal taxa by the Mallemkonda sacred grove region tribal communities is considerably different and also neglected by the previous researchers. Collected plants were identified, and preserved in the departmental herbarium of Vikrama Simhapuri University College, Kavali, Nellore district. Crosschecking of the inventory with the Dr. Duke's Phytochemical and Ethnobotanical database exhibited the ethno-medicinal significance of Mallem konda sacred grove.

Keywords: Inventorization, Mallem Konda, Sacred Groves, Sciophytes, Ethnobotanical,

INTRODUCTION

Archaic times onwards people are using plants in their day to day life. Ethnic people are well known for their knowledge to treat various ailments by using plant based medicines. These concerns of ethnic groups are defined as

Ethnobotany which deals with the study of interactions between plants and humans (Savithramma *et al*. 2017). The ethnic people depend on the nature and natural resources as well as they protect them with their spiritual, religious beliefs for future uses (Savithramma *et al*. 2014; Rao *et al*. 2015; Savithramma *et al*. 2015; Rao *et al*. 2016; Koda, 2021 and Sharma and Kumar, 2021). The sacred groves are one of such patches of forest lands which are safeguarded by the people by their religious beliefs. In India, groves are managed by the government (Forest, revenue and endowment departments) and many are conserved by the local people and non government organizations (22). Though studies were conducted in many sacred groves of Eastern Ghats, so far, no work related to study of medicinal plants was carried out in Mallem konda sacred grove of Andhra Pradesh. Hence, this study is the first attempt to document medicinal plants used by tribals of Mallem konda region.

Etimology

Mallem Konda is named after the Hindu God Lord Shiva. According to the local tribals who know about the sacred grove, Lord Shiva is living in this grove as a saint and the local people find him few times when they came to feed their cattle to this grove. One day several cattle were died and the locals suspected that sheeps were dead due to the saint who is roaming around and they caught the saint and tied him with the *"epi nara"* (the bark fibre of *Hardwikia binata*). The saint disappeared and all the cattle became live. Later, tribals found a statue in the area where the saint was tied. With this incident, the tribals believed that the Lord Maha Shiva was protecting them in and around this grove without any problems.

MATERIALS AND METHODS

Study area

The study area was situated at the boarders of Nellore and Kadapa districts in deep forest of Eastern Ghats, Andhra Pradesh, India. It lies between Latitude 14.5691^{o}N and Longitude 79.2345^{o}E and at an Altitude ranging from 1950 ft MSL. The vegetation type is mixed dry deciduous. Lord Shiva temple on the name of Mallem Kondaiah Swamy is located in this sacred grove. Map depicting the study area is given in Figure 1.

Threat to the grove

In the current situation the Mallem konda sacred grove is under serious threat due to reduction in the religious beliefs caused by interaction with civilized people, anthropogenic involvement through pilgrimages and nature tours; infrastructure development in sacred grove. Introduction of Ornamental flora and Invasive Alien Species, grazing by cattle are the other threats.

Identification and herbarium preparation of the Medicinal plant taxa

Majority of the plants are identified in the field itself up to species level, but few are identified by their genus further identifications were done in S.V. University and V.S. University herbarium. Specimens are collected, mounted and preserved after poisoning with 1% Mercuric Chloride ($HgCl_2$). Specimens are identified initially by Miss. Lalithamba (Modulator e-flora India) later on conformed and authentified by Dr. K. Madhava Chetty, Assistant professor (Rtd) in department of Botany, Sri Venkateswara University, Thirupati. Identification of specimens in lab as well as in field was done with the help of Gamble and Mathew volumes, Flowering plants of Chittoor district, Andhra Pradesh, India (5th volume, 2018) and Forest trees of Andhra Pradesh (a pictorial guide, 2020). Inventory was cross checked with the Dr. Duke's Phytochemical and Ethnobotanical database.

RESULTS

Information procured from repeated and periodic field trips was provided in the tabular form with the details of Botanical name, family, vernacular name, habit, plant part used and ethno medicinal uses (Table 1). Photographs of some important medicinal plants of Mallem konda are shown in Plate 1 and 2.

DISCUSSION

This study revealed that the tribals of Mallem konda sacred grove used 80 medicinal species belonging to 37 families. The predominant families are Fabaceae, Rubiaceae, Combretaceae and Malvaceae (Fig 2). The habit of plants that are utilized as medicine in this grove exhibited trees (24), shrubs (13), herbs (37), lianas (02) and vine (01) (Fig.3). Herbs are mostly used. In nearly thirty percent of the medicinal plants, whole plant is used in the preparation of medicine. The usage of different plant parts for medicine preparation is shown in the venn diagram (Fig 4). Among the plant parts, roots (16.5%) were used followed by leaf (15.25), stem bark (13.34%), fruits (4.85%), and seeds (3.75%). Different parts of the same medicinal plant in many taxa are used to treat different ailments. Both root and leaf of the same plant is used in 12.5%, Leaf and Stem bark (6.66%); Fruit and stem bark (6.66%); root, fruit and leaf (3.75%); root, leaf and seed (1.25%). The study indicated that Mallem konda area has abundant medicinal plants (Table 1) to treat a wide range of human ailments such as diabetes, fever, cough, ulcer, wound healing, kidney stones, jaundice, skin allergies, stomach disorders and for poisonous bites.

CONCLUSION

Mallem konda is a treasure trove of medicinal plants. Plants have been used as traditional medicine from time immemorial. Plants consist of secondary metabolites and phytochemicals that have potential to act as therapeutics. Information provided in this inventory may help in developing new leads to the drugs. In addition, unexplored plants and their compounds help to find more effective and non toxic natural drugs that can provide health care benefits to large population.

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Table 1: List of medicinal plants used by Tribals of Mallem konda

S.No.	Botanical name of the Taxa	Family	Vernacular name	Habit	Part used	Ethno botanical uses
1	*Achyranthes aspera L.*	Amaranthaceae	Uttareni	Herb	Whole plant	Whole plant- Leucorrhoea, Jaundice, piles, diarrhoea, scorpion sting, anaemia, amenorrhoea, ear ache, dropsy, stomach disorders, tooth ache.
2	*Adenia wightiana (Wall. ex Wight and Arn.) Engl.*	Passifloraceae	Rogam gadda	Tendrillar climbing herb	Root and leaf	Root- Tonic; Leaf- Tonic, skin troubles.
3	*Ageratum conyzoides (L.) L.*	Asteraceae	Pumpulla	Herb	Whole plant, leaf and root	Whole plant- Nervine tonic, dysentery; Leaf- Styptic, vulnerary, wounds, sores, skin diseases, boils; Root- Root- Anthelmintic, Ophthalmia.
4	*Albizia odoratissima (L.f.) Benth.*	Fabaceae	Konda sigara	Tree	Fruit	Fruit- Erysipelas, wounds, cough, dyspnoea, leprosy.
5	*Allophylus serratus (Hiern) Kurz*	Sapindaceae	Kaki beera/Guvva gutti	Tree	Root, leaf and fruit	Root- Diarrhoea; Leaf- Colic; Fruit- Anthelminthic.
6	*Amorphophallus margaritifer (Roxb.) Kunth*	Araceae	Adavi-kanda or Adavi chaama	Herb	Whole plant, Rhizome	Rhizome-Used to cure scabies. Arthritis; Asthma; Bronchitis; Cancer; Cough; Dysentery; Stomachache; Toothache; Urinary troubles; Vomiting; Piles
7	*Andrographis glandulosa Nees*	Acanthaceae	Nelavemu	Herb	Whole plant	Cancer treatment; cold; flu; upper respiratory infections
8	*Anisochilus carnosus (L.f.) Wall.*	Lamiaceae	Ritchu-rodda	Herb	Whole plant and leaf	Whole plant- Diaphoretic, stimulant, expectorant, hepatic

						disorders, cough and cold; Leaf- Cough, dropsy, indigestion, sores in the leg fingers.
9	*Anogeissus latifolia (Roxb. ex DC.) Wall. ex Guillem. and Perr.*	Combretaceae	Sirimanu	Tree	Whole plant and stem bark	Whole plant- Cancer; Stem bark- Bitter tonic, astringent, polyuria, anaemia, piles, leprosy, snake bite and scorpion sting;
10	*Barleria mysorensis B.Heyne ex Roth*	Acanthaceae	Bantalagobbi, Nallamulugoranta, Erramulugoranta	Herb	Whole plant, Leaf	This plant is used as Febrifuge (lowering of temperature during fever), and relieving of stomach pain. Leaf decoction is given for the treatment of cough. Leaf paste applied externally as an antidote for scorpion bite.
11	*Bridelia retusa (L.) A.Juss.*	Phyllanthaceae	Kora maddi, peyamaddi, mulamaddi	Tree	Fruit	Fruit- Sciatica, lumbago.
12	*Canscora alata (Roth) Wall.*	Gentianaceae	Kancakura	Herb	Whole plant	Epilepsy, Enlargement of the Scrotum, Pain, Skin diseases, Ulcer, Worms, Abdominal disorders
13	*Chloroxylon swietenia DC.*	Rutaceae	Billudu	Tree	Stem bark, leaf, wood and gum	Stem bark- Astringent; Leaf- Rheumatism, wounds, mosquito repellent; Wood- toy making; Gum- Diabetes;
14	*Cissampelos pareira L.*	Menispermaceae	Vishaboddi	Climbing shrub	Root	Root- Antiperiodic, diuretic, dropsy, urinary disorders, anorexia, coryza, purgative, febrifuge, stomachic, diabetes.

15	*Cissus repens Lam.*	Vitaceae	Nellaboddu	Woody climber (Liana)	Leaf and stem	Leaf- Used as vegetable, swelling, fever, stupefying fish. Stem- used as cordage.
16	*Corchorus aestuans L.*	Malvaceae	Nela beera	Herb	Seed	Seed- stomachic.
17	*Curculigo orchioides Gaertn.*	Hypoxidaceae	Nelathadi	Herb	Tuberous root	Tuberous root- Skin troubles, demulcent, diuretic, tonic, diarrhoea, piles, jaundice and asthma.
18	*Cyanthillium albicans (DC.) H.Rob.*	Asteraceae	Tella sahadevi	Herb	Whole plant	Whole plant- Leucorrhoea, stomatitis, skin diseases.
19	*Cyanthillium cinereum (L.) H.Rob.*	Asteraceae	Nalla sahadevi	Herb	Whole plant and root	Whole plant- Coryza, vomiting, skin diseases, leucorrhoea; Root- Eye diseases, anthelmintic, blood disorders, fevers.
20	*Decalepis hamiltonii Wight and Arn.*	Apocynaceae	Maredu kommulu	Woody climber (Liana)	Root	Root- Appetizer, depurative, haemorrhage, thirst, urticaria, jaundice, gout, diabetes.
21	*Decaschistia crotonifolia Wight and Arn.*	Malvaceae	Konda gogu	Shrub	Leaf	Leaf- Hydrocele.
22	*Desmodium pulchellum (L.) Benth.*	Fabaceae	Deyyapu mokka	Shrub	Stem bark	Stem bark- Diarrhoea, haemorrhage, aphrodisiac, eye problems.
23	*Didymocarpus tomentosus Wight*	Gesneriaceae	Rathi Thamara	Herb	Tuber, Leaf	cure for kidney stone, Treatment of fever, skin allergy and inflammation
24	*Dioscorea pentaphylla L.*	Dioscoreaceae	Monyakku pendalam	Twining herbs (Vines)	Tuber	Tuber- swellings, urinary calculi, polyuria, aphrodisiac, dysentery, diarrhoea, leprosy, piles ulcers, abortifacient.

25	*Drosera burmanni Vahl*	Droseraceae	Beda sundari	Herb	Whole plant	Whole plant- Rubefacient
26	*Ehretia microphylla Lam.*	Boraginaceae	Bavana bure, Pithchika beera	Shrub	Leaf	Skin allergies, Psoriasis, Scabies, stomach pain, Wound healing
27	*Erythroxylum monogynum Roxb.*	Erythroxylaceae	Billudu	Tree	Leaf, Stem bark and wood	Stem bark and wood- stomachic Leaf- refrigerant
28	*Euphorbia hirta L.*	Euphorbiaceae	Nanubalu, pachabotlu aaku	Herb	Whole plant and leaf	Whole plant- Urinary disorders, constipation, leucorrhoea, asthma, cough, colic, dysentery, purgative, dyspnoea, urino-genital tract diseases; Leaf- bowel movements
29	*Ficus arnottiana (Miq.) Miq.*	Moraceae	Kala ravi	Tree	Stem bark and leaf	Stem bark and leaf- Cutaneous infections, diabetes.
30	*Ficus mollis Vahl.*	Moraceae	Kala juvvi	Tree	Stem bark and leaf	Stem bark- Diabetes; Leaf- Diabetes, jaundice.
31	*Galactia tenuiflora (Willd.) Wight and Arn.*	Fabaceae	Sanna kandi	Twinning herb	Seed	Seed- Edible
32	*Gardenia gummifera L.f.*	Rubiaceae	Bikki	Tree	Gum	Gum- Ulcers, veneral diseases, constipation, skin diseases, liver disorders.
33	*Getonia floribunda Roxb.*	Combretaceae	Putangi teega	Straggling shrubs	Leaf, Flower, and fruit	Leaf- Astringent, colic, dysentery, malarial fever, anthelmintic, laxative, ulcers, depurative; Flower- Worm infections, scorpion sting, snake bite, thirst, leprosy, diarrhoea. Fruit- Jaundice, ulcers.
34	*Glycosmis pentaphylla (Retz.) DC.*	Rutaceae	Gonji	Tree	Root, stem	Root- Dandruff; Stem bark-

					bark and leaf	Anorexia, burning sensation in eyes; Leaf- Febrifuge, diabetes, liver complaints, vermifuge,eczema, rheumatism and mosquito repellent;
35	*Gymnopetalum cochinchinensis (Lour.) Kurz*	Cucurbitaceae	Avaguda	Climber	Fruit	Fruit- Diabetes
36	*Gymnosporia montana (Roth) Benth.*	Celastraceae	Dantansi	Small Tree	Leaf	Leaf - Jaundice
37	*Habenaria roxburghii Nicolson*	Orchidaceae	Chukka dumpa	Herb	Whole Plant	Whole Plant – Wound healing, Ulcers, diabetes
38	*Haldinia cordifolia (Roxb.) Ridsdale*	Rubiaceae	Rudra ganapa	Tree	Stem bark	Stem bark- Liver disorders, febrifuge, anaemia, leprosy.
39	*Hardwickia binata Roxb.*	Fabaceae	Yepi chettu	Tree	Stem bark and wood	Stem bark- Hats , bags and rope making; Wood- Gonorrhoea;
40	*Helicteres isora L.*	Malvaceae	Gooba tada	Tree	Root bark, leaf and fruit	Root bark- Diabetes; Leaf- Diabetes, stomach infections; Fruit- Diarrhoea, dysentery, flatulence, appetite, colic.
41	*Hemidesmus indicus (L.) R. Br. ex Schult*	Apocynaceae	Sugandapala	Twining herbs	Root	Root- Cardio tonic, cooling beverage, haemorrhage, gout, dysuria, urinary disorders, rheumatism, skin troubles, jaundice, diabetes.
42	*Hibiscus platanifolius (Willd.) Sweet*	Malvaceae	Konda patti	Tree	Leaf	Leaf- Diabetes, hair growth, rheumatism.
43	*Indigofera linnaei Ali*	Fabaceae	Chela pachi	Herb	Whole plant	Whole plant- Leucorrhoea, oliguria, fever, diuretic, antiscorbutic, epilepsy, insanity.
44	*Ipomoea eriocarpa R. Br.*	Convolvulaceae	Puriti teega	Twining Herb	Whole plant	Whole plant – Fever, rheumatism,

						epilepsy, ulcers,
45	*Kohautia aspera (B.Heyne ex Roth) Bremek.*	Rubiaceae	Sanna Parapatam	Slender Herb	Whole plant	Whole plant – Stomachic, diarrhoea
46	*Kopsia fruticosa (Roxb.) A.DC.*	Apocynaceae	Gutti ganneru	Shrub	Latex	Latex- Arrow poison, sores and syphilis.
47	*Lagerstroemia parviflora R oxb.*	Lythraceae	Chennangi	Deciduous Tree	Stem, Leaf	Stem – Wound healing; Leaf - Purgative
48	*Leucas stricta Benth.*	Lamiaceae	Konda Tummi	Hairy Herb	Whole plant, Leaf	Leaf – Jaundice Whole Plant – Cold, cough, insecticide
49	*Merremia tridentata (L.) Hallier f.*	Convolvulaceae	Mududantla Aku	Prostate Herb	Whole plant	Whole Plant – Facial Paralysis, laxatives, piles
50	*Mollugo pentaphylla L.*	Molluginaceae	Pichichatraku	Herb	Whole plant, Leaf	Whole plant – Emmenagogue, Stomach ache, Ear ache Leaf - sprue and mouth infections
51	*Morinda tinctoria Roxb.*	Rubiaceae	Togaru/Maddi	Tree	Root and fruit	Root and fruit- Eczema, glandular swellings, ulcers, digestive disorders.
52	*Mucuna pruriens (L.) DC.*	Fabaceae	Dulagondi/adavi ulava	Vine	Root and seed	Root- Tonic, stimulant, purgative, diuretic, renal affections, failariasis; Seed- Aphrodisiac, tonic, Parkinsonism, eczema, dropsy, leucorrhoea, menorrhagia, hemiplegia, diarrhoea.
53	*Oldenlandia affinis (Roem. and Schult.) DC.*	Rubiaceae	Chinna parapatakam	Herb	Whole plant	Whole plant- Gonorrhoea, cancer.
54	*Oldenlandia ovatifolia (Ca v.) DC.*	Rubiaceae	Parapatakam	Herb	Whole plant	Whole plant - Wound healing, rheumatic pains
55	*Pavetta tomentosa Roxb. ex Sm.*	Rubiaceae	Tella papidi chettu	Tree	Root bark	Root bark- Dropsy, Jaundice.
56	*Phyllanthus amarus Schu mach. and Thonn.*	Phyllanthaceae	Nelausiri	Herb	Whole plant, leaf and latex	Whole plant- Jaundice, diabetes, eye diseases, skindiseases, menorrhagia, urinary diseases,

						diuretic, astringent, dropsy, colic; Root- Galactagogue, Jaundice Latex- Sores.
57	*Phyllanthus maderaspatensis L.*	Phyllanthaceae	Nallausiri	Herb	Leaf and seed	Leaf- Headache, diaphoretic; Seed- Carminative, diuretic, laxative, hepatotonic.
58	*Phyllanthus virgatus G.Forst.*	Phyllanthaceae	Gadha usiri	Herb	Whole plant	Whole plant- Diabetes, eye problems, urinary disorders, jaundice, thurst, leprosy.
59	*Physalis minima L.*	Solanaceae	Budda bhushada	Herb	Root, leaf and fruit	Root- Fever, bronchial asthma; Leaf and fruit- Tonic, purgative, diuretic, gonorrhoea.
60	*Polygala elongata Klein ex Willd.*	Polygalaceae	Nela janumu	Herbs	Whole plant, root and leaf	Whole plant- Skin eruptions and urticaria; Root- Bronchitis; Leaf- Biliousness, constipation;
61	*Pouzolzia zeylanica* (L.) Benn.	Urticaceae	Yeddu moothi dumpa	Shrub	Leaf	Leaf-lactagogue
62	*Pterocarpus marsupium Roxb.*	Fabaceae	Yegisa	Tree	Heart wood and stem bark	Heart wood- Worm infestation, diabetes, leprosy, wounds, polyuria, leucoderma; Stem bark- Leucorrhoea, emetic, cough, dental disorders, skin problems, wounds;
63	*Pterocarpus santalinus L.f.*	Fabaceae	Erra chandanamu	Tree	Heart wood	Diabetes, ulcers, skin diseases, anthelmintic, bilious infections, astringent, tonic, diaphoretic, diarrhoea inflammations and head ache;

64	*Rhus mysurensis B. Heyne ex Wight and Arn.*	Anacardiaceae	Sita sundari	Shrub	Leaf and fruit	Leaf- Dysentery, diabetes, allergy and rashes; Fruit- Dysentery.
65	*Sida cordifolia L.*	Malvaceae	Chiru benda	Shrub	Root and leaf	Root- Diuretic, diabetes, tonic, cystitis, astringent, haematoria, sciatica, facial paralysis, galactogogue, leucorrhoea; Leaf- Cardiotonic, demulcent, dysentery and febrifuge.
66	*Spermacoce articularis L.f.*	Rubiaceae	Madana kattu	Herb	Root, leaf and seed	Root- Coryza, fever; Leaf- Gall bladder stones, hemorrhoids; Seed- Diarrhea, dysentery, demulcent.
67	*Syzygium alternifolium (Wight) Walp.*	Myrtaceae	Mogi/ Konda neredu	Tree	Stem bark and fruits	Stem bark- Diabetes; Fruit- Diabetes, duodenal ulcers, gastric ulcers, joint pains, burni g sensation in stomach.
68	*Taxillus tomentosus (B. Heyne ex Roth) Tiegh.*	Loranthaceae	Tella nugu badanika	Shrub	Whole plant, leaf flower and fruit	Whole plant- Skin cancer; Leaf- Leprosy, skin diseases; Flower and fruit- Mental disorders.
69	*Terminalia tomentosa Wig ht and Arn.*	Combretaceae	Nalla maddi	Tree	Stem bark and fruit	Stem bark- Fever , boils, pruritus, styotic, cardio tonic; Fruit- Piles, vertigo, constipation, chronic dysentery, diarrhoea, rheumatic pains, diabetes.
70	*Tylophora fasciculata Buch .-Ham. ex Wight*	Apocynaceae	Kaakapaala	Twinning Herb	Root and Leaf	Root- Rejuvinator, febrifuge, purgative; Leaf- Emetic, ulcers, wounds.
71	*Vigna radiata var. sublobat a (Roxb.) Verdc.*	Fabaceae	Pesalu	Climbing herb	Seed	Seed- Galactagogue, vertigo, diuretic, gout, epilepsy, fever,

						polyuria.
72	*Vitex altissima L.f.*	Lamiaceae	Nemali adugu	Tree	Fruit	Fruit- Cardiac diseases, stomatitis, anorexia, digestive, carminative and leprosy.
73	*Waltheria indica L.*	Malvaceae	Nalla benda	Herb	Whole plant and leaf	Leaf- Internal haemorrhage, thirst; Whole plant- Febrifuge, purgative, emollient.
74	*Wrightia tinctoria R.Br.*	Apocynaceae	Reppala	Tree	Bark, latex and seed	Bark- Bilious problems, diabetes, flatulence, skin disorders, psoriasis; Latex- Mouth ulcers, cough, cold, fevers; Seed- Anthelmintic, aphrodisiac.
75	*Pseudarthria viscida* (L.) Wight and Arn.	Fabaceae	Nayaku ponna	Shrub	Root	Root- Rheumatism, asthma, cardiac problems, piles, biliousness, diarrhoea, worm infestation.
76	*Phyllanthus airy-shawii* Jean F. Brunel and J. P. Roux	Phyllanthaceae	Kanduru	Herb	Whole plant	Whole plant- Measles, jaundice, diuretic
77	*Terminalia pallida* Brandis	Combretaceae	Tella karaka	Tree	Stem bark and fruit	Stem bark- Diuretic, swellings; Fruit- Antipyretic, purgative, diuretic, diarrhoea, peptic ulcers, diabetes, veneral diseases, dysentery, fissures and cracks.
78	*Terminalia chebula* Retz.	Combretaceae	Nalla karaka	Tree	Stem bark and fruit	Stem bark- Diuretic, cardio tonic; Fruit- Rejuvenator, coryza, spure, piles, jaundice, cough, dyspnoea, carcinoma, tympanitis, diabetes, anorexia, laxative;
79	*Terminalia bellirica* (Gaertn.) Roxb.	Combretaceae	Tani kaya	Tree	Stem bark,	Stem bark- Diuretic and cardio tonic;

					fruit and gum	Fruit- Astringent, diabetes, tonic, digestive, laxative, rheumatism, swellings, bronchial asthma, trachyphonia, venereal diseases, hyper tension, dyspnoea; Gum- demulcent and purgative;
80	*Dodonaea viscosa* (L.) Jacq.	Sapindaceae	Banderu	Shrubs	Twigs and leaf	Twigs- used to construct house fences; Leaf- Bone fractures, antipyretic, burns, wounds, swellings.



Figure 1. Map showing Mallem konda, the study area.

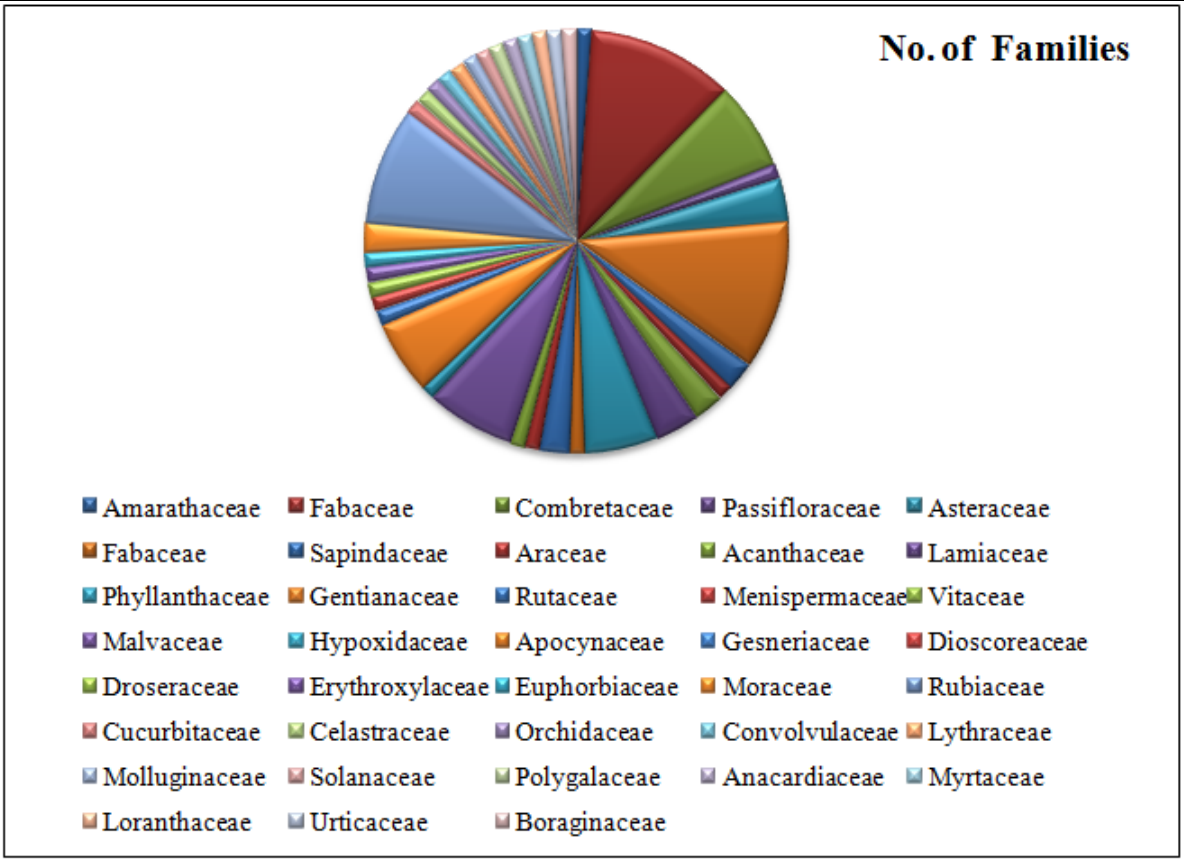


Figure 2: Family wise distribution of medicinal plants used by Tribals of Mallem konda

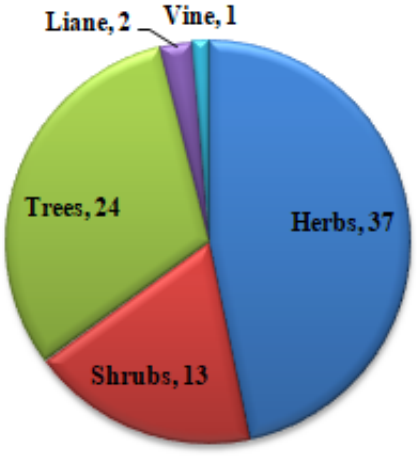


Figure 3: Life form of medicinal plants used by tribals of Mallem Konda

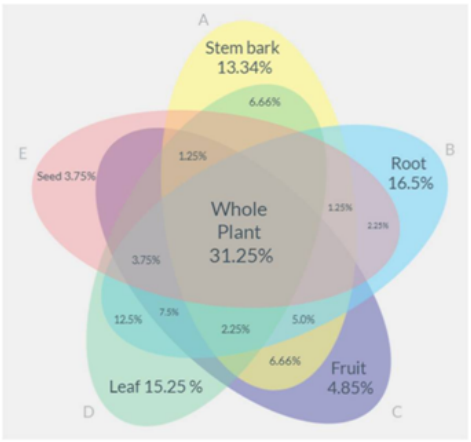
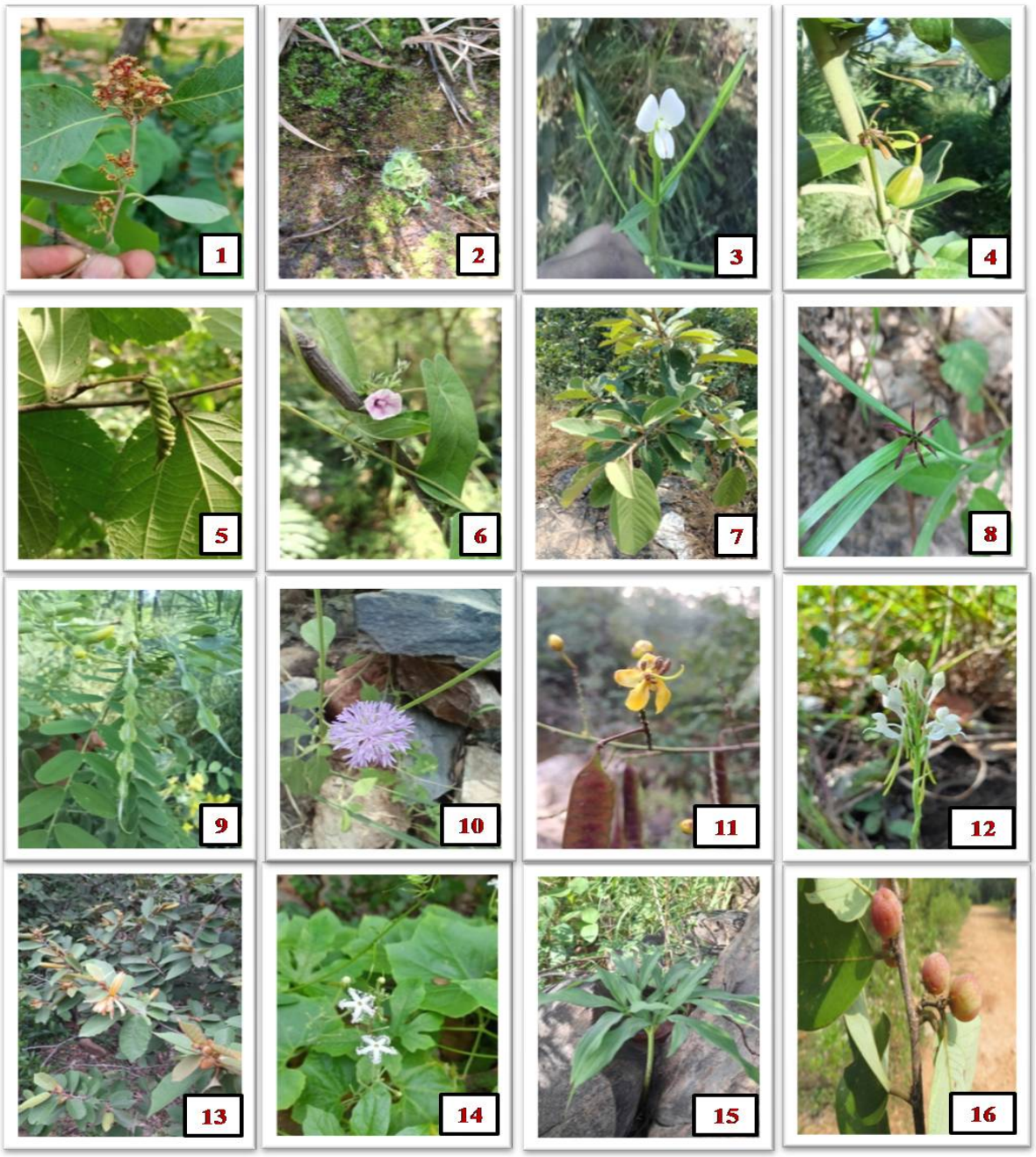
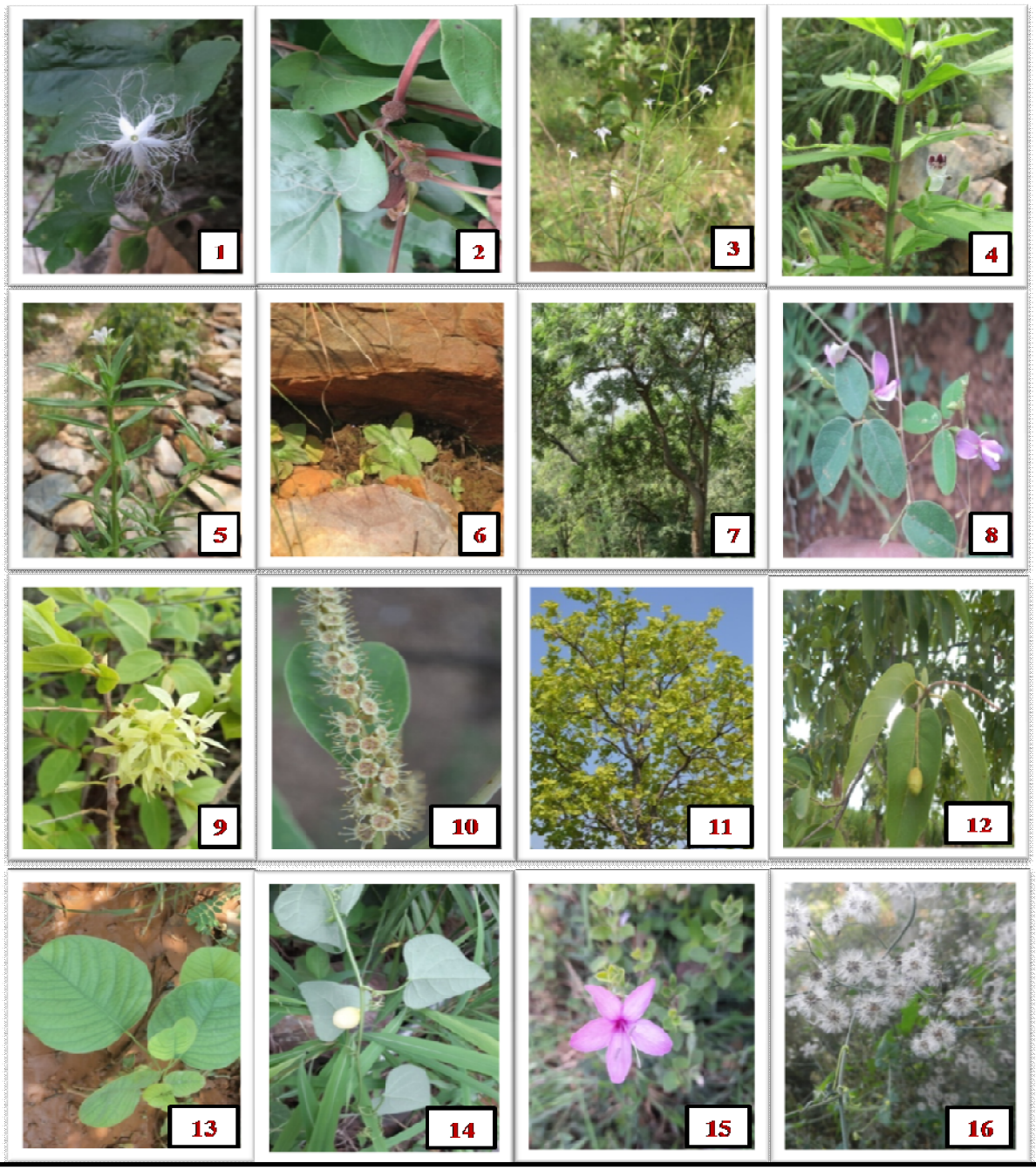


Figure 4: Venn diagram showing the usage of different plant parts as medicine



1. Anogeissus latifolia; 2. Drosera burmanni; 3. Canscora alata; 4. Aristalochia indica; 5. Helicteres isora; 6. Ipomoea eriocarpa; 7. Ficus mollis; 8. Iphigenia indica; 9. sophora interrupta; 10. Cyanthillium albicans; 11. Cassia Montana; 12. Habenaria roxburghii; 13. Pterospermum xylocarpum; 14. Gymnopetalum cochinchinensis; 15. Amorphophallus margaritifer; 16.Bridelia retusa;

Plate 1



1. Trichosanthes cucumerina; 2. Haldinia cordifolia; 3. Oldenlandia affinis ; 4. Andrographis glandulosa; 5. Oldenlandia umbellata; 6. Didymocarpus tomentosus; 7. Chloroxylon swietenia; 8. Galactia tenuiflora; 9. Getonia floribunda; 10. Terminalia bellirica; 11. Terminalia pallida; 12. Terminalia chebula; 13. Pterocarpus santalinus; 14. Adenia wightiana; 15. Barleria mysorensis; 16. Cyanthillium cinereum;

Plate 2

RESEARCH ARTICLE

Studies on Morphological Modification of Nano Hydroxyapatite Ceramic Powders through Organic and Inorganic Additives

C. Elavarasi[1], R. Mary Sinthiya[2], V. Thamizharasi[2], A. Arokia Nepolean Raj[1], S. Bharathi Bernadsha[3] and V. Collins Arun Prakash[1]*

[1]Assistant Professor, Department of Chemistry, Sacred Heart College (Autonomous), Tirupattur, (Affiliated to Thiruvalluvar University, Vellore), Tamil Nadu, India.
[2]Research Scholar, Department of Chemistry, Sacred Heart College (Autonomous), Tirupattur, (Affiliated to Thiruvalluvar University, Vellore), Tamil Nadu, India.
[3]Assistant Professor, Department of Physics, Sacred Heart College (Autonomous), Tirupattur, (Affiliated to Thiruvalluvar University, Vellore), Tamil Nadu, India.

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***Address for Correspondence**
C. Elavarasi
Assistant Professor,
Department of Chemistry,
Sacred Heart College (Autonomous), Tirupattur,
(Affiliated to Thiruvalluvar University, Vellore),
Tamil Nadu, India.
E.Mail: elavarasi@shctpt.edu

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ABSTRACT

Hydroxyapatite (HAP) with the chemical formula $Ca_{10}(PO_4)_6(OH)_2$ has been used extensively in the field of dental and orthopaedics for implant fabrication owing to its biocompatibility with human bone and teeth. The hydroxyapatite nanoparticle is mainly used in orthopaedic and dental applications due to its properties of bioactivity, osteoconduction, biocompatibility and non-toxicity. In this paper, we report the synthesis of organic and inorganic incorporated HAP powder by hydrothermal method. FTIR, XRD, SEM and TGA techniques were performed to analyse the chemical composition, crystallite structure and uniform morphology. The results indicated that the dextrin assisted alumina substituted hydroxyapatite nanoparticle has a uniform spherical morphology, smaller particle size with no impurities. The cytotoxicity was evaluated using MG-63 cell line study.The developed nano-HAP powders using organic and inorganic additives may be potentially used as bone substitute applications.

Keywords: Hydroxyapatite, Biocompatibility, Hydrothermal Method, Orthopaedic, Nanoparticle.

INTRODUCTION

Hydroxyapatite [HAP, $Ca_{10}(PO_4)_6(OH)_2$] is one of the most attractive materials for bone implant due to its compositional and biological similarity to natural bone. However, due to lower strength of pure HAP, it has been difficult to use it as bone implant for a long time. It is because the mechanical strength of this material mainly depends on grain size, grain size distribution, porosity, and other microstructural defects. In addition to this, the bioactivity of the synthetic hydroxyapatite has been found to be strongly affected by structural crystallinity [1]. The commonly used methods to prepare HAP mainly include hydrothermal, precipitation, solvothermal, spontaneous combustion, micro-emulsion, ultrasonic synthesis, bionic and solid state reaction, wet and dry methods [2]. Studies have found that the shape and size of hydroxyapatite can be regulated by changing the hydrothermal temperature, hydrothermal time and reaction concentration. According to this method, researchers have prepared high-purity hydroxyapatite of different shapes, such as nano spherical, rod-like, needle-like, flake, hexagonal prism, small plate, spine-like, etc [3-6]. Composite materials are formed by combining two or more materials that have quite different properties, and they do not dissolve or blend into each other. The different materials in the composite work together to give the composite unique properties [7]. Owing to excellent wear and corrosion resistance, alumina is widely used for composites in load bearing applications. It improves the mechanical properties of hydroxyapatite and is also helpful in retaining the biocompatibility [8]. Furthermore, addition of alumina into hydroxyapatite increases the compressive and bending strength of the composites [9-11]. Also, the in vitro compatibility evaluation of HAP–Al_2O_3nanocomposites prepared under microwave assisted synthesis and co-precipitation method shows similar biochemical activity like natural hydroxyapatite in bone [12]. The bio-inertness of alumina has no harmful effect on cells and tissues [13]. Another additive dextrin is a low-cost, broadly available raw material derived from starch, widely used in many industrial applications and accepted as a generally-recognized-as-safe (GRAS) food ingredient [14]. Dextrin is available in medical grade, has high solubility in water and DMSO, and holds hydroxyl groups suitable for bioconjugation, which has prompted this glucose polymer for several biomedical applications [15], including as hydrogels [16-18]. Studies have shown that they have synthesized successfully a nanocomposite system incorporating dextrin into nano HA/chitin matrix via co-precipitation route for the application in bone tissue engineering [19-25]. In the present study, hydrothermal synthesis was employed to synthesize HAP–nanocomposites using dextrin and alumina, as this method facilitates the formation of tunable nanostructures of hydroxyapatite with uniform morphological distribution, especially when assisted by dextrin and alumina. The resulting composite powders were subjected to structural, morphological, elemental and thermal characterization. Additionally, their biocompatibility was evaluated using the MTT assay on MG63 human osteosarcoma cell lines.

MATERIALS AND METHODS

Chemicals and Reagents

All chemicals used in this study were of analytical grade and the chemicals used to synthesize hydroxyapatite were purchased from Merck, Germany.Calcium nitrate tetrahydrate ($Ca(NO_3)_2.4H_2O$: 99%) and Diammonium hydrogen phosphate ($(NH_4)_2HPO_4$), Dextrin, Alumina, ammonia and ethanol were used without further purification. Deionized water was used throughout the synthesis.

Synthesis of Hydroxyapatite Nanoparticles

Hydroxyapatite was synthesized via the hydrothermal method using calcium nitrate tetrahydrate ($Ca(NO_3)_2{\cdot}4H_2O$) and diammonium hydrogen phosphate ($(NH_4)_2HPO_4$) as the calcium and phosphate precursors, respectively. Deionized water served as the solvent for both precursors. Solutions of 0.04 M $Ca(NO_3)_2{\cdot}4H_2O$ and 0.024 M $(NH_4)_2HPO_4$ were prepared to maintain a stoichiometric Ca/P molar ratio of 1.67. The phosphate solution was added dropwise into the calcium solution under constant stirring. Dextrin, used as an additive, and alumina, acting as a growth-regulating agent, were each dissolved in 50 mL of deionized water and added dropwise to the precursor mixture. The pH was adjusted to 11 using ammonia solution. The resulting mixture was stirred on a magnetic stirrer at 600 rpm for 1 hour. Subsequently, the entire solution was transferred into a Teflon-lined autoclave and subjected to

hydrothermal treatment in an oven at 120 °C for 3 hours. The resulting precipitate was washed repeatedly with deionized water and ethanol, then dried at 100 °C for 3 hours. The dried powder was further subjected to calcination at 600 °C and sintering process at 800°C to obtain phase-pure hydroxyapatite. The final product was characterized using various analytical techniques.

Sample Characterization

Powder X-ray diffraction (XRD) analysis was performed using a Bruker D2 Phaser instrument using Cu-Kα radiation (k = 1.5418 Å).FT-IR spectra of synthesized samples were also recorded on a Perkin Elmer spectrum two FTIR spectrometer in the 4000 to 350 cm^{-1} range and the spectra were recorded withS/N ratio: 2000:1 ppm for 1 minute scans. HRSEM investigations were performed by using Carl ZeisEVO 10 model. Thermogravimetric analysis (TGA) was performed using Perkin Elmer STA 6000. Measurements were performed in the nitrogen flow and the temperature was raised from 25°C to 800°C with a heating rate of 20°C min^{-1}.

Potential Application – MTT assay

The MG63, human osteosarcoma cell line was obtained from the National Centre for Cell Science (NCCS), Pune, India**.** Cells were cultured in Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS) and 1% antibiotic-antimycotic solution**.** Cultures were maintained at 37 °C in a humidified incubator with 5% CO_2 and 18-20% O_2**.** Cells were sub-cultured every three days to ensure optimal growth and viability.

RESULT AND DISCUSSION

XRD Analysis

The development of crystalline HAP phase was confirmed by XRD technique. In Fig. 1(a–d),XRD pattern of the synthesized hydroxyapatite powders obtained from hydrothermal method with various amounts of alumina and dextrin concentration were shown. Fig. 1 shows XRD pattern of HAP powder (a) HAP, (b) Dx-1.5% and Al-0.15 g, (c) Dx-2% and Al-0.1 g and (d) Dx-2% and Al-0.15g. All the four samples exhibited, the major characteristic peaks observed at 2θ values of 25.6, 28.7, 31.5, 33.8, 39.3, 46.3 and 49.1 corresponding to (002), (210), (211), (202), (212), (222) and (213) planes of hexagonal HAP. It was clear that, all the major peaks observed could be accredited to hydroxyapatite structure and no additional crystalline phases were seen. The high and narrow peaks show high crystallinity of the obtained HAP. The obtained pattern is in good accordance with the standard JCPDS data (09–0432). The average grain size of the HAP powders were calculated using Scherrer equation which is shown in Table. 1.

FT-IR Analysis

Figure 2 shows the FTIR spectrum of all the HAP samples. The functional groups present in the samples can be easily identified by the FTIR peaks. The HAPobtained with and without additive shows the absorption peaks (Fig.2 a)at 563, 603, 960, 1010–1130, 1400, 1635, 3000–3600, and 3554cm^{-1}. The peaks for HAP observed at 474cm^{-1} (ν_2 bending mode), 601 and 571cm^{-1} (ν_4 bending mode), 1096–1035cm^{-1} (ν_3 vibration mode) and 963cm^{-1}($\nu_1$stretching) clearly reveal the occurrence of the PO_4^{3-} group. The existence of the OH^- group observed at 3573cm^{-1} by the stretching vibration mode was distinct. The broad peak detected at 3423 cm^{-1} is assigned to the adsorbed lattice water. Furthermore, there was existence of CO_3^{2-} ions, which was confirmed by vibrational peaks observed at 1400cm^{-1}. The presence CO_3^{2-} ions might be due to its occurrence in the reaction solution used for the HAP synthesis [26].FTIR spectral assignment of the functional groups (Table 2).

SEM Analysis

Figure 3 (a–d) illustrates the surface morphology of the synthesized spherical hydroxyapatite (HAP) prepared via the hydrothermal method, using varying concentrations of dextrin and alumina. The pure HAP exhibits a network-like structure, as seen in Fig. 3(a). When synthesized with Dextrin-1.5% and Alumina-0.15g, the HAP particles appear

spherical, uniform, and free from agglomeration (Fig. 3b). In contrast, the sample with Dextrin-2% and Alumina-0.1 g displays a rice grain-like morphology Fig. 3(c), while the combination of Dextrin-2% and Alumina-0.15g results in significant agglomeration Fig. 3(d). Thus it was observed that the addition of dextrin and alumina has significantly reduced the particle size of HAP with uniform spherical morphology [27].

Thermal Analysis

The thermal stability of the prepared hydroxyapatite was analysed through thermogravimetric analysis and shown in Fig. 4. The thermogravimetric analysis (TGA) plots reveal the thermal stability of the hydroxyapatite (HAP) samples obtained with and without the additive to track their chemical decomposition as temperature increases. In Fig. 4(a) The initial weight loss observed below 120°C is attributed to the release of adsorbed surface water and the removal of any lattice water present within the HAP structure [28, 29]. Further heating up to 650°C results in weight loss due to the decomposition of carbonate species that is incorporated in the HAP [30].HAP is generally stable up to around 700 °C, with minimal weight loss due to moisture and decomposition. Beyond this temperature, no significant weight loss is observed up to 800°C, indicating the high thermal stability of the synthesized HAP powder. On adding dextrin and alumina, the thermal stability of the powders was found to increase (Fig. 4 b and c) 1%. This is due to the rapid densification of the HAP powders brought by the additives.

Cytotoxicity test by MTT Assay

The MTT assay was employed to evaluate the in vitro biocompatibility and proliferation of MG-63 cells in the presence of the dextrin and alumina HAP composite which was supported by microscopic images (Fig. 5). The assay was conducted with different concentrations such as 15.62µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml and 250 µg/ml. The composite exhibited increased cell viability with higher concentration. The cell viability obtained for 15.62µg/ml, 31.25 µg/ml, 62.5 µg/ml, 125 µg/ml and 250 µg/ml were 98.24, 95.13,89.97,84.23 and 78.2 % respectively is shown in Table 3. Compared to higher concentrations, the lower concentration exhibited significantly higher cell viability. The number of MG-63 cells observed on the samples indicated that as the concentration decreased, cell viability increased, suggesting effective attachment and proliferation of MG-63 cells on the synthesized composite which is shown in Fig. 6. This result provides strong evidence of the composite's favourable biocompatibility.

CONCLUSION

In this work, dextrin-alumina assisted HAP was prepared by a hydrothermal method. The functional groups and diffraction patterns of the synthesized HAP powders were characterized using FT-IR and XRD techniques. The product formation was well supported by FTIR evidences which showed no impurity peaks. The HAP powder was well crystallized with hexagonal structure with no impurity phases was obtained as evident from the XRD results.The SEM micrograph revealed that the synthesized size of the HAP powder was well controlled and the morphology was spherical.TGA analysis has proved the highthermal stability of the HAP powdersupto 800°C.The MTT assay showed non-cytotoxicity of the nanoparticles against MG-63 cells, demonstrating the good biocompatibility of the HAP powders. Hence these bioceramic powders could be well used for potential biomedical applications.

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Table 1: Calculated average grain size of HAP powders using Scherer's equation

Sample code	hkl values	Average grain size (nm)
HAP	(002), (210), (211), (202), (212), (222), (213)	39.4
Dx-1.5% and Al-0.15g	(002), (210), (211), (202), (212), (222), (213)	14.8
Dx-2% and Al-0.1g	(002), (210), (211), (202), (212), (222), (213)	32.3
Dx-2% and Al-0.15g	(002), (210), (211), (202), (212), (222), (213)	16.4

Table 2: FTIR spectral assignment of the functional groups.

Sample	OH		Phosphate			
	Stretching	Lattice Water	υ_1	υ_2	υ_3	υ_4
HAP	3640 and 631	3441	963	474	1089 and 1041	601 and 571
HAP, Dx-1.5% and A1-0.15	3567 and 637	3423	963	474	1089 and 1041	601 and 571
HAP, Dx-2% and Al-0.1 g	3573 and 637	3435	963	468	1096 and 1041	601 and 571
HAP, Dx-2% and Al-0.15g	3573 and 637	3435	963	474	1096 and 1035	601 and 571

Table 3: Proliferation Analysis on MG63 cell line on 24 hours

Sample	Concentration (μg/ml)	Cell viability (%)
Dextrin-1.5% and Alumina-0.15g	15.62	98.24
	31.25	95.13
	62.5	89.97
	125	84.23
	250	78.21

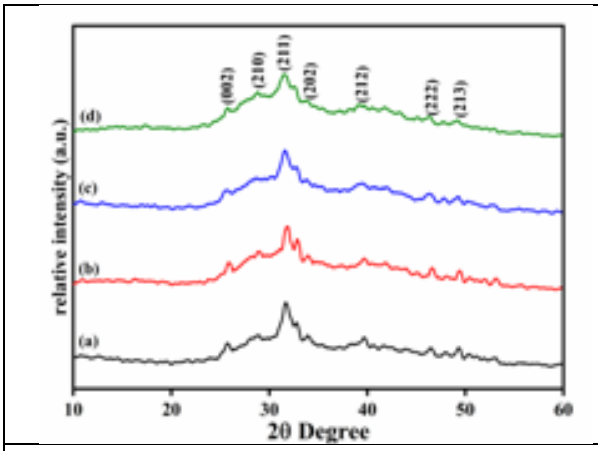


Fig. 1: XRD pattern of HAP powder (a) HAP, (b) Dx-1.5% and Al-0.15 g, (c) Dx-2% and Al-0.1 g and (d) Dx-2% and Al-0.15g.

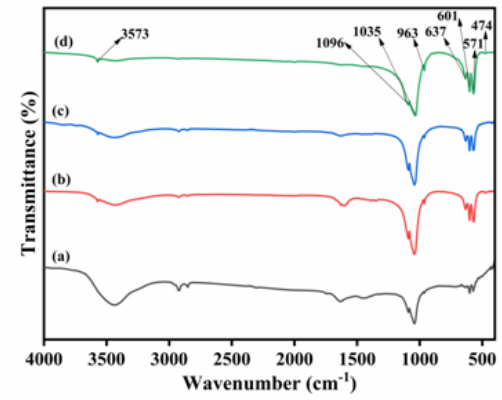


Fig. 2: FTIR spectra of HAP powder (a) HAP, (b) Dx-1.5% and Al-0.15 g, (c) Dx-2% and Al-0.1 g and (d) Dx-2% and Al-0.15g.

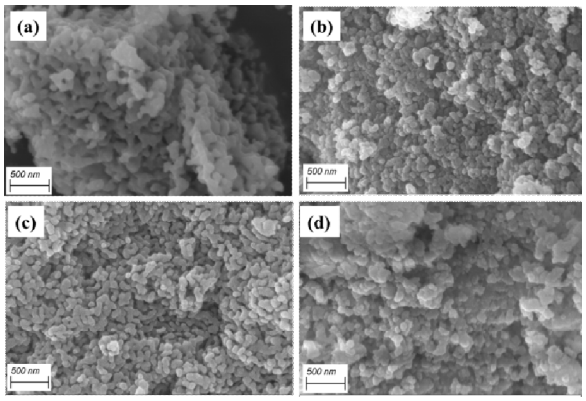


Fig. 3: SEM micrographs of HAP powder (a) HAP, (b) Dx-1.5% and Al-0.15 g, (c) Dx-2% and Al-0.1 g and (d) Dx-2% and Al-0.15g.

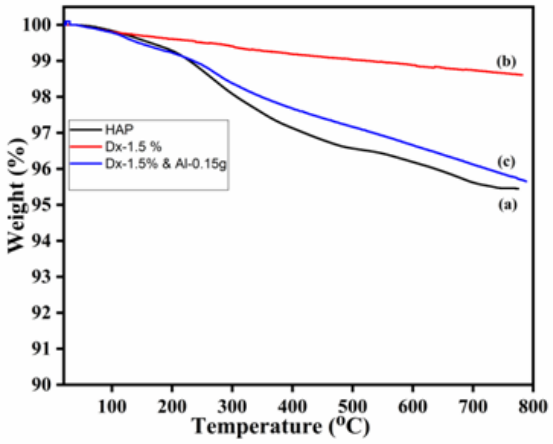


Fig. 4: TGA graph of (a) HAP, (b) Dx-1.5% and (c) Dx-1.5% and Al-0.15g

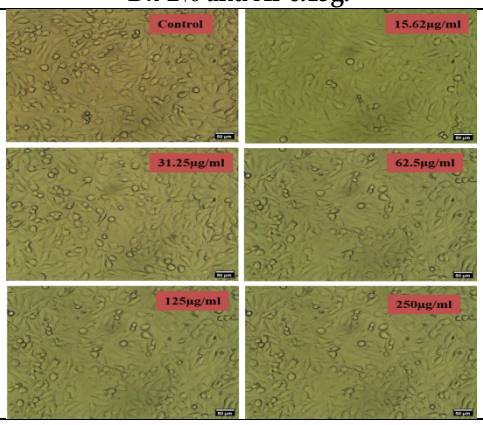


Fig. 5: Proliferation analysis of Dx-1.5% and Al-0.15g assisted HAP on MG-63 cell line

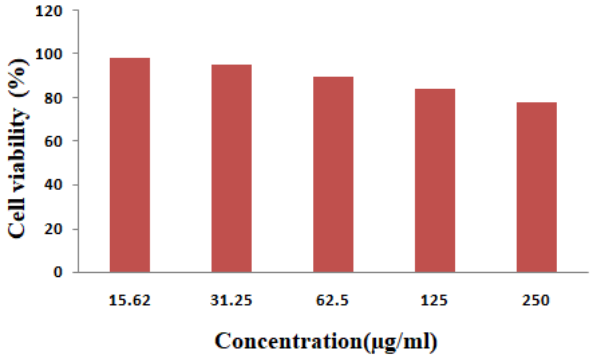


Fig. 6: Cell viability (%) of MG-63 cells at different concentrations of Dx-1.5% and Al-0.15g

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RESEARCH ARTICLE

Physicochemical Analysis and Potability Status of Traditional Naula Aquifers in District Champawat, Uttarakhand

Lata Kharkwal[1] and Dharmendra Kumar[2*]

[1]Research Scholar (Reg. No:. SSJURS20240176), Department of Zoology, Government P.G. College Maldevata (Raipur), (Affiliated to Sridev Suman Uttarakhand University Badshahithol, Tehri Garhwal), Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Zoology, Government P.G. College Maldevata (Raipur), (Affiliated to Sridev Suman Uttarakhand University, Badshahithol, Tehri Garhwal), Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Dharmendra Kumar
Assistant Professor,
Department of Zoology,
Government P.G. College Maldevata (Raipur),
(Affiliated to Sridev Suman Uttarakhand University, Badshahithol, Tehri Garhwal),
Dehradun, Uttarakhand, India.
E.Mail: oxidation01@gmail.com

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ABSTRACT

Naulas, the traditional groundwater harvesting structures of Uttarakhand, are vital sources of potable water in hilly regions and possess both ecological and cultural significance. This study evaluates the physicochemical quality and seasonal potability of water from seven Naula aquifers in the Champawat district. Monthly water sampling was conducted over a two-year period (April 2022 to April 2024) in accordance with BIS [IS 3025 (Part 1):1987] guidelines using pre-cleaned 500 mL polypropylene bottles. A total of 24 physicochemical parameters were analyzed using standard BIS methods, including pH, temperature, colour, odour, taste, electrical conductivity (EC), total dissolved solids (TDS), total hardness (TH), calcium, magnesium, alkalinity, turbidity, chloride, residual chlorine, dissolved oxygen (DO), biochemical oxygen demand (BOD), chemical oxygen demand (COD), cadmium, chromium, lead, iron, arsenic, mercury, and zinc. The results showed that all tested parameters were within the permissible limits prescribed by BIS, confirming the potability of Naula water. Seasonal Water Quality Index (WQI) values were 12.59 (Winter), 15.24 (Summer), and 17.53 (Monsoon), all falling under the "Excellent" category (WQI < 25), with a slight decline during the monsoon season due to surface runoff. These findings confirm that Naula water remains consistently safe for drinking throughout the year, highlighting the need for protection and sustainable management of these traditional water sources.

Keywords: Naula, Groundwater Quality, BIS Standards, Water Quality Index

INTRODUCTION

Water is a fundamental natural resource essential for the survival of all life forms on Earth. It plays a crucial role in maintaining ecological balance, supporting agricultural and industrial activities, and fulfilling domestic needs. Although nearly 70% of the Earth's surface is covered by water, only about 3% constitutes freshwater. Potable water is typically obtained from surface water bodies such as rivers, lakes, and reservoirs, as well as extracted from groundwater sources like wells, aquifers, and handpumps. However, increasing pollution due to urban expansion, industrial effluents, and agricultural runoff has severely affected surface water quality, resulting in greater reliance on groundwater sources for drinking purposes. Groundwater exists in underground layers of soil and rock and is accessed through various traditional and modern techniques. In rural and hilly regions of India, traditional methods are still widely practiced. For example, the 'Kul' irrigation system in Himachal Pradesh and underground tunnel methods in other regions are used to extract groundwater (More, 2020). These sources are not only essential for water supply but also hold deep cultural, religious, and historical value. In many Indian communities, such water sources are considered sacred and are associated with traditional beliefs, rituals, and customs. These traditional groundwater sources are known by different names across India, reflecting the country's cultural richness. In Uttarakhand, they are referred to as *Naulas*, while in Nagaland, similar systems are called *Zabo*. Naulas are small, stone-lined springs typically found in the hilly terrain of the Kumaon region (Rawat, 2021). These traditional structures are often preserved and worshipped by local communities, highlighting their ecological and spiritual significance. In this context, the present study aims to analyze the water quality of Naulas—traditional natural aquifers—in the Champawat district of Uttarakhand. As groundwater plays an increasingly vital role in meeting drinking water needs, assessing the quality of these sources is essential for protecting public health and ensuring the sustainable management of water resources.

MATERIALS AND METHODS

Sampling locations: Seven groundwater aquifers were selected for physicochemical analysis in the Champawat district, and the GPS coordinates of the sampling sites are provided in Table 1, with the sampling area shown in Figure 1. Water samples were collected in accordance with BIS guidelines [IS 3025 (Part 1):1987] using pre-cleaned 500 mL polypropylene bottles to ensure sample integrity. Data collection was conducted monthly over a two-year period, from April 2022 to April 2024, during morning hours to minimize variations due to diurnal changes. This consistent sampling schedule facilitated a comprehensive assessment of the potability and physicochemical characteristics of the selected aquifers. All laboratory analyses were performed at the laboratory of S.V. Government P.G. College, Lohaghat. Figure 2 illustrates the sampling locations along with the laboratory procedures carried out for the analysis of collected water samples.

Sample analysis: A total of 24 parameters were tested following the standard protocols prescribed by the Bureau of Indian Standards (BIS). The tested parameters and their respective BIS codes are: pH [IS 3025 (Part 11):1983], Temperature [Part 9:1984], Odour [Part 5:1983], Taste [Part 8:1984], Colour [Part 4:2021], Electrical Conductivity (EC) using a digital EC meter, Biochemical Oxygen Demand (BOD) [Part 44:1993], Chemical Oxygen Demand (COD) [Part 58:2006], Dissolved Oxygen (DO) [Part 38:1989], Residual Chlorine [Part 26:1986], Total Hardness [Part 21:2009], Calcium [Part 40:1991], Magnesium [Part 46:1994], Total Dissolved Solids (TDS) [Part 16:1984], Alkalinity [Part 23:1986], Turbidity [Part 10:1984], Chloride [Part 32:1988], Cadmium [Part 41:1992], Chromium [Part 52:2003], Lead [Part 47:1994], Iron [Part 53:2003], Arsenic [Part 37:1988], Mercury [Part 48:1994], Zinc [Part 49:1994]. For the seasonal potability analysis, the Water Quality Index (WQI) method was employed. Based on the regional climatic pattern, the year was categorized into three distinct seasons: Winter (November–February), Summer (March–June), and Monsoon (July–October).

RESULT AND DISCUSSIONS

The physicochemical analysis of Naula water across the seven sampling sites revealed that the water quality parameters largely conformed to the permissible limits set by the Bureau of Indian Standards (BIS). The pH values ranged from 7.0 to 7.4, indicating neutral to slightly alkaline nature, with a mean of 7.2 ± 0.15, well within the BIS range of 6.5–8.5. Temperature varied slightly across sites (9.4–10.8 °C), reflecting minimal thermal variation. Colour values were generally stable at 5 Hazen, except at Site 1 (2.3 Hazen), with an overall mean of 4.6 ± 0.9, meeting the standard limit of 5 Hazen. Electrical conductivity (EC) ranged between 306.85 and 502.5 µS/cm (mean: 392.1 ± 55.8 µS/cm), indicating moderate ionic presence. BOD and COD values (mean 2.1 ± 0.2 mg/L and 3.7 ± 0.7 mg/L, respectively) suggest low levels of biodegradable and chemical organic pollutants. Dissolved oxygen (DO) was high across all sites (7.1–8.3 mg/L), with a mean of 7.9 ± 0.4 mg/L, indicative of good water quality. Chloride concentrations remained low (mean: 26.2 ± 6.2 mg/L), far below the BIS threshold of 250 mg/L.Total hardness (TH) was within acceptable limits, ranging from 149.3 to 170.4 mg/L (mean: 157.1 ± 7.9 mg/L), with corresponding calcium (mean: 36.2 ± 1.9 mg/L) and magnesium (mean: 15.9 ± 1.4 mg/L) concentrations well under BIS limits. Total dissolved solids (TDS) were also within safe levels, with values between 180.3 and 208.3 mg/L (mean: 192.5 ± 9.5 mg/L). Alkalinity showed moderate variability (mean: 122.3 ± 12.1 mg/L), indicating adequate buffering capacity. Turbidity values remained low (mean: 2.2 ± 0.1 NTU), suggesting clear water. Residual chlorine averaged 0.08 ± 0.03 mg/L, below the upper limit of 0.2 mg/L, indicating the absence of excessive chlorination. Heavy metals, including chromium (0.0048 ± 0.0003 mg/L), lead (0.0043 ± 0.0009 mg/L), iron (0.05 ± 0.01 mg/L), and zinc (0.05 ± 0.008 mg/L), were detected in minimal concentrations, well below the permissible limits. Cadmium, mercury, and arsenic were below detection limits at all sites. Sensory attributes such as odour and taste were found to be acceptable across all samples.Overall, the results suggest that the Naula water is of good quality and fit for consumption, with all parameters falling within the BIS permissible limits. These results are summarised in Table 2 and Figure 3 represents the graphical illustration of the mean values of all tested water quality parameters. Similar findings have been reported by several researchers that support the results of the present study. Kotlia and Bakshi (2023) observed a slightly alkaline pH (7.637) in groundwater of Udham Singh Nagar, which aligns with the pH range of 7.0–7.4 recorded in the current Naula water analysis. Nayak *et al*. (2022) also reported comparable electrical conductivity (412.34–478.25 µS/cm) and TDS values (298.34–384.21 mg/L), which are in agreement with the EC (306.85–502.5 µS/cm) and TDS (183.9–208.3 mg/L) values in this study. Similarly, chloride concentrations (13.2–22.5 mg/L) and turbidity levels (0.24–1.11 NTU) noted by Nayak *et al*. were close to those found in Naula water, confirming low salinity and clear appearance. Patni *et al*. (2022) reported total hardness between 104–208 mg/L, calcium in the range of 16.67–37.19 mg/L, and magnesium from 14.37–34.98 mg/L in primitive water sources of Champawat district, which are comparable to the present study's values of TH (149.3–170.4 mg/L), Ca (33.5–40.4 mg/L), and Mg (14.3–18.9 mg/L). Furthermore, their findings of satisfactory DO levels and moderate EC also corroborate this study's observations. These consistent results across multiple studies strengthen the conclusion that the groundwater in this hilly region remains within safe and potable limits. Patni *et al*. (2022) also did not observe the presence of hazardous heavy metals like uranium in their analysis of primitive water sources in Champawat, reporting concentrations well below the BIS permissible limit. This observation is consistent with the present study, where all tested heavy metals—including chromium, lead, iron, and zinc—were found within safe limits, and toxic elements like cadmium, mercury, and arsenic were below detection levels. These similarities further confirm that groundwater in this region is largely free from heavy metal contamination and remains safe for human consumption.

Seasonal water potability analysis

For the seasonal assessment of water quality, the Water Quality Index (WQI) was computed following the method proposed by Brown *et al*. (1972). The study period was categorized into three distinct seasons based on regional climatic trends: Summer (March–June), Monsoon (July–October), and Winter (November–February). WQI serves as a comprehensive numerical indicator that synthesizes multiple physicochemical parameters to provide an overall evaluation of water quality. Each parameter was assigned a specific weight (Wn) according to its relative importance to human health, and a quality rating (Qn) was derived for each.

The WQI was calculated using the formula
WQI = (ΣQnWn) / (ΣWn). Table 3 presents the classification of water quality based on WQI values, while Table 4 summarizes the seasonal WQI values computed for each sampling site.
A total of 18 physicochemical parameters were selected for the Water Quality Index (WQI) analysis to ensure a comprehensive assessment of seasonal water quality. The Water Quality Index (WQI) values calculated for different seasons reveal noticeable seasonal variation in water quality. The WQI for the Winter season was found to be 12.59, followed by 15.24 during Summer, and the highest value of 17.53 was observed during the Monsoon season. According to the WQI classification (Table 3), all seasonal values fall within the "Excellent" water quality category (0–25). However, the gradual increase in WQI from winter to monsoon indicates a relative decline in water quality, likely due to increased surface runoff and contamination during the monsoon. Overall, the analysis suggests that water quality remains suitable for drinking throughout the year, with the best quality recorded in winter and relatively lower quality in the monsoon season. A comparable trend in water potability was also observed by Patni *et al*. (2022), who assessed aquifers in the Champawat region and reported that these groundwater sources remained within potable. These sources are considered suitable for drinking purposes and can serve as reliable alternative water sources, especially during the summer season when surface water availability is limited.

CONCLUSION

The comprehensive analysis of water quality from these traditional groundwater sources confirms that they are physiochemically fit for potable use, with all tested parameters, including heavy metals, falling within permissible limits set by national standards. The Water Quality Index (WQI) analysis indicates only minor seasonal fluctuations, yet values consistently remained within the potable range, highlighting the overall stability of water quality throughout the year. These ancient and culturally significant water sources, though once central to local water supply systems, are now facing neglect due to reduced popularity, low maintenance, and structural degradation. Despite their age, these sources still possess the capacity to meet the potable water demands of the region, especially during water-scarce periods like summer. Therefore, it is crucial to implement protection, restoration, and regular maintenance measures to preserve these heritage water systems. Ensuring their sustainability could contribute significantly to addressing the ongoing water scarcity challenges in the area.

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Table 1. Location Details and GPS Coordinates of Groundwater Aquifer Sampling Sites

Sampling Site (Aquifer)	Longitude (°E)	Latitude (°N)
Katreshwer Naula	80.066473	29.413312
Taxi Stand Naula	80.089116	29.402927
Rukmani Naula	80.087683	29.39951
Risheshwar Naula	80.08935	29.395988
Chauri Naula	80.094708	29.391366
Khetigarh Naula	80.112118	29.374101
Gaudi Naula	80.123208	29.361777

Table 2: Physico-Chemical and Heavy Metal Profile of Naula Water (2022–2024) Across Seven Sites

Parameter	Unit	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7	Mean	St. Dev	BIS Limit
pH	–	7.3	7.1	7.3	7.1	7.0	7.4	7.0	7.2	0.15	6.5–8.5
Temperature	°C	10.5	9.8	10.8	9.4	9.9	10.5	10.2	10.2	0.4	NS
Colour	Hazen	2.3	5.0	5.0	5.0	5.0	5.0	5.0	4.6	0.9	5
EC	µS/cm	306.85	360.6	364.6	502.5	416.6	395.04	399.0	392.1	55.8	NS
BOD	mg/L	1.8	2.1	2.2	1.9	2.4	2.0	2.4	2.1	0.2	NS
COD	mg/L	2.8	4.21	3.3	3.3	4.9	3.2	4.6	3.7	0.7	NS
DO	mg/L	8.2	8.2	7.5	8.3	8.05	7.9	7.1	7.9	0.4	NS
Chloride (Cl^-)	mg/L	12.07	23.8	30.6	26.6	28.5	30.9	30.95	26.2	6.2	250
Total Hardness (TH)	mg/L	149.3	151.3	153.9	152.3	168.2	170.4	154.2	157.1	7.9	300
Calcium (Ca)	mg/L	33.5	36.05	36.5	35.5	34.9	40.4	36.8	36.2	1.9	75
Magnesium (Mg)	mg/L	15.9	14.3	15.2	15.4	18.9	16.9	14.9	15.9	1.4	30
TDS	mg/L	183.9	180.3	186.1	189.0	208.3	200.7	199.5	192.5	9.5	500
Alkalinity	mg/L	96.6	124.5	114.7	118.6	138.2	130.7	132.5	122.3	12.1	300
Turbidity	NTU	2.1	1.9	2.4	2.0	2.4	2.4	2.1	2.2	0.1	5
Residual Chlorine	mg/L	0.04	0.13	0.1	0.05	0.07	0.1	0.07	0.08	0.03	0.2
Chromium (Cr)	mg/L	0.004	0.004	0.005	0.005	0.005	0.004	0.004	0.0048	0.0003	0.05
Lead (Pb)	mg/	0.006	0.004	0.004	0.004	0.004	0.005	0.003	0.00	0.00	0.01

	L								43	09	
Iron (Fe)	mg/L	0.06	0.05	0.06	0.05	0.05	0.07	0.01	0.05	0.01	0.3
Zinc (Zn)	mg/L	0.06	0.04	0.06	0.05	0.05	0.06	0.05	0.05	0.008	5
Cadmium (Cd)	mg/L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	0.001
Mercury (Hg)	mg/L	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	-	-	0.001
Arsenic (As)	mg/L	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	<0.005	-	-	0.05
Odor	-	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	-	-	
Taste	-	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	Acceptable	-	-	

Table 3: Water Quality Index (WQI) Classification and Corresponding Water Quality Status

WQI table	
WQI	**Status**
0-25	Excellent
26-50	Good
51-75	Poor
76-100	Very Poor
Above 100	Unsuitable for drinking

Table 4: Seasonal WQI of tested samples (2022-2024)

S. No.	Parameter	Winter W_iQ_i	Summer W_iQ_i	Monsoon W_iQ_i
1	pH	0.24704	0.252975	0.2564
2	Colour	0.4437	0.443701	0.5042
3	EC	0.00061	0.000693	0.0007
4	BOD	0.42017	0.560229	0.7843
5	COD	0.07437	0.092522	0.1184
6	DO	0.86723	0.796646	0.726
7	Cl	0.00137	0.001588	0.0019
8	TH	0.00396	0.004412	0.0048
9	Ca	0.01497	0.016224	0.0175
10	Mg	0.03938	0.044538	0.05
11	TDS	0.00176	0.001939	0.0021
12	Alkalinity	0.00688	0.007694	0.0085
13	Turbidity	0.16135	0.211767	0.2823
14	R. Chlorine	3.15129	5.042062	6.9328
15	Cr	4.73954	4.840379	4.9412
16	Pb	1.15967	1.184884	1.1596
17	Fe	1.26052	1.73671	1.7367
18	Zn	0.00545	0.005345	0.0065
	WQI Result	12.59	15.24	17.53

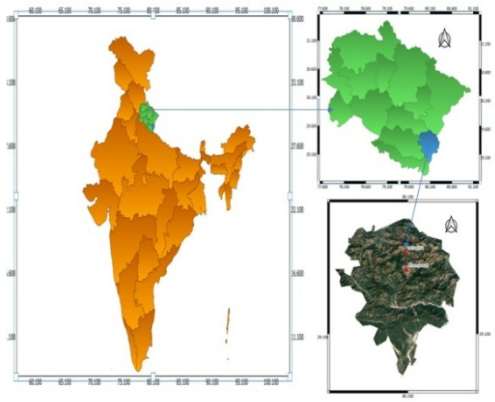


Figure 1: Geographical Map of the Study Area in Champawat District



Figure 2: Representative images of traditional groundwater aquifer sources (Naulas) from selected sampling sites, alongside laboratory procedures undertaken for the physicochemical analysis of collected water samples.

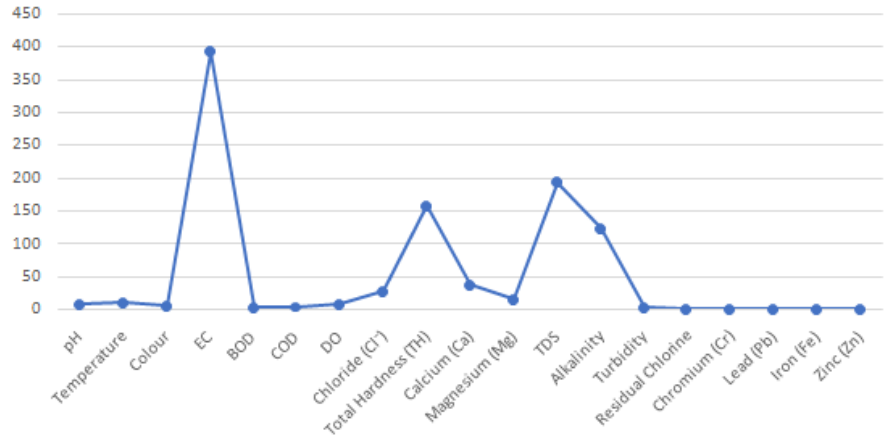


Figure 3: Graphical Representation of Mean Values of All Tested Water Quality Parameters

RESEARCH ARTICLE

Solanum trilobatum Linn.: An Herbal Guardian against Oxidative Damage, Infections and Cancer - An *In vitro* Approach

Rejitha L. R[1]*, Suju Skaria C[1] and Merlin S[2]

[1]Assistant Professor, Department of Botany, Mar Ivanios College, (Affiliated to University of Kerala, Palayam) Thiruvananthapuram, Kerala, India.
[2]Student, Department of Botany, Mar Ivanios College, (Affiliated to University of Kerala, Palayam) Thiruvananthapuram, Kerala, India.

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***Address for Correspondence**
Rejitha L. R
Assistant Professor,
Department of Botany,
Mar Ivanios College,
(Affiliated to University of Kerala, Palayam)
Thiruvananthapuram, Kerala, India.
E.Mail: rejitha.lr@mic.ac.in

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ABSTRACT

Solanum trilobatum Linn. commonly known as Purple Fruited Pea Eggplant or Thuthuvalai in traditional Indian medicine, is a well-known medicinal plant in Ayurveda and Siddha medicine. It is valued for its various pharmacological properties. The plant is known for its effectiveness in treating respiratory ailments like asthma, bronchitis, and cough, it also possesses anti-inflammatory, antidiabetic, and liver-protective benefits. The rich in bioactive compounds present in the plant, helps boost immunity, improve digestion, and combat infections. Additionally, its potential in cancer prevention and neuroprotection makes it a promising natural remedy. In the present study, antioxidant, antibacterial and anticancer efficacy was carried out in aerial parts of petroleum ether extract of *S. trilobatum.* The antioxidant activity was done by using DPPH and was observed that, extract showed low IC_{50} value in comparison with the standard ascorbic acid, thereby making it an effective antioxidant agent. Further, antibacterial assay conducted against the bacterial strains *Staphylococcus aureus* and *E. scherichia coli* showed good activity against both the strains. Also, anticancer studies conducted via MTT assay also proved the plant extract as a good anti-cancer agent. Further using thin layer chromatographic studies, major compounds in the fraction were also identified.

Keywords: *Solanum trilobatum* Linn., Antioxidant, Antibacterial, Anticancer, Petroleum extract

INTRODUCTION

Plants as a source of medicinal compounds have continued to play a dominant role in the maintenance of human health since ancient times. According to the World Health Organization plant extracts or their active constituents are used as folk medicine in traditional therapies. Over 50% of all modern clinical drugs are of natural product origin (Kirbag *et al.,* 2009). Phytochemicals such as vitamins, carotenoids, terpenoids, flavonoids, polyphenols, alkaloids, tannins, saponins, pigments, enzymes and minerals have antimicrobial and antioxidant activity (Madhuri and Pandey, 2009). The specific function of many phytochemicals is still unclear; however, a considerable number of studies have shown that they are involved in the interaction of plants/pests/diseases. Medicinal plants contain some organic compounds which produce definite physiological action on the human body and these bioactive substances include tannins, alkaloids, carbohydrates, terpenoids, steroids and flavonoids (Edoga *et al*., 2005). They are of great importance to the health of individuals and communities. Many of these indigenous medicinal plants are used as spices and food plants. Phenolics have been known to possess a capacity to scavenge free radicals. The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors. Phenolics are especially common in leaves, flowering tissues and woody parts, such as stems and barks. The Solanaceae family comprises 3000–4000 species that are classified in approximately 90 genera. The family is highly diverse, includes perennial trees as well as herbaceous annual species and occupies a wide range of terrestrial habitats from deserts to rainforests (Knapp *et al.*, 2014). Many species of *Solanum* have pharmaceutical as well as horticultural importance. Isolation of solasodine was reported from *S. aculeatissimum*, *S. neocardensaii* (Rejitha and Ashalatha, 2014). The presence of Solasodine, Solasonine, Diosgenin were identified and extracted from *Solanum* species. *Solanum trilobatum* Linn. has been given supreme importance in the folk and traditional medicines. The plant is commonly available in Southern India. *Solanum trilobatum* is reported to cure numerous diseases viz., tuberculosis, respiratory problems and bronchial asthma and was reported to have hepatoprotective activity (Pratheebha *et al.,* 2013). Phytochemical screening showed the presence of carbohydrates, saponins, phytosterols, tannins, flavonoids and cardiac glycosides as major phytochemical group (Govindan *et al.,* 1999), treatment for cancer (Kanchana and Balakrishnan, 2011), (Swapnalatha and Kannabiran, 2006) and liver infection (Shajahan *et al.,* 2004). Owing to the great demand for the medicinal plants in global levels for the advancement in the drug and phytochemical preparations for curing many diseases, our goal of the present study was to determine the specific bioactivity of total steroidal fraction obtained from the crude petroleum ether extract of *Solanum trilobatum.*

MATERIALS AND METHODS

Phytochemical analysis

Petroleum ether extraction

Aerial part of *Solanum trilobatum* was cleaned, chopped, dried and powdered. Weighed amount of the plant powder were filled in a soxhlet extractor and extracted exhaustively with petroleum ether (60-80°C) for 10 hours. The extracts were allowed to evaporate and maintained as a stock in refrigerator.

Isolation of steroid rich component from petroleum ether extract

A part of the residue of the petroleum ether soluble portion was saponified by refluxing for 30 minutes with 0.5N alcoholic KOH, cooled and diluted with distilled water. Transferred the extract into a separatory funnel and extracted with diethyl ether (5 to 6 times) after extraction, transferred to a conical flask and added anhydrous sodium sulphate, tightly stoppered, kept overnight and filtered repeatedly with diethyl ether. The extract was concentrated. The concentrated steroid rich component was further fractionated. Characterization of the steroids was done by using Olympus stereomicroscope.

Antioxidant activity

Quantitative measurement of radical scavenging property of extract was carried out according to the method of (Mensor *et al.,* 2001). A methanolic solution of 1 mL of DPPH (0.1mM) was added to 1mL of different concentrations

(50- 1000 µg/mL) of the extract and allowed to react at room temperature for 30 min in the dark. Absorbance was measured at 517nm. Methanol served as the blank, DPPH in methanol without the extract served as the control and ascorbic acid was taken as the reference standard. Lower absorbance of the reaction mixture indicated higher free radical scavenging activity. The capacity to scavenge the DPPH radical was calculated using the following equation

% radical scavenging activity = $\left[\frac{Abs.control-Abs.sample}{Abs.control}\right]\times100$. The IC_{50} values (concentration of sample required to scavenge 50% of free radicals) were calculated from the regression equation of the graph.

Antimicrobial activity

Disc diffusion method as described in European pharmacopeia with slight modification was used for antimicrobial testing (Tahseen, 2015). Sterility checked Nutrient Agar plates were used for the test. Bacterial stock cultures of *Staphylococcus aureus* (MTCC 96) and *Escherichia coli* (ATCC 25922) maintained at the Department of Biotechnology, University of Kerala was used for the test.Freshly sub-cultured bacterial strains were suspended in 1 mL nutrient broth and incubated at 37°C for 2 hours to obtain log phase cultures, opacity was checked with 0.5 McFarland turbidity standards. About 100 µL of the pure cultures of test strains were swabbed uniformly using a sterile swab on the surface of nutrient agar to obtain an even inoculum. The plates were allowed to dry for 5 minutes. Sterile discs loaded with extracts, DMSO (negative control) and Streptomycin (positive control-standard) were placed on to the microbe swabbed nutrient agar. The plates were kept for incubation at 37°C. The zone of inhibition marking the antibacterial activity was noted in mm diameter.

Anticancer activity

The extract was tested for anti-proliferative activity and presence of any cytotoxic property by using MTT test for the cell lines. 4T1 mouse mammary tumor cells were seeded in 96 wells plate and after 24 h of incubation they were treated with different concentrations of isolated compound to further incubate for 72 h. After that incubation media was replaced with MTT solution (0.5 mg/mL DMEM media) and incubated for 2 h at 37 °C in 5 % CO_2 and 100 % humid environment. MTT solution was then discarded and resulting formazan crystals were solubilized in 200 µL of DMSO by continuous shaking. The optical density was read at 595 nm using Multiskan PLUS ELISA plate reader (Thermo Fisher Scientific). For each concentration of isolated compound to be tested three replicate wells were used in three independent experiments and the percentage of cell viability was calculated as follows

Percentage of cell viability (%) = ((A0-A1))/A0×100

where; A0 is the absorbance wavelength of control and A1 is the absorbance wavelength of test compound/standard solution.

Preparation of analytical TLC plates and identification of lead compounds

Thin Layer Chromatographic plate was prepared using silica gel G 60 (Merck, India) as the adsorbent. A slurry of silica gel G was prepared by mixing with distilled water 1:2 (w/v) and spread on 5mm thick glass plate. The plate was dried at room temperature (32°C). Hexane, diethyl ether and acetic acid (85:15:2) was used as the solvent system for TLC. Rectangular glass chromatographic chambers (30X20X25cm) were saturated. Concentrated samples of known quantity of steroid component were dissolved in minimum volume of DMSO and spotted on TLC plates, 2 cm from the bottom of the plate using a capillary tube. The spots were dried in air and the plates were placed in the chromatographic chamber and sealed tightly with a glass lid. The solvent was allowed to run until the solvent front reached three fourth of the total length of the plate. The TLC plate was then removed from the chamber, the solvent front was marked and allowed to dry in air. The three different fractions were obtained and detected using iodine crystals. The Rf value of different fractions was calculated.

RESULTS

Antioxidant activity

The antioxidant activity of the aerial part of *Solanum trilobatum* was studied by carrying out DPPH (1-1-diphenyl-2-picryl hydrazine) Free Radical Scavenging Assay. The results were explained on the basis of the percentage of inhibition for radical scavenging activity of standard (Ascorbic Acid), petroleum ether extract and total steroids (Table I).For the standard, ascorbic acid the concentration taken was 10, 25, 50, 75 and 100 µg/mL and the percentage of inhibition was 45.03, 71.61, 85.12, 94.1, and 98.99 respectively. The standard shows low IC_{50} value since it is an analytical standard. It is clear that as the concentration increases the percentage of inhibition also increases. A percentage inhibition of 98.99 was observed in 100µg/mL and the minimum value 45.03 for 10µg/mL. At a concentration of 62.5µg/mL the percentage inhibition for the petroleum ether extract was 13.7% and that of steroid rich component was 18.18%. For the petroleum ether extract at 1000µg/mL it was 78.64% and 84.82% for the steroid. The concentrations taken for petroleum ether extract and steroid rich component were 62.5, 125, 250, 500 and 1000µg/mL. The % inhibition of petroleum ether extract was 13.7, 36.22, 47.76, 62.05, 78.64% and that of steroid rich component was 18.18, 40.4, 54.55, 71.57, 84.82% respectively. Graphs were plotted for these values.The IC_{50} value of the standard ascorbic acid is 11.24µg/mL. The IC_{50} value of the petroleum ether extract is 278.66µg/mL and that of steroids is 212.72µg/mL. In pure steroid component IC_{50} value was very low and is more effective in antioxidant activity. Lower the IC_{50} values the more effective will be the sample with reference to the standard. Since steroids show lower IC_{50} value it is further analyzed for anticancer activity.

Antibacterial activity

Antibacterial activity of the *S.trilobatum* was evaluated *in vitro* against the bacterial strains of gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The antibacterial activity test revealed that the inhibitory effect is found in both the bacterial strains. The petroleum ether extract taken for antibacterial activity for both *Staphylococcus aureus* and *Escherichia coli* were 250mg and that of steroids were 25mg. Bacterial zone of inhibition was observed at a concentration of 100µL. The total steroids isolated from the petroleum ether extract was white needle like crystals and was dissolved in DMSO, 100µL positive control streptomycin (0.1mg/mL) was taken as the positive control and DMSO taken as the negative control (100µL). The zone of inhibition for both *Staphylococcus aureus* and *Escherichia coli* is shown in Fig.II. For the gram positive bacteria *Staphylococcus aureus* the zone of inhibition of petroleum ether extract was 20mm and that of steroids was 15mm while the positive control streptomycin showed zone of inhibition 25mm. In case of gram negative bacteria *Escherichia coli* the zone of inhibition of petroleum ether extract was 15mm and that of steroids was 14mm while the positive control streptomycin showed zone of inhibition 20mm. The negative control showed no zone of inhibition in both the cultures. In *Staphylococcus aureus* the petroleum ether extract showed the zone of inhibition of 20mm and for the steroid was 15mm (Table II). But the concentration used for the petroleum ether extract was 250mg and the steroids were 25mg. In *Escherichia coli* the zone of inhibition of petroleum ether extract was 15mm and that of steroids was 14mm. As the concentration taken for steroids was only 25mg the activity can be compared with 250mg of the petroleum ether extract. The isolated steroids can produce remarkable antibacterial activity even though the concentration used was 25mg.

Anticancer activity

The anticancer activity steroid extract of *Solanum trilobatum* was studied by carrying out MTT Assay. The results were explained on the basis of values of percentage viability and percentage cytotoxicity summarized (Table III). A graph was plotted with percentage of cytotoxicity against concentration. The IC_{50} values were calculated from the regression equation of the graph. The IC_{50} value was 90.92µg/mL. As the concentration increases percentage viability decreases and as the concentration increases percentage cytotoxicity increases. For concentrations 6.25, 12.5, 25, 50,100 and 200µg/mL the percentage viabilities are 89.83, 83.60, 75.83, 58.72, 45.50 and 38.11 and their percentage cytotoxicities are 10.17, 16.40, 24.17, 41.28, 54.50 and 61.89 respectively. In a higher concentration of steroid it shows high % cytotoxicity.

TLC preparation

For the phytochemical screening of *S.trilobatum* sterol rich fraction on separation by TLC clearly showed that the showed seven fractions. The R_f values for each fraction was calculated and designated as FI- FVII for the studies (Fig.III) (Table IV).

DISCUSSION

Traditional use of medicinal plants to cure specific ailments had been practiced in ancient times. Although synthetic drugs and antibiotics brought a revolution in controlling different diseases, herbal medicine occupied a significant role as raw material for some important drugs (Hong, 2011). Over the past dozen years, increasing attention has accordingly been paid to natural products in the search for novel drugs in combination with new technology (Nago *et al*., 2013). There is a continuous search for potent and cheaper raw material in the drug industry. The actions of the herbs depend on the interaction and synergy of many unknown biochemical components. The traditional herbs should be subjected to a thorough analysis towards standardization. The present investigation is an attempt to identify the antimicrobial, antioxidant and the anticancer activity of crude petroleum ether extract and the total steroids isolated from the petroleum ether extract. The plant *Solanum trilobatum* is widely seen in warmer parts of the Asian continent as inhabitant of scrublands, mainly distributed throughout the Southern India. It has been used in the areas where it is cultivated for treating various ailments. For phytochemical studies appropriate selection of the solvent is an essential pre-requisite for the extraction of active principle from plants. Various alkaloids present in the family Solanaceae have already been identified and described (Evans and Ramsey, 1983). Cytotoxic potential of different solvent extracts from *S. trilobatum*has been reported and "Sobatum,"- the partially purified component of the plant obtained from the petroleum ether extract, contained betasitosterol as the active principle and exhibited anti-tumor activity (Mohanan and Devi, 1997). Presence of a steroid, solasodine (glycoalkaloid), was also reported from *Solanum trilobatum*. Various chemical constituents are reported from *Solanum* species, which includes alkaloids, phenolics, flavanoids, sterols, saponins and their glycosides.

Alkaloids such as soladunalinidine and tomatidine were isolated from leaf and stem of *Solanum* species. This plant has been reported to possess a broad spectrum of antibiotic, antibacterial and anticancer activities (Mohanan and Devi, 1996). Owing to the increased demand for the phytosterols in curing various diseases, the attention was given to meet the hike in the demand. Reports claim that phytosterols have effects in the treatment of cancer, rheumatoid arthritis, allergies and stress related illness (Balakrishnan *et al*., 2015). Petroleum ether, chloroform, methanol and ethanol were the commonly used solvents for extraction (Salie *et al*., 1996). Chloroform which showed low efficiency in the extraction of steroids, petroleum ether was used as the solvent for extraction and is agreeing with the reports of Eong and Achance (2001). Saponification was carried out for the isolation of steroids from petroleum ether extract using ethanol and KOH. Similar results were also obtained by Chern *et al*., (1999) while isolating a hormone of *Phytophthora parasitica*. Isolation of steroids had already been reported from the extracts of aerial parts and roots of *Buddleja cordata* (Avila *et al*., 1999) *Rubus impertialis* (Kanegusuku *et al*., 2002) and *Agave attenuate* (da Silva *et al*., 2002). Needle like crystals were obtained from the isolation and concentration of the steroids. In the antioxidant activity, the aerial part of *Solanum trilobatum* was studied by carrying out DPPH free radical scavenging activity. Lower absorbance indicated higher free radical scavenging activity. This method is based on the reduction of an alcoholic DPPH solution in the presence of a hydrogen donating antioxidant due to the formation of non-radical form DPPH-H by the reaction. Certain plant metabolites have been reported to have strong antioxidant activities which help to protect cells against oxidative damage by free radicals. In the DPPH radical scavenging assay, the results obtained from the extracts of *Solanum trilobatum* were compared with ascorbic acid. In the present study, highest DPPH scavenging activity was shown by the total steroids, 212.72µg/mL.

Ascorbic acid inhibited 50% of free radicals at 11.24µg/mL concentration. For both ascorbic acid and the sample, the percentage of inhibition increased as their concentration increased. In the study of berberine the IC_{50} value of berberine was reported as 42.7µg/mL (Shirwaikar *et al*., 2006). In the present study the purified component of

Solanum trilobatum show higher antioxidant activity also due to the low IC_{50} activity. The *in vitro* antioxidant studies of chloroform extracts from *Solanum trilobatum* were also studied (Sini and Devi, 2004).The chloroform extract of *Solanum trilobatum* exhibited less scavenging effect on DPPH radicals. The antibacterial efficacy of the *Solanum trilobatum* extract was tested. The antibacterial activity test revealed that the overall inhibitory effect is found in the tested bacterial species. The extract at 100µg for gram positive bacteria (*S. aureus*) and 100µg for gram negative bacteria (*E. coli*). The tested sample exhibited a higher level of inhibitory effect on *S. taphylococcus aureus*. Similar results were obtained in butanol extract of *S. trilobatum* against *E. coli* and *S. aureus.* Methanol extracts of flowers also showed inhibition over *E. coli* and *S. aureus* whereas stem extract showed inhibition only against *S. aureus* (Trease and Ewan, 1989). Clinical studies have reported by Govindan *et al*., (2004) in *S. trilobatum* was found to be very effective for antimicrobial activity against organism. The results of *S. nigrum* showed highest antibacterial activity against *E.coli* (Jimoh *et al.,* 2010). *S. schimperianum* showed their highest antibacterial activity against *E.coli* and *S. aureus* (Al-Oqaila *et al.,* 2012). In the present study, *S. aureus,* the gram-positive bacteria was more sensitive to the samples than gram-negative bacteria *E. coli.*

The probable factor for the higher susceptibility of *S. aureus* observed in the present study is that some extracts had a good potential for therapeutic use against the bacterial pathogens their activity is not related to the differences in the cell wall structure, as the gram-positive bacteria has thicker cell wall than gram-negative bacteria. The qualitative screening of phytochemical compounds in *S. trilobatum* revealed the presence of saponins and tannins in leaves, stem, flowers and fruits. Flavonoids, phenolic compounds and cardiac glycosides were present in all extracts. Carbohydrates were also present only in leaves and stem. These findings reveal that antimicrobial activity of *S. trilobatum*extracts is mainly due to the presence of these phytochemicals. The anticancer activity of *Solanum trilobatum* was studied by carrying out MTT assay. It is a sensitive, quantitative and reliable colorimetric assay that measures viability, proliferation and activation of cells. This assay is based on the capacity of cellular mitochondrial dehydrogenase enzyme in living cells to reduce yellow water-soluble substrate (MTT) into dark blue / purple formazan product which is insoluble in water (Mosmann, 1983). In an MTT assay that is being conducted in *Solanum nigrum* it was found that % growth inhibition increases with increasing concentration and IC_{50} value of this assay was 265µg/ml. In present study conducted in *Solanum trilobatum* also % cytotoxicity increases with increase in concentration and the IC_{50} value of this assay was 90.92µg/ml. For the identification of the active principles further fractionation of total steroids was done by thin layer chromatography. Three TLC fractions were obtained with different R_f values.

CONCLUSION

Solanum trilobatum, a medicinal plant commonly available in South India, is an extensively used Indian traditional medicine to cure various human ailments. *Solanum trilobatum* was reported to cure numerous diseases such as tuberculosis, respiratory problems and bronchial asthma. The extracts were proved to have antibacterial, antioxidant and anticancer activity. The antibacterial activity was demonstrated by considerable zone of inhibition against gram positive *S. aureus* and gram negative *E.coli*. The isolated steroids of 25mg showed higher antibacterial activity since it is a pure component. It also showed significant results of 15mm in gram positive and 14mm in gram negative bacteria. The antioxidant activity was detected against extracts and standard Ascorbic acid. Since the IC_{50} value of steroids were less it showed higher antioxidant activity. The anticancer activity was done against steroids. The steroid component showed high percentage cytotoxicity of 61.89µg/mL. TLC plates were also made to obtain various fractions for the further isolation of compounds. Thus our results support the view that the purified part of *Solanum trilobatum* is a promising source of potential antibacterial, antioxidant and anticancer properties. Further our work includes the identification and isolation of specific active principle which causes these properties through compound identification method such as IR, NMR.

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Table 1: DPPH Free Radical Scavenging activity of extracts

Concentration (µg/mL)	% inhibition of petroleum ether extract	% inhibition of steroids
62.5	13.7	18.18
125	36.22	40.4
250	47.76	54.55
500	62.05	71.57
1000	78.64	84.82

Table 2: Antibacterial activity of *Solanum trilobatum*

Samples	Zone of Inhibition (mm in diameter)	
	***Staphylococcus aureus* (G+ve)**	***Escherichia coli* (G –ve)**
Petroleum ether	20	15
Steroid	15	14
Positive control (streptomycin)	25	20
Negative control (DMSO)	No Inhibition	No Inhibition

Table 3: Anticancer activity of steroid extract of *Solanum trilobatum*

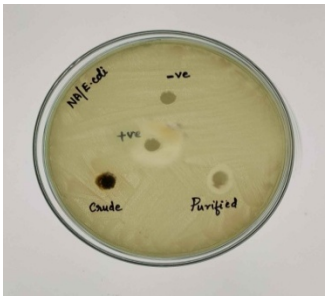
Concentrations (µg/mL)	% Viability	%Cytotoxicity
6.25	89.83	10.17
12.5	83.60	16.40
25	75.83	24.17
50	58.72	41.28
100	45.50	54.50
200	38.11	61.89

Table 4: Fractionation of steroids in *Solanum trilobatum*

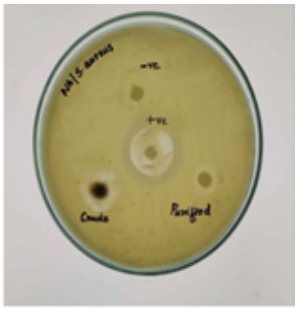
Fraction	*Solanum trilobatum*
F-I	0.090
F-II	0.151
F-III	0.243
FIV	0.315
F-V	0.455
F-VI	0.576
F-VII	0.939



Fig 1: *Solanum trilobatum* L.



***Escherichia coli* L.**



***Staphylococcus aureus* L.**

Fig 2: Diameter of zone of inhibition against the different test bacteria

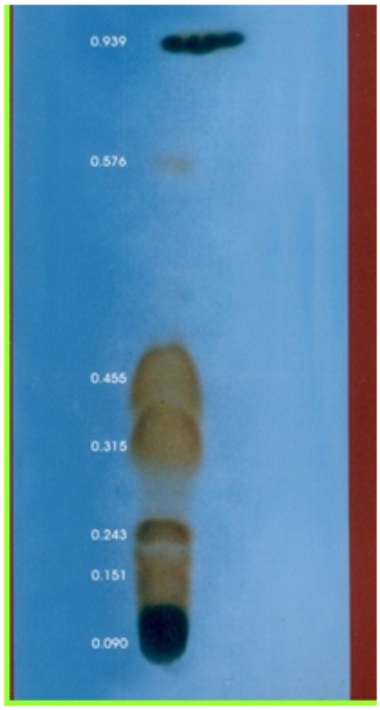


Fig 3: Steroid rich fractions isolated from *Solanum trilobatum* L.

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RESEARCH ARTICLE

Biomass Waste into Wealth: Advanced Processing for Sustainable Applications

Dibyarupa Pal[1], Ritam Paul[2], Gayatri Das[3], Avishek Kaser[4] and Pranabi Maji[5]*

[1]Assistant Professor, Department of Biosciences, JIS University, Kolkata, West Bengal, India.
[2]M.Sc Student, Department of Biosciences, JIS University, Kolkata, West Bengal, India.
[3]B.Sc Student, Department of Biosciences, JIS University, Kolkata, West Bengal, India.
[4]M.Sc Student, Department of Physics, JIS University, Kolkata, West Bengal, India.
[5]Assistant Professor, Department of Physics, JIS University, Kolkata, West Bengal, India.

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***Address for Correspondence**
Pranabi Maji
Assistant Professor,
Department of Physics,
JIS University, Kolkata, West Bengal, India.
E.Mail: pranabi.ism@gmail.com

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ABSTRACT

Rapid population expansion, urbanization and escalating consumption has led to global increase in waste biomass with an estimate of 2.24 billion tonnes in the year 2020. Mismanagement and unregulated waste biomass disposal create health issues, ecosystem damage, climate change and safety hazards. The impact of unsustainable waste management is disproportionate among nations but severely affects people of developing countries. Biomass wastes are of different kinds sourced from industrial, municipal to agriculture and agro-forestry. Their minimisation is of absolute importance to lessen the menace of hazardous, non-recyclable and infectious waste. Recyclable biomass waste helps to recover costly and rare elements and derive energy from it whereas reusable waste helps in resource savings. Valorization of the waste into biogas, biofuel, biomethane are some of the foremost areas by which a circular economy can be generated through waste recirculation. Different conventional and advanced treatment mechanisms of waste biomass removal are pragmatic but associated with its challenges and constraints. Based on these findings, the study has been designed to focus on different source generation of lignocellulosic waste and different modern avenues for generation of ways for waste valorization.

Keywords: Waste, Wealth, Valorisation, Biogas, Biomethane, Landfill

INTRODUCTION

Population explosion and modernization has long been an unescapable concern for the ecosphere pertaining to the huge amount of waste being generated. Globally, the waste generation is approximately 2.02 billion tons, which can escalate to 3.4 billion tons in the year 2050. More people invariably leads to more generation of waste and management and disposal of these wastes has raised candid concern among ecologists, governments and other stakeholders. This biomass trash remains the huge source of threat to the ecosystem, environment and human health [1,2]. Open dumping is the major sight of un-engineered or mismanaged landfills and simultaneously contributes heavily to environmental health hazards which should be mitigated speedily. Considering the disastrous state of waste management in poor socio-economic countries, there urgently, a huge paradigm shift towards waste management is needed. The generated waste is recognised as a resource wealth than a liability. To address the issue of 'biomass waste to wealth', modern techniques like anaerobic fermentation, pyrolysis, dark fermentation, torrefaction and bioelectrochemical systems are utilized to convert organic waste into energy and fuel mainly. Certain biomass elements need active pre-treatment processes like grinding, pelletizing, chemical treatment and others toenhance the conversion process efficiently. Pre-treatment processes are done with an objective to augment the effectiveness of lignocellulose hydrolysis as it increases the accessibility to cellulose rich fractions [3]. Biological treatment with microbes or their derivatives also help in this pre initialization processes. Again leachate and the landfill are the two main by-products of the landfill system which can be valorization to generate cost effective and sustainable solutions for waste management. When water percolates through MSW, it produces leachate, a liquid byproduct that results from the physiochemical and biological treatment of solid waste. It is typically produced in landfills, composting sites, and incineration facilities. It is distinguished by the presence of rich organic matter, inorganic compounds, and nitrogen and phosphorus elements. It also contains hazardous components, but it can be converted into energy and biofuel through value-added resources. Leachate recirculation through the landfill can aid in the recovery of important nutrients, the maintenance of a higher moisture content inside the trash disposal, and the reduction of environmental contamination in ground water. Increased moisture content promotes the growth of anaerobic bacteria, accelerating the waste breakdown process and increase the rate of methane production. Waste biomass refers to organic material derived from living, or recently living organisms that is discarded as waste. It includes agricultural residues, forestry remnants, animal manure, industrial waste, municipal solid waste, sludge waste, landfill gas, food waste and other biodegradable materials. It is the primary energy source, accounting for 10-14% of global demand and in developing countries, rural sectors rely on 90% of their energy supply from biowaste conversion [4].Rather than letting it decompose in landfills, waste biomass can be converted into energy through different processes providing an eco-friendly alternative to fossil fuels. By capturing and utilizing waste biomass, biofuels, electricity and heat, can be produced, reducing greenhouse gas emissions and dependence on non-renewable energy sources. Figure 1displays the waste composition in municipal landfill.

Important avenues for Waste biomass utilization

Renewable energy source: Waste biomass can be converted into bioenergy, including biofuels like biohydrogen, biogas and bioelectricity. This reduces dependence on fossil fuels and contributes to a cleaner, more renewable energy supply.

Waste Reduction: Utilizing waste biomass helps manage agricultural, industrial, and household waste, preventing landfill overuse, reducing greenhouse gas emissions, and supporting better waste management practices.

Environmental Impact: Waste biomass conversion helps decrease methane emissions from organic waste in landfills, lowering the overall carbon footprint. By producing biochar, it also supports carbon sequestration, which can enhance soil quality and reduce atmospheric carbon.

Economic Growth: The waste biomass industry can generate jobs in bioenergy production, processing and technology development, to support a circular economy concept.

Valorization of Biomass: By-products like biochar, created from biomass waste, can improve soil fertility, increase crop yields and reduce reliance on chemical fertilizers, fostering more sustainable agricultural practices. Current sustainable waste treatment tactics focus on transforming waste into viable and utilizable sources like energy or fuel generation and converting into value-added products, by systematically harnessing biomass resources. This method not only provides an efficient use of organic biomass waste but also represents a cleaner and more environmentally friendly solution. By adopting such practices, these strategies contribute significantly to the development of a lignocellulosic biomass-based circular bioeconomy, promoting resource efficiency and sustainability within a closed-loop system [5].

Types of Waste Biomass

Based on the origin of the waste sources and their different properties like moisture content, volatile organic content, heating value, ash content and others, they are categorized into different sections[2,6,7].

Agricultural waste

Agricultural waste consists of the leftover materials generated during various farming activities, including crop harvesting, processing and livestock rearing. While often discarded or burned openly, they create huge air pollution by greenhouse gas emissions, impacts biodiversity and leads to degradation of soil. Contrarily agricultural waste holds substantial potential as a resource to improve soil health and thus crop productivity. Again with effective management, it can be transformed into bioenergy, organic fertilizers and even resources for construction or manufacturing. Technologies like composting, anaerobic digestion and biochar production allow for recycling and repurposing waste, by reducing emissions and enhancing soil fertility. Biochar of sugarcane bagasse, rice husks or other biomass from agrowaste has been utilized for remediation of various toxic pollutants from environment like heavy metals, toxic dyes, paints, organic pollutants. Even biofuels like biogas, biomethane, biohydrogen and bio-oil are generated by pyrolysis or torrefaction of the waste [8]. Administrators, scientists, farmers and industries are increasingly exploring sustainable waste management practices towards carbon neutrality, understanding that utilizing agricultural by-products can play a crucial role in achieving environmental sustainability and supporting the circular economy[9-12].

Forest Residues

Forest residue refers to the organic material left behind after forest management activities, such as logging, thinning or natural events like storms and fires. This residue includes branches, leaves, needles, bark, flowers and stumps, which are rich in carbon, fiber and polymeric content. This composition makes them highly suitable as a starting material for designing and developing advanced adsorbents for remediation purposes [13]. Often seen as waste, forest residue can actually play a vital role in the ecosystem by providing habitats for wildlife, enriching soil nutrients and aiding in carbon sequestration. Agricultural waste valorization, leads to environmental cleanup, socioeconomic development, promotes resource conservation, recovery and enhances energy security. For instance, forestry bio-waste has high organic matter content which is repurposed as edible biocoating for fruits and vegetables, prolonging their shelf life and incorportaed in the edible packaging materials, providing biodegradable alternatives to synthetic plastics. In water purification processes, plant extracted polysaccharides function effectively as flocculants, aiding in removal of various waste [14,15]. Forest residues are also valorised by growing edible fungus like oyester and button mushroom after sterilization, and the remaining spent mushroom substrate are reutilized for composting, second substrate, organic fertilizer, animal feed and biofuel generation[5,16,17].

Industrial waste

Industrial waste refers to the by-products generated from manufacturing textiles, paper, oil refineries and food processing units. Mining operations, construction, and power generation activities also contribute significantly to industrial waste generation. These wastes include chemical by-products, metal scrap, packaging materials, sludge, ash, debris and other residues. Industrial waste can be hazardous like toxic and flammable, or non-hazardous but can still be an environmental threat. Improper disposal and processing of this biomass waste has caused environmental

issues like drinking water source contamination, respiratory difficulties and soil health depletion affecting agriculture and productivity[5].

Municipal solid waste

The waste materials (recyclable or non-recyclable residues) obtained from municipality zone like households, communities, commercial buildings, institution and small scale industry are known as municipal solid waste (MSW). The MSW waste composition varies depending on the urban or rural source, season, economic status, processing patterns and geographical location. Usually both biodegradable and non-biodegradable fractions are present and 15% of the total waste are recycled while the remaining is disposed to landfills or dumped openly. Mismanaged MSW creates severe environmental pollution albeit conversion into value-added products could offer solution towards the energy scarcity challenges and ecological waste management. Landfilling, pyrolysis, gasification, anaerobic digestion and composting are usual methods of disposal, recycle, or convert into energy resources like biogas or biofuel. For instance, 1 metric ton of MSW roughly release 1.3 metric tons of CO_2 equivalents close to the CO_2emissions from power plants. Valorising CO_2 into biogas or upgrading into biomethane can significantly reduce environmental pollution and the generated biofuel can serve as alternatives to fossil fuel creating avenues for uplifting economy of the country [5,18].

Animal Manure

Animal manure, a natural byproduct of livestock, is an effective organic fertilizer used to enhance soil fertility and boost plant growth. Composed of macro and micro nutrients like nitrogen, phosphorus and potassium, manure enriches the soil by supplying essential elements that stimulate crops and improves soil structure. For example, poultry manure is particularly rich in nitrogen, making it beneficial for crops that demand high nitrogen levels. Proper composting of manure is essential to ensure pathogens are destroyed and nutrient levels are stabilized, making it safer for agricultural use. Using manure as a fertilizer is environmentally pleasant, as it recycles animal waste that might otherwise contribute to pollution and affect local ecosystems by leaching. By incorporating manure into farming practices, growers can reduce reliance on synthetic fertilizers, leading to more sustainable agriculture[19-21].

Sewage and waste water

Sewage and wastewater refer to the water that contains human waste, food scraps, oils, soaps, and chemicals from households, laundry, industries or commercial activities. If not properly treated, it can be harmful to both human health and the environment. Sewage systems are designed to collect and transport this water to treatment facilities. These facilities employ physical, biological, and chemical processes to remove contaminants and purify the water. The goal is to make the treated water safe for discharge into water bodies or reuse in irrigation and industrial processes. Untreated sewage can lead to water pollution, causing harm to aquatic life and spreading diseases. Modern sewage treatment includes primary, secondary, and sometimes tertiary treatment stages. The primary stage separates and suspends the solids, the secondary uses microbial break down of organic material and the tertiary stage further purifies the water. Sewage sludge is highly filled with nutrients which can be valorised for biogas and biofuel production[22,23].

Food waste

Food waste refers to edible food that is discarded or unused at various stages, from production to consumption. It occurs due to overproduction, poor storage, improper handling, or consumer behavior like buying more than needed. It is a significant global issue with social, economic and environmental implications. From the wasted food landfills releases methane, a potent greenhouse gas. Food waste also raises ethical concerns, given that millions of people worldwide face hunger and food insecurity. Efforts to reduce food waste focus on better management across the supply chain with improved harvesting methods, good storage facilities and adopting efficient distribution strategies. Addressing food waste requires to implement sustainable practices, create pathway for efficient valorisation like converting the waste into biogas or biofuel through advanced treatment processes. Different

conversion pathways such as physicochemical (pelletization), biochemical (anaerobic digestion, bio-methanation) and thermochemical (pyrolysis) methods can successfully utilize and manage these biomass wastes.

Characteristic properties of waste biomass

Usually biomass mainly consists of carbon, hydrogen, oxygen, traces of sulphur, nitrogen and other elemental compounds. These constituents made up the primary organic compounds in plant biomass which are cellulose, hemicellulose and lignin. Cellulose, major polysaccharide and the primary structural component of plant cell walls, makes up 30-50% of biomass. It provides a source of fermentable sugars. Hemicellulose, the second complex carbohydrate constitutes 20-30% of biomass. It is more easily degradable than cellulose and releases various sugars upon breakdown. Lignin, third complex aromatic polymer that gives rigidity to plant cells, accounts for 15-30% of plant biomass (Table 1). It has high calorific value but not easily degradable. The ratio and proportion of these components influence the formation of value added products from these compounds. For example agricultural waste normally has 10-15% moisture, while animal manure has more moisture content. Biomass density also impacts the end product conversion along with transportation and storage. Chopped agricultural waste, shredded and powder form has high surface area for enhanced availability of treatment[12,24].The inorganic mineral constituents of biomass turn to ash after combustion but high ash content reduces energy efficiency and can cause operational issues in boilers. Among the chemical properties, high calorific value (for biofuel) is influenced by the proportions of carbon, hydrogen and oxygen of the waste biomass. Influences microbial digestion during biogas production like low pH can inhibit working of anaerobic bacteria. Carbon-Nitrogen (C/N) ratio(20:1 to 30:1) of the biomass is optimal for microbial activity and influences biogas production. Understanding these physical and chemical properties is critical in optimizing waste biomass for energy recovery processes and biofuel production. Proper assessment leads to more efficient energy conversion technology and better waste-to-energy programs [3,25].

Pre-treatment procedures of biomass waste

Agricultural waste is primarily constituted of lignocellulosic biomass that comprises of complex molecules such as-cellulose, hemicellulose and lignin. These are complex, compact matrices and need to be degraded to simple monomers before being converted into other products. Cellulose fibres are enclosed by hemicellulose and it also connects the cellulose fibres to lignin. It is composed of diverse assembly of branched polysaccharides. Monomers of galactose, glucose, arabinose, xylose, mannose and glucuronic acid are the structural components of hemicellulose. It is amorphous, hydrolyzed quite easily by weak acids, bases, enzymes, and not much resistant to chemicals. Comparatively, hemicelluose is not as mechanically strong as cellulose. Lignin is a non-carbohydrate complex aromatic polymer composed of unevenly placed phenylpropane units which renders them structurally rigid, water resistant and resilient to microbial attack. Owing to this complex structure, the breakdown of lignocellulosic biomass is a difficult task[3,12,26-28].The goal for pretreatment processes on agricultural wastes and other lignocellulosic biomass are-

- reduce consumption of energy during conversion
- cost reduction
- formation of sugars directly from biomass
- pre-treatment methods also preserves sugar produced from degradation
- reduced formation of inhibitory products

On the basis of the physical and chemical nature of biomass, the preferred pre -treatment method needs to be chosen.

Physiochemical methods

Steam explosion: This process separates cellulose from lignocellulose by disrupting the β-O-4 bond in lignin and destroying the cell wall configuration of biomass by subjecting it to strict temperature and pressure. An optimal pressure range of 0.5–5 MPa and temperatures of 160–250 °C, is needed. It was reported that 80% lignin was removed if steam explosion was applied to pineapple leaf fibres at conditions like 215 ºC, 2Mpa for five minutes. Despite this, the disadvantages of this method is that it consumes high energy, inhibitory by-products are generated and extreme reaction conditions are needed.

Alkali heat pre-treatment: Utilizes alkali and heat for lignin decomposition, and to reduce the crystalline nature of lignocellulose, and maximize the surface area of contact for it. NaOH, Na_2CO_3, and alkaline peroxide are some of the alkalis preheated between 75°C and 125°C for removal of lignin and hemicellulose. Although effective, this process is complex and the maintenance of high temperature is expensive.

Ammonia explosion fibre pre-treatment: Here, ammonia and oxy-hydrogen ions are prepared from liquid ammonia, at high pressure, thereby causing an abrupt temperature rise. This decomposes ester and ether bonds between lignin and hemicellulose and then reduces the cellulose crystals. This has proven to be very efficient for lignin degradation and improved the glucose yield of barley straw and corn stover. Conditions are simple but need much energy, environment friendly and does not produce inhibitory by-products.

Extrusion pre-treatment: This involves combination of thermal and mechanical pre-treatment methods. This changes lignocellulose physically and chemically. Here, force is applied on agricultural wastes, kept in an airtight vessel in order to increase temperature and pressure. NaOH is added to the process. This removes hemicellulose and lignin efficiently and sugar yield is increased by significant amount[29,30].

Green solvent- based

Biochemical pre-treatment: This method compensates for the low efficiency and the time consuming biological pre-treatment methods and to reduce the large volume of inhibitory compounds formed. But it is costly pre-treatment method and mot utilized in cases of wheat straw conversion into biomass.

Ionic liquid pretreatment: Molten salts or liquid electrolytes are referred to as ionic liquids. They are comprised of cations(imidazole, choline, pyridine) and ions (chloride and acetate). Their interaction with lignocellulose materials leads to broken β-O-4 bonds and formation of ion-dipole bonds. This interrupts the crystalline composite structure of lignocellulose, decomposes the structure, and subsequently separates lignin and hemicellulose from these cellulose-rich materials. They offer almost 99% reliability in conversion of lignocellulosic biomass to bioethanol, but are cost operative.

Deep eutectic solvents: These resemble ionic liquids and are constituted of hydrogen bond donors like urea, amides, alcohols, acids, etc. and hydrogen bond acceptors. These hydrogen bond donor and acceptor interface causes a slight acid or base catalytic blend and helps to degrade the ether bond present between lignin and hemicellulose during lignocellulose pre-treatment. A 96% lignin removal efficiency was reported by Ma *et al*., [31]as they pre- treated poplar wood with such solvents. Examples of deep eutectic solvents are choline chloride, ethylene glycol. This method is cost effective, has low toxicity and high recovery.

Supercritical fluid: They behave like liquids at above critical temperature (304.25 k) and pressure (7.36 MPa),for example H_2O and CO_2. They are not costly, safe, non-toxic, non-combustible, eco-friendly and renewable. If lignocellulose is dissolved in supercritical carbon dioxide (SC CO_2), it results in the change of structure and decomposition of the chemical bonds between lignin and hemicellulose. Pre-treating green coconut fibre under 20–30 MPa for 6–72 h resulted in an increased efficiency of 101% of sugar production. This method, if applied to microalgal and lignocellulosic biomass pre-treatment results in a significant increase in the generation of reducing sugar [32].

Biological methods

Fungal: In this process of biological pre-treatment, various types of fungi like white-rot fungi, brown rot fungi, soft rot fungi and other fungi are used for lignin degradation. Hydrolyases and oxidative ligninolytic enzymes of White-rot fungi of class Basidiomycetes like *Phanerochaete chrysosporium, Trametesversicolor* and others are used for biodegradation of lignin. A recent study on white-rot fungus, *Peniophora incarnata* for the pretreatment of waste wood, reduced the percentage of hemicellulose and lignin by 48% and 70%, respectively. The water content and particle size of the feedstock, reaction temperature, and residence time highly influenced the reaction parameters[33,34]. *Termites:* They secrete enzymes to digest lignocellulose efficiently. Many classes of termites like

Microcerotermesparvus, *Termeshospes* and *Nasutitermese phratae* can secrete enzymes for degradation of lignocellulose[35]. *Microbial consortium*: Microbes from soil, wastewater, etc. are agents of pre-treatment for agricultural wastes. They are able to decompose both the cellulose and hemicellulose present in agricultural wastes through their enzymes like cellulases and hemicellulases. It was seen from research that microbes sourced from landfills, decomposing straws and wastewater could pre-treat cassava wastes to produce 96% biogas. Cassava pulp, sugarcane bagasse, corn stover, Napier grass, rice straw, etc. are some examples that have been successfully pre-treated with enzymes to be effectively converted to biobutanol[36]. The cellulolytic bacterium *Acetobacter orientalis* was used by Chen *et al*., [37] and achieved about 76.24% cellulose degradation of banana residue.

Augmentation of Bio-chemical methods

Bioventing

An in situ bioremediation procedures in which air or pure oxygen is introduced into the unsaturated zone of the soil by gas injection to assure aerobic biodegradation of contaminant chemicals. This technique increases the activity of indigenous bacteria present in the landfill subsurface while also convert aerobically degradable substances such as gasoline, oil and petroleum to simpler molecules. Along with oxygen, nutrients such as nitrogen and phosphorus are introduced into the contaminated site to promote microbial development, and their distribution is determined by the soil's physical qualities. Bioventing is more successful if the water table is deep below the surface and the area has high temperatures [38,39].

Biosparging

In Biosparging, pressurized air is injected into the groundwater in order to raise the oxygen concentration to aid in aerobic microbial breakdown of pollutants. Volatilization of certain compounds are also facilitated by this technique, but necessary to determine the soil's permeability and texture before injecting the oxygen. Injecting oxygen into the polluted site requires careful pressure management to avoid releasing volatile materials into the air. Mainly the sites contaminated with petroleum products like diesel or jet fuel are ideal for biosparging, but not for lighter petroleum products like gasoline which volatilize more easily [39].

Bioaugmentation

In order to improve the breakdown of trash, microorganisms with appropriate metabolic capabilities are introduced to the polluted site. To make sure that the microbes already there can break down chlorinated ethenes like tetrachloro ethylene and trichloroethylene into harmless ethylene and chloride, bioaugmentation is employed at areas where these pollutants have leached into the soil or groundwater.

Bioleaching

Bioleaching is a sustainable approach of leaching metals from landfill leachate sources using diverse live microbes [40]. The agents like nitric, sulphuric and hydrochloric acids dissolve heavy metals in soil or else the metabolic ability of acid-producing microbes dissolve them. Many fungi and bacteria, including sulfur-oxidizing bacteria and *Aspergillus* and *Penicillium* species, are used to bioleach metals from polluted soil. The most prevalent bioleaching archaea and bacteria are chemolitho autotrophic acidophiles, which contribute to leaching by oxidizing iron and reduced inorganic sulphur compounds. These acidophiles, functions best at temperatures and pH values below 3.0 and temperature range from 15 °C to 80 °C [40].

Thermal treatment

Pyrolysis

It is the most common method to produce biochar. In this method organic biomass is heated in the absence of oxygen at high temperatures ranging from 250°C to 900°C.In this process the waste biomass is converted into biochar, syngas and bio-oil. Here pyrolysis can be classified as fast and slow pyrolysis processes depending on the heating rate, temperature, residence time and pressure of the reactor. Different types of reactors such as paddle kiln, bubbling fluidized bed, wagon reactors are used for biochar production. It has been reported that lower temperature pyrolysis

favors more biochar production than the treatment of higher temperature pyrolysis. But higher temperature enhances carbon stability and surface area of the biochar[6].

Gasification

It is a partial combustion process where biomass is converted into syngas as major product and biochar as byproducts at higher temperatures (>800°C).Though this process produces lower biochar yields but it is energy efficient and also suitable for energy recovery.

Hydrothermal Carbonization (HTC)

In this method biomass is treated with water at high pressure and moderate temperatures (180–250°C).This process is mainly effective for wet biomass and it yields hydrochar, a material similar to biochar. The resulting hydrochar used to obtained via different chemical processes like polymerization, intramolecular dehydration and liquefaction/condensation. This mechanism is complex because of the high molecular mass and intricate structure of lignin. During pyrolysis, complex organic polymers or lignins remain unblended under liquefaction conditions that actually forms hydrochars.

Torrefaction

Torrefaction is a comparatively modern technique. The methodology is known as median pyrolysis having a lagging heating rate. Under anaerobic conditions at a heating rate<122 °F/min, a residence time of less than half an hour and a temperature increase from 300 °C to 500 °C torrefaction is considered as a form of fast pyrolysis. The running mechanism of torrefaction is divided into several steps such as heating, dehydrating, torrefying and condensing. This process is critical in the formation of biochar because it thermally alters biomass to improve its properties for soil amendment, carbon sequestration.

Purposeful products from biomass

Biochar

Biochar is a carbon-rich material, produced from the thermochemical conversion or pyrolysis of organic biomass under limited oxygen conditions. Pyrolysis is the most common method to produce biochar. In this methodorganic biomass is heated in the absence of oxygen at high temperatures ranging from 250-900°C and converted to biochar, syngas and bio-oil. It displays certain physicochemical properties such as it contains high surface area, high porosity and high product retention capacity like Carbon sequestration for addressing environmental challenges. These properties make biochar widely applicable for improving soil health to enhance crop productivity, reducing greenhouse gas emissions, detoxifying waste water, remediating polluted environment and for many other purposes. Biochar blended with green waste can help grow mushroom and significantly increases the nutrient content of the compost. Global focus on sustainability and the circular economy has also positioned biochar as an advanced tool for converting agricultural as well as organic wastes into value-added products[6,41].

Landfill gas/Biogas

Landfill gas is produced when organic waste in landfills decomposes anaerobically, releasing a mixture of gases, primarily methane (CH_4) and carbon dioxide (CO_2), along with small amounts of other compounds. Methane, being a potent greenhouse gas, can contribute significantly to global warming if released into the atmosphere. The final product from anaerobic digestion of various biomass wastes result in Biogas with multiple potential, including upgrading to biomethane (CH_4) and then using it to generate electricity, fuel, or heat. The leftover solids or digestate are used as biofertilizer for crop and plants. Hydrolysis, acidogenesis, and methanogenesis are processes in which bacterial populations engage through syntrophic pathways during anaerobic reaction. But challenges are prevalent when anaerobic reaction in the landfill are guided by a number of variables, like the feedstock composition, the microbial diversity, the pH and temperature of reaction process. To mitigate this, many landfills install gas collection systems capture methane and use it to generate electricity or heat, turning a harmful by-product into a resource. Biogas on the other hand, is produced in controlled environments, such as anaerobic digesters. These digesters break down organic materials like agricultural waste, food scraps, and sewage sludge under anaerobic conditions,

generating a similar mixture of methane and carbon dioxide. Biogas can be refined to produce biomethane, a renewable energy source that can replace natural gas for heating, electricity, and even as vehicle fuel. Both landfill gas and biogas capture methane emissions, reducing environmental impacts and providing a renewable energy source[23,42,43].

Biomethane

Again biogas can be upgraded to biomethane which lessens our reliance on fossil fuels and increases the fuel power and efficiency. Through adsorption procedures, biogas can be enhanced to produce methane by rerouting the CO_2 pathway to generate more high-quality biomethane (97% CH_4). In this context, CO_2 is removed from biogas by a variety of physiochemical processes, including adsorption and membrane penetration. Thermal treatment like Carbonization or pyrolysis of waste biomass ensues at high temperatures (400-850°C) with limited oxygen. Biomass converted to porous carbons has great affinity to absorb a lot of CO_2andimprove biomethane production efficiency. Again, the adsorption approach is relatively inexpensive, dependable and sustainable, with the only requirement of large adsorption surface area and an efficient adsorbent. Furthermore, during physical activation process, an oxidant like air steam of N_2, NH_3,O_2 and CO_2 or gaseous mixture at high temperatures (600-1000°C) can add several surface functional groups to aid in the adsorption process[43,44].

Biofuel

Biofuel are derived in three different form like solid, liquid and gaseous state. Solid biofuels originate from municipal and animal waste biomass to generate energy, heat, and cooking fuel. Liquid biofuels like bioethanol, biodiesel and bio-oil are also derived from biodegradable biomass sources but have higher energy density than solid/gaseous biofuels, making them better for storage and transit. For instance, Biodiesel, is utilized as a fuel for compression-ignition engines by blending with petroleum diesel at a certain amount, and the most common mixture, B20 consist of 20% biodiesel and 80% petroleum fuel. Biohydrogen, and bio syngas, are example of gaseous fuel which are of low-density, obtained through pyrolysis or gasification process[11].

Cellulose deconstructive enzymes

Cellulases, the main enzyme for cellulose degradationison high demand in the global industrial market and developing low-cost cellulases from biomass waste are the most sustainable and promising way to lower their cost. Cellulases comprise three sub-enzyme groups of endoglucanases, exoglucanases and β-glucosidases. Microbial enzymes have specific interaction with the lignocellulosic substrate and thus produce enzymes differently. Aerobic bacteria release enzymes externally as free cellulose molecules whereas anaerobic bacteria produce cellulases into the cell surface as a protein complex called a cellulosome. Previous investigations has shown that fungi, rather than bacteria, are the most effective cellulase producers from organic waste like wheat straw, paddy straw, sugarcane bagasse, and other lignocellulosic components. For example, *Trichoderma* and *Aspergillus* through solid-state or submerged fermentation process has been successfully producing cellulases valorizing the organic biomass waste [45].

Challenges of biomass valorization

Diversity in waste feedstock

Cellulose, hemicellulose, lignin, and a trace amount of inorganic substances make up waste biomass. Different proportions in the biomass determines the degree to which it will decompose during pyrolysis and how resistant it is during hydrolysis. Because of their structural stability, these three main components take different time and method to decompose and thus there is no such constant methodology applicable.

Efficiency in conversion

When turning waste materials into biofuels, complex processes like hydrolysis, anaerobic digestion and thermochemical conversion requires step optimization. Inhibitory byproduct generation, high energy inputs and poor conversion efficiency, need to be minimized and regulated that will increase the economic feasibility of valorized production.

Cost efficacy

The cost of waste valorization is critical to their economic sustainability to generate market demand and investment. It considers feedstock costs, conversion methods, operating expenses, and distribution infrastructure. Advances in technology, scale, and feedstock availability can all help to reduce production costs and increase competitiveness.

Opportunities of biomass valorization

The safety of energy resources

Generation of opportunity to reduce reliance on fossil fuels is one way in which biofuels contribute to greater energy security. The renewed energy systems from waste feedstocks can ameliorate economy of the country.

Utilization of refuse

Waste valorization has the potential to repurpose various types of waste, including those from agriculture and forestry, instead of sending them to landfills and worsening environmental impacts. This method opens the door to reducing waste and making better use of available resources.

Creating jobs and fostering rural development

Rural economies could be enriched by providing jobs in sectors of feedstock sorting, processing and distribution which will open up new avenues of income for the rural people.

CONCLUSION

Waste accumulation is of grave concern today which can be only mitigated by advanced technologies and waste valorization. To tap the full potential of waste biomass, improved and sustainable technologies for large-scale commercialization needs to be achieved by further investigations. Utilization of renewable resources from waste biomass, is essential for reducing emissions of greenhouse gases and increasing energy security. In the current scenariomore emphasis is on thermochemical and biochemical conversion processes to improve biomass conversion into power, heat and biofuels. Furthermore, it contributes significantly to the advancement of a circular economy by establishing closed-loop systems that reduce reliance on non-renewable resources while producing value from waste. This strategy is a significant step toward more sustainable integrated waste management with value generation.

Conflicts of Interest

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Table 1. highlights the differentcomposition of lignocellulosic waste form agricultural sources.

Substrate	Lignocellulosic composition		
	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Wheat straw	40	39	13
Rice straw	41	45	14
Sugarcane bagasse	35-40	20-25	18-24
Areca Leaf	37.50	14-15	36.20
Leaf Sheath	26.40	16-17	38.68
Areca Husk	41-42	17-18	43-44
Rapseed	51.3	17.3	44

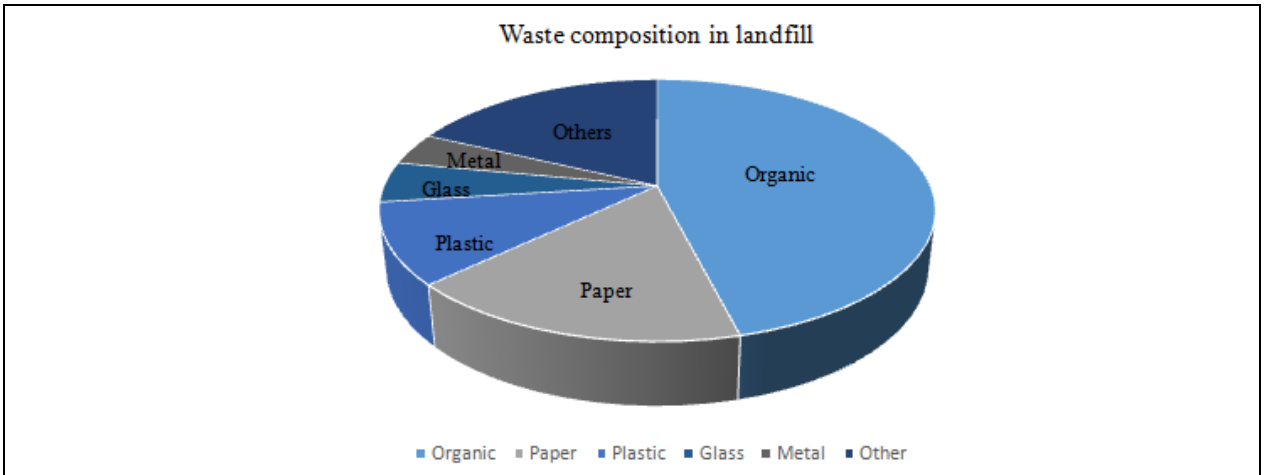


Fig. 1: Waste biomass composition in municipal landfill

REVIEW ARTICLE

A Review Article on Management and Outcomes of UTIs Patients : A Retrospective Study

Harsh Arya, Anshu Kumar Singh, Zeba Naj, Navdeep Singh, Umesh Kumar and Pankaj Kishor Mishra*

Subharti College of Allied and Healthcare, Faculty of Allied Health Sciences, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

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***Address for Correspondence**
Pankaj Kishor Mishra
Subharti College of Allied and Health Sciences,
(Affiliated to Swami Vivekanand Subharti University),
Meerut, Uttar Pradesh, India.
E.Mail: pkjbiotech@gmail.com

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ABSTRACT

Urinary tract infections (UTIs) are amongst the most frequent infections. Although other microbes like fungus and some viruses have occasionally been implicated in UTIs, bacteria are the primary cause of these illnesses. The most frequent cause of both uUTIs (uncomplicated) and cUTIs (complicated) is Uropathogenic *Escherichia coli* (UPEC), which is followed by other harmful microbes such Staphylococcus species, *Klebsiella pneumoniae*, Proteus mirabilis, and *Enterococcus faecalis*. Uropathogens' high recurrence rates and growing resistance to antibiotics pose a serious danger to the illnesses' financial burden. Many patients will receive better clinical care if UTIs are diagnosed accurately and treated with evidence-based practices, which will also reduce the overuse of antibiotics. The degree of immunosuppression and the genitourinary anatomy of the patient also influence the therapy strategy. Therefore, a specialist and interdisciplinary therapeutic approach may be necessary for individuals who have kidney transplants or urological blockage.

Keywords: UTIs, Antimicrobials, Management, Outcomes, Uropathogens, Virulence Factors

INTRODUCTION

One of the most prevalent bacterial illnesses in the world, urinary tract infections (UTIs) can occur in both public and medical settings. Most UTIs are often treated empirically, despite the fact that the clinical symptoms of UTIs vary widely and can range from simple (uUTIs) to complex (cUTIs). Although other microbes like fungus and some viruses have occasionally been implicated in UTIs, bacteria are the primary cause of these illnesses. The most frequent cause of both uUTIs and cUTIs is Uropathogenic *Escherichia coli* (UPEC), which is followed by other harmful microbes such Staphylococcus species, *Klebsiella pneumoniae*, Proteus mirabilis, and *Enterococcus faecalis*. The urinary

system consists of the kidneys, ureters, bladder, and urethra, and its main function is to filter blood by removing waste products and excess water. The urinary system plays a key role in removing the waste products of metabolism from the bloodstream. Other important functions performed by the system are the normalization of the concentration of ions and solutes in the blood and the regulation of blood volume and blood pressure[1]. Numerous pathogens, including both Gram-positive and Gram-negative bacteria and fungi, can cause urinary tract infections (UTIs). Women, children, and elderly patients who are otherwise healthy are usually the ones that suffer from simple UTIs. Indwelling catheters, anomalies of the urinary system, immunosuppression, or antibiotic exposure are typically linked to complicated UTIs[2]. Urinary tract infections (UTIs) are extremely common, cause significant patient morbidity, cost health care systems a lot of money, and are among the most frequent causes of antibiotic use globally[3]. UTIs can have an impact on pregnant women's outcomes. There is a positive link between lower birth weight, preterm birth, and perinatal mortality, according to several research examining the consequences of UTI in pregnant women. Two categories are usually used to classify UTIs. The lower urinary tract is affected by acute cystitis, which is the first. Acute pyelonephritis, which affects the upper urinary system, is the second[4]. Fever, vague symptoms, or poor growth in infants can all raise suspicions of a urinary tract infection, as can urinary symptoms in older children[5]. Diagnosing and managing upper and lower UTI have always been a challenge to physicians, given its high prevalence, risk of recurrence and improper treatment, and the fact of worldwide increase in antibiotic resistance, necessitating implementation of a proper antibiotic stewardship[6].

In order to educate and increase knowledge of the proper diagnosis and management of UTIs, a patient awareness campaign was also launched during the Congress. This campaign involved patients, patient organizations, and doctors in managing bacterial infections in accordance with EAU guidelines. a video that promotes the usage of antibiotics, UTIs, and the campaign[7]. One of the main causes of antimicrobial resistance (AMR) is the treatment of UTIs. The most common uropathogen is still *Escherichia coli*. According to international reports, uropathogen resistance rates are rising, which is linked to an increase in the number of fatalities and disability-adjusted life years. [8]. Urinary tract infections (UTIs) are characterized by pain or discomfort when urinating, frequent urination, a feeling of urgency, blood or mucus in the urine, lower abdominal cramps or ache, discomfort during sexual activity, and pain, pressure, or tenderness in the bladder area. Back discomfort, chills, fever, nausea, and vomiting are some symptoms for patients whose bacteria have gone to their kidneys[9]. The identification of a quantifiable measurement of ≥10,000 CFU/mL of multiple bacterial species in a urine sample, independent of the presence of pyuria, is also referred to as asymptomatic bacteriuria[10]. On the other hand, clinical observations of dysuria, urgency, frequency, hematuria, suprapubic pain, and uterine contractions, along with the presence of bacteriuria exceeding a count of 10,000 colony-forming units per milliliter (CFU/mL), are used to determine a symptomatic lower urinary tract infection (UTI). (11)UTIs are frequently brought on by bacteria that invade the genitourinary and lower gastrointestinal tracts and progress upward[12]. Regardless of a female's reproductive status, the agents that cause urinary tract infections (UTIs) belong to the same taxonomic group and have similar virulence traits. Among the bacteria that are frequently observed are *Escherichia coli*, *Klebsiella pneumoniae*, Proteus species, Acinetobacter species, *Staphylococcus saprophyticus*, Group B Streptococcus (GBS), and *Pseudomonas aeruginosa*[13]. Pregnant women frequently get both symptomatic and asymptomatic UTIs, which can have detrimental effects on the mother, pregnancy, and unborn child. These alterations occasionally lead to symptomatic infection and raise the prevalence of asymptomatic bacteriuria (ASB). Such infections can have serious repercussions for the developing fetus as well as the expectant mother. Moreover, a number of factors may enhance pregnant expectant moms' susceptibility to UTIs, including advanced maternal age, parity, diabetes, sickle cell anemia, prior UTI occurrence, urinary tract abnormalities, and immunological inadequacies. (14)

Classification

According to the site of infection, UTIs are called urethritis, ureteritis, or inflammation of the urethra, and cystitis or pyelonephritis, or inflammation of the kidney and bladder, respectively.

Cystitis Bladder infections are more common in women than in men. In female patients, sexual activity usually precedes uncomplicated cystitis. Asymptomatic bacteriuria can be distinguished from dysuria when symptoms such

as urgency, frequency, nocturia, hematuria, and suprapubic discomfort are present. Significant bacteriuria and bladder mucosal invasion are its defining features. Women who are afebrile and show no symptoms of a systemic disease have these markers. (15)

Pyelonephritis When a midstream MSSU culture reveals at least 100,000 bacteria/mL of a single uropathogen along with inflammation of the pelvis, calices, and renal parenchyma, it is a disease suspected in the presence of systemic illness. If therapy is not administered, pyelonephritis can result in maternal sepsis, preterm labor, and early birth. Nausea, vomiting, chills, pyrexia, stiffness, flank or renal angle pain, and hip pain are all signs of pyelonephritis. There may or may not be signs of a lower tract infection, such as frequent urination and lack of thirst. (16)

Urethritis It refers to a urethral infection caused by bacteria, viruses, protozoa, or fungi. This occurs when these organisms gain access to both the periurethral glands in the male urethra and the complete female urethra. Numerous sexually transmitted Pathogens such *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, *Chlamydia trachomatis*, and herpes simplex virus are commonly responsible for urinary tract infections in both sexes.(17)

Management of UTIs

Antibiotic treatment

Complicated UTIs

Multi organ involvement and severe, potentially fatal sepsis are possible presentations of a UTI. Resuscitation frequently comes before final treatment. It may be necessary to deliver broad-spectrum antibiotics and vigorous fluid resuscitation to the extremely septic patient in the emergency room. The patterns and recommendations of local bacterial resistance should always govern the selection of antibiotics[18]. The incidence of UTIs in women seems to be decreased by postcoital treatment of ciprofloxacin or trimethoprim/sulfamethoxazole (TMP/SMX). TMP/SMX, norfloxacin, and nitrofurantoin are relatively effective, according to observational data; nevertheless, findings are constrained by the study design[19]. Patients with diabetes may be more susceptible to UTIs due to a number of possible reasons specific to the disease. 38 Increased urine glucose levels could encourage the growth of harmful microorganisms. High amounts of glucose in the renal parenchyma provide an ideal environment for bacteria to develop and multiply[20]. The same species and virulence factors are responsible for UTIs in women, pregnant or not. *Escherichia coli*, *Klebsiella pneumonia*, Proteus, Acinetobacter, *Staphylococcus saprophyticus*, Group B Streptococcus (GBS), and *Pseudomonas aeruginosa* are among the bacteria that are frequently isolated. Preterm deliveries were more common in women with a UTI during pregnancy than in those without one (c2=7.092; p=0.007). While pyelonephritis was comparatively uncommon in this group (1.45%), recurrent UTI was noted in 26.6% of women with UTI. Pregnancy-related UTI and LBW did not significantly correlate (c2=0.097; p=0.756). *Escherichia coli* (30.9%) and Group B Streptococcus (GBS, 31.3%) were the most frequently isolated bacteria from women with UTIs. They had a broad spectrum of antibiotic sensitivity[21]. Preventive treatment is necessary since recurrent infections are prevalent during pregnancy. Intrapartum preventive medication should be administered to pregnant women who have a urinary group B streptococcal infection[22]. Education regarding the link between recurrent UTIs and the frequency of sexual activity and the use of spermicide-containing products should be part of strategies to prevent recurrent UTIs in young women[23]. Particularly for pregnant women from low socioeconomic backgrounds, the study suggests health education regarding personal hygiene and repeated urine cultures in the first and third trimesters of pregnancy. Prescriptions for certain antibiotics are necessary to prevent unfavorable pregnancy outcomes[24]. Giving an update on UTIs in immunocompromised patients was the main goal. The most frequent clinical cases that urologists see are kidney transplant (KT), diabetes mellitus (DM), and chronic kidney disease. A summary of diagnosis, treatment, and future research needs is provided. Autosomal dominant polycystic kidney disease (ADPKD) individuals had a 21–75% UTI prevalence. Although their efficiency is trending downward due to rising resistance rates, lipophilic antibiotics are still effective in treating infected cysts. With a reported rate of 45-72%, UTIs are the most prevalent illness among KT recipients. Both KT and ADPKD patients who get UTIs have diagnostic difficulties[25].

Uncomplicated UTIs

One of the most frequent bacterial illnesses seen in clinical practice is an uncomplicated urinary tract infection (UTI). They mostly impact the bladder and related structures in the lower urinary system. Prompt and adequate care of simple UTIs depends on a prompt and correct diagnosis. Reducing UTIs through preventative measures is essential to lessening the strain on medical resources. Health care providers can enhance patient treatment and improve overall results by being aware of the essential features of simple UTIs[26]. Numerous bacterial virulence factors have been found in UTI-causing pathogens. The most significant inhibitory variables in urine include low pH, high urea, high organic acid, and very high or low osmolality. Numerous novel antimicrobial drugs have been developed that aid in the eradication of germs, particularly those microbes that were resistant to earlier antibiotics[27]. For simple illnesses, E. coli predominates in 80–90% of cases. Acute infection may be caused by microorganisms that have the capacity to colonize the uroepithelium (fimbriae/pili) and to cytotoxically harm cells and tissue (hemolysin)[28]. The effectiveness of trimethoprim-sulfamethoxazole (TMP-SMX) in treating uncomplicated UTIs caused by TMP-SMX-resistant (TMP-SMX-R) microorganisms was assessed in this study. Pyuria and bacteriuria were evaluated in healthy non-pregnant premenopausal women with lower urinary tract infections; if either was found, a urine sample was obtained and TMP-SMX was recommended[29]. As people age, their incidence rises, and the lack of novel treatments and antibiotic resistance make therapy more difficult. We looked into how susceptible the current urine isolates in Europe were to fosfomycin and other antibiotics[30]. Our study's findings support our recommendation that fosfomycin be used as the first line of treatment for simple lower urinary tract infections because of its wide spectrum, minimal uropathogen resistance, excellent safety profile, and good compliance[31]. It is suggested that phytolysin be used in the combined treatment of acute, uncomplicated cystitis and as a way to stop the illness from returning[32]. Because of the higher risks of resistance, the empirical treatment of UTIs with fluoroquinolones should be limited[33].

Symptoms of UTIs

UTI symptoms include a strong and frequent urge to urinate, burning while passing urine, lower abdominal pain even when passing little urine, blood when passing urine, and a stronger than usual urine odor. Symptoms of upper urinary tract infections typically include fever (>100°F), chills, flank pain, nausea, vomiting, discomfort in the costovertebral angle, and/or cystitis.3. Fever is typically linked to more complex types of UTIs and is rare in lesser UTIs. Common UTI symptoms include fever, burning, itching, blistering in the vaginal area, pain in the suprapubic region, and pyuria. Inflammation and a urine white blood cell (WBC) count of more than 8 cells/ mL are signs of a symptomatic infection. The disorder is known as pyuria or leucocyturia, and the urine may seem cloudy. If patients have a history of recurrent UTIs, this likelihood rises to 84%–92%[34]. The most prevalent cause of recurrent UTIs, which manifest as dysuria or irritative voiding symptoms, is reinfection with the original bacterial isolate in young, healthy women who do not have any urinary tract anatomical or functional abnormalities. The most reliable indicator of recurrent UTIs in people with recurrent dysuria is the frequency of sexual activity[35]. Young, sexually active females and patients exhibiting additional signs and symptoms typical of these illnesses should be evaluated for other causes of dysuria, such as urethritis and vaginitis brought on by venereal disease[36]. The microbiological diagnosis is frequently challenging, despite the fact that the clinical diagnosis is straightforward and mostly depends on symptoms and urine sediment microscopy. The primary causative agent, *Escherichia coli*, is responsible for about 80% of urinary tract infections[37].

Diagnosis of UTIs

A new technique for identifying bacteria is surface-enhanced Raman spectroscopy (SERS). There aren't many documented uses of SERS in clinical diagnosis, though. Urine pathogens in patients with urinary tract infections (UTIs) were identified in this investigation using cylindrical SERS chips[38]. The majority of isolates exhibited multidrug resistance, while nitrofurantoin, amikacin, and gentamicin had comparatively greater action against isolates. Numerous bacterial uropathogens were found to be present in cultures of around one-third of the individuals with urinary tract infections who received a clinical diagnosis. Bacterial infections were more common in inpatients and hypertensive patients. The percentages of bacterial isolates that were susceptible to the tested antibiotics varied[39]. The UriSed analyzer's sensitivity value was poor, despite its specificity falling within

reasonable bounds. As a result, UriSed urinalysis results are not reliable indicators of culture results[40]. With a low percentage of false-negative results and great sensitivity, the UriSed appears to be a useful tool for UTI screening[41]. An algorithm for diagnosing UTI in children under five was developed using the findings of the systematic review. The review's findings about best practices are reflected in this algorithm. Not enough information was available to suggest an algorithm for the additional study of UTI in children less than five. The quality assessment pointed out a number of areas that future research on diagnostic accuracy could focus on improving[42]. It is reasonable to rule out UTI if a clean void pee, bag, or diaper/pad specimen is dipstick negative for both LE and nitrite, or if microscopic analysis is negative for both pyuria and bacteriuria. Without the necessity for a confirmation culture, these patients can thus be rationally eliminated from additional research. In a similar vein, a series of positive tests could be used to rule out a UTI and initiate additional research[43]. For identifying UTI in children, Gram stain and dipstick analysis for nitrite and LE work similarly and are better than microscopic examination for pyuria[44]. In situations of suspected urinary tract infections, the results of urinalysis, especially the leukocyte esterase and nitrite tests, are frequently utilized to decide whether therapy is required or whether a culture will be conducted[45]. A positive nitrite test result has a 96% positive predictive value. The nitrite test's diagnostic accuracy was not increased by include the leukocyte esterase test results[46]. Women who exhibit symptoms related to their urinary tract are diagnosed with UTIs. A weak sign that an infection is not present is vaginal discharge. The most accurate method for identifying UTIs was the urine dipstick test[47].

Outcomes of UTIs

The majority of patients who received carbapenem treatment recover quite well[48]. Table 2 and Figure 2 present the results of hospitalizations with CAUTI and non-CA cUTI. Although overall mortality was low, admissions with CAUTI (3.4%) had a mortality rate that was almost 20% greater than that of patients with non-CA cUTI (2.8%)[49]. The prevalence of CRE in patients with sepsis, pneumonia, or Enterobacteriaceae UTI was similar to other national estimates in this sizable US database. A four-fold higher probability of acquiring IET was linked to CRE infection, which raised mortality, length of stay, and expenses[50]. One in eight hospitalized individuals with cUTI develop TR. Particularly in the context of non-CAUTI, it is linked to a slight attributable lengthening of LOS, an increase in overall expenditures, and an increased risk of IET exposure[51]. The most common cause of cUTI was CA-UTI, which primarily affected elderly individuals. Although not directly linked to the infection, patients with CA-UTI had a high mortality rate[52]. The purpose of the study is to learn more about the epidemiology, clinical management, outcome, and healthcare costs of individuals with cUTI, particularly in nations where MDR GNB is highly prevalent[53]. Compared to patients who do not need such devices, patients with CAUTI have a higher frequency of resistant bacteria, utilize more resources, and have worse clinical results. Stronger controls should be put in place to reduce its use in both inpatient and outpatient settings[54]. If at least 70% of respondents assessed an outcome as critical (rated 7–9), it was included in round 3. In order to prevent other groups from overshadowing public-prioritized outcomes, round 3 also included any outcome with an average score of 7 or above among those who have had a UTI or have cared for someone who has[55]. The outcomes from various treatment approaches must be comparable across trials if clinical trial results pertaining to UTI treatment interventions are to influence policy and practice[56]. The disparity in results currently reported makes it difficult to compare the results of trials looking into simple UTI treatment. Establishing a minimal number of consistent outcomes that ought to be recorded in every study would enhance consistency in outcome reporting[57]. A significant number of outcomes were found. These objectives were narrowed down to a core set of six outcomes using strong consensus techniques, which should be assessed and reported in randomised trials and systematic reviews of treatments for people with uncomplicated UTIs at the very least[58]. The symptom diary is a promising tool for future use because it measures a number of outcomes, such as the degree of symptoms, their bothersomeness, and their influence on daily activities. It is necessary to conduct additional validation research, which includes evaluating structural validity[59].

DISCUSSION

The management and outcomes of UTIs depend on various factors, including the severity of the infection, patient characteristics, and whether the infection is complicated or uncomplicated. In this discussion, we will explore the decision-making process for managing UTI patients, considering the treatment options, factors influencing the course of the disease, and potential outcomes. The data used in this study was not intentionally gathered for research purposes; rather, it was analyzed as part of ordinary clinical primary care practice. In conclusion, there are opportunities to optimize antibiotic prescribing for UTIs that could potentially reduce the risk of antibiotic resistance. Most patients currently diagnosed with lower UTI in primary care receive same-day antibiotic treatment, without microbiological investigation. In order to minimize the development of antibiotic resistance to medications used for first- and second-line therapy, management of UTIs may need to take recent antibiotic use into account.

CONCLUSION

"In conclusion, the management of UTIs requires a multifaceted approach that incorporates accurate diagnosis, effective treatment, and preventive strategies. Antibiotic therapy remains the cornerstone of UTI treatment, but it is essential to consider the rising threat of antimicrobial resistance and to adopt antibiotic stewardship practices. Additionally, non-antibiotic treatments, such as pain management and behavioral modifications, can play a crucial role in alleviating symptoms and improving patient outcomes.

To optimize UTI management, healthcare providers should
Stay up-to-date with the latest guidelines and recommendations, adopt a patient-centered approach that takes into account individual needs and preferences, encourage patients to practice good hygiene and behavioral habits to prevent UTI recurrence, consider the use of alternative treatments, such as cranberry products or probiotics, in conjunction with conventional therapies. By adopting a comprehensive and evidence-based approach to UTI management, healthcare providers can improve patient outcomes, reduce the risk of complications, and mitigate the spread of antimicrobial resistance."

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RESEARCH ARTICLE

Exploring the Ethno Medicinal Antioxidant Properties of *Musa Paradisiaca* Latex through Biochemical Assays

R. Rakkimuthu*

Assistant Professor and Head, PG and Research Department of Botany, Nallamuthu Gounder Mahalingam College, Pollachi, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
R. Rakkimuthu
Assistant Professor and Head,
PG and Research Department of Botany,
Nallamuthu Gounder Mahalingam College, Pollachi,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: biorakki@gmail.com

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ABSTRACT

The present study investigates the antioxidant potential of methanolic extracts of *Musa paradisiaca* through various in vitro assays, including DPPH, ABTS, nitric oxide scavenging, and total antioxidant capacity. The DPPH assay revealed moderate radical scavenging activity with an IC_{50} value of 349.3 µg/ml, whereas the ABTS assay demonstrated significantly stronger activity, with 99.32% inhibition at 1000 µg/ml and an IC_{50} of 36.73 µg/ml. The extract also showed promising nitric oxide scavenging activity (73.42% at 1000 µg/ml), though with a higher IC_{50} of 417.04 µg/ml. The total antioxidant capacity increased in a concentration-dependent manner, achieving 69.08% inhibition at the highest tested concentration. When compared to the standard ascorbic acid, the extract showed lower potency but consistent activity across all assays. The results indicate that *M. paradisiaca* is a potential source of natural antioxidants and supports its ethnomedicinal relevance. Further research is recommended to isolate and characterize the active constituents responsible for the observed activity.

Keywords: *Musa paradisiaca*, antioxidant activity, DPPH, ABTS, nitric oxide, total antioxidant capacity, methanolic extract.

INTRODUCTION

Banana (*Musa* spp.) is a tropical flowering plant widely cultivated for its edible fruit and ranks among the most important staple foods globally, following rice, wheat, and maize. Beyond its nutritional value, bananas have long been recognized for their medicinal properties in both traditional and scientific contexts [1]. Various parts of the

banana plant—including the pseudo-stem, leaves, sap, and flowers—have demonstrated a range of pharmacological activities such as anti-snake venom[2], anti-gastric ulcer[3], antimicrobial[4], antidepressant[5], antihypercholesterolemic and antioxidant[6], antidiarrheal [7], and antidiabetic effects [8]. In traditional medicine, the banana plant's latex or sap is valued for its astringent properties and has been used to treat a wide spectrum of ailments, including leprosy, hysteria, fever, digestive disorders, hemorrhage, epilepsy, hemorrhoids, and insect bites [9]. Latex, a milky secretion derived from the stem, peel, and unripe fruit, is particularly rich in secondary metabolites such as phenolics, flavonoids, and terpenoids [10]. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defenses, is a major contributor to the pathogenesis of chronic conditions including cancer, diabetes, and cardiovascular diseases [11]. Due to growing concerns over the safety of synthetic antioxidants, there has been increased interest in identifying effective natural alternatives [12]. In this context, banana latex, with its reservoir of bioactive compounds, presents promising antioxidant potential. However, its antioxidant efficacy remains relatively underexplored. Therefore, the present study was undertaken to evaluate the antioxidant activity of banana latex through various in vitro assays. The investigation aims to determine its potential as a natural source of antioxidants. Previous studies by Pothavorn *et al.* [13] and Nguyen *et al*. [14] on the phytochemistry of banana stem juice laid the foundation for this exploration into the bioactive potential of banana latex.

MATERIALS AND METHODS

Collection of plant latex

Musa paradisiaca latex was collected from the surrounding area of Pollachi in Coimbatore district.

Extraction of latex

Musa paradisiaca leaf and stem latex were collected aseptically and extracted using an organic solvent (methanol). These were centrifuged for 5–10 minutes. The extracted material was collected and stored in an airtight container.

***In vitro* Radical Scavenging activity**

Sample Preparation

The methanol leaf extract of *Musa paradisiaca* latex 50gm was dissolved in 5 ml of DMSO. From the above 1000, 500, 250, 125 and 62.5 mg/ml concentration of sample was prepared and used for different assays. Ascorbic acid was used as standard for comparing the activities.

DPPH radical scavenging activity

The free radical scavenging capacity of the evaporated sample of *Musa paradisiaca* extract was determined using DPPH by Blois [15]. DPPH solution was prepared in 95% methanol. Different concentration (62.5-1000mg/ml) of methanol extract of *Musa paradisiaca* latex was reacted with freshly prepared 3.0 ml DPPH solution (0.1mM) and incubated at room temperature. After 30 minutes, the absorbance was taken at 517 nm using a spectrophotometer (Labman UV – Visible spectrophotometer). Ascorbic acid was used as a reference standard. The control was devoid of the sample. Standard ascorbic acid was treated similar to the extracts. The percentage of inhibition was calculated by following formula.

$$\% \text{ of DPPH radical scavenging activity} = \frac{(\text{A of control} - \text{A of sample})}{\text{Aofcontrol}} \text{X100}$$

ABTS radical scavenging assay

ABTS (2, 2′-azinobis-3-ethylbenzothiozoline-6-sulphonicacid) assay is based on the scavenging of light by ABTS radicals. An antioxidant with an ability to donate a hydrogen atom will quench the stable free radical, a process that is associated with a change in absorption. The relatively stable ABTS radical was green and it was quantified spectrophotometrically at 734 nm. Fresh ABTS solution was prepared for each assay. Different concentration (62.5-1000mg/ml) of *Musa paradisiaca* latex extract was allowed to react with 1 ml of the ABTS solution and the absorbance

was taken at 734 nm after 7 minutes using a spectrophotometer [16]. The percentage of inhibition was calculated by following formula.

% of ABTS radical scavenging activity $= \frac{(\text{A of control} - \text{A of sample})}{\text{A of control}} \text{X}100$

Nitric oxide scavenging activity

The nitric oxide scavenging capacity of the extract of *Musa paradisiaca* was determined using nitric oxide generated by oxidation nitroprusside [17]. Sodium nitroprusside (10 mM, 2ml) in phosphate buffer saline was incubated with different concentration of methanol extract of latex (62.5-1000mg/ml) at room temperature for 30 minutes. After 30 minutes, 0.5 ml of the solution was mixed with 1ml of Griess reagent. The absorbance was measured at 546 nm. Ascorbic acid was used as a reference standard. The percentage of inhibition was calculated by following formula.

% of inhibition $= \frac{(\text{Aofcontrol} - \text{Aofsample})}{\text{Aofcontrol}} \text{X}100$

Total antioxidant capacity by phosphomolybednum assay

The spectrophotometric measurement of total antioxidant capacity (TAC) was based on the reduction of Mo (V1) to Mo (V) by antioxidant compound and the formation of green phosphate / Mo (V) complex at acidic PH[18]. Different concentrations (62.5-1000mg/ml) of methanolic latex extracts were combined with 3 ml of reagent solution (0.6 sulfuric acid, 28 Mm sodium phosphate and 4Mm ammonium molybdate). The tubes containing the reaction solution was incubated at 95 C for 90 min. Then the absorbance of the solution was measured at 695 nm. Methanol (0.3ml) in the place of extract was used as the blank. Ascorbic acid was used as a reference standard.

RESULTS AND DISCUSSION

The present study evaluated the DPPH radical scavenging activity of the methanolic leaf extract of *Musa paradisiaca* and compared its antioxidant potential with that of ascorbic acid as a standard. The results demonstrated a concentration-dependent increase in free radical scavenging activity, which aligns with earlier finding reported by Subashree [19]. At the highest tested concentration (1000 µg/ml), the methanolic extract exhibited 77.42% inhibition of DPPH radicals, with an IC_{50} value of 349.3 µg/ml. In comparison, ascorbic acid showed superior scavenging ability with an IC_{50} of 30.93 µg/ml, confirming its strong antioxidant activity. Subashree reported a high percentage inhibition (98.19%) at 10 mg/ml for *M. paradisiaca* peel extracts, suggesting potent antioxidant properties at higher concentrations [19]. In contrast, the current study observed substantial antioxidant activity at a relatively lower concentration, indicating the leaf extract's effectiveness. The antioxidant activity observed can be attributed to the presence of bioactive compounds such as flavonoids and phenolics, which are known to donate hydrogen atoms or electrons to neutralize free radicals. This is in line with the findings of Sharma *et al*. [20] who reported significant antioxidant activity in the methanolic and ethanolic extracts of various parts of *Musa paradisiaca* and *Musa acuminata*, particularly in the unripe pulp and peel. In their study, the methanolic extract of unripe pulp of *Musa acuminata* showed the highest DPPH scavenging activity (85.38%), whereas our results suggest moderate activity in latex extracts. DPPH radical scavenging of *Musa paradisiaca* latex (Table 1). The ABTS radical scavenging activity of the methanolic extract of *Musa paradisiaca* latex was investigated and compared with the standard antioxidant, ascorbic acid. The results, presented in Table 2, indicate that the extract possesses strong antioxidant potential in a concentration-dependent manner. At the highest tested concentration (1000 µg/ml), the methanolic extract achieved 99.32% inhibition of ABTS radicals, with an IC_{50} value of 36.73 µg/ml. Ascorbic acid, used as a positive control, showed slightly higher radical scavenging activity with 99.63% inhibition at 50 µg/ml and an IC_{50} of 3.57 µg/ml. Although ascorbic acid exhibited stronger potency, the methanolic extract demonstrated a comparable radical scavenging effect, especially at higher concentrations. These findings are consistent with previous studies. Okonogi *et al*.[21] reported a similar concentration-dependent ABTS radical scavenging activity for ethanolic extracts of banana peels, with a maximum inhibition of 97.66% observed at 10 mg/ml. The present study shows even higher inhibition (99.32%) at a considerably lower concentration (1000 µg/ml), suggesting the superior effectiveness of *M. paradisiaca* latex extract in ABTS radical neutralization. Additionally, Fidrianny *et al*. [22] examined various banana peel extracts (raja bulu, muli and ambon lumut) and classified their antioxidant strength based on IC_{50} values. Their study found

IC_{50} values for ethyl acetate and ethanolic extracts ranging from 1.91 to 11.63 ppm, indicating very strong antioxidant properties. Although the IC_{50} of the current methanolic extract (36.73 µg/ml or 36.73 ppm) is slightly higher, it still falls within a range that signifies moderate to strong antioxidant activity, especially when considering its natural origin and simplicity of extraction. The ascorbic acid in Irda's study demonstrated an IC_{50} of 1.27 ppm, reaffirming its role as a benchmark antioxidant. The nitric oxide (NO) scavenging activity of the methanolic extract of *Banana* was investigated to assess its potential activity. As shown in Table 3, the extract exhibited a dose-dependent increase in NO inhibition, with the highest activity (73.42 ± 11.82%) observed at 1000 µg/ml. The IC_{50} value was determined to be 417.04 µg/ml, indicating a moderate nitric oxide scavenging capacity. In comparison, the standard antioxidant ascorbic acid demonstrated significantly higher NO inhibitory activity, with 83.45 ± 4.80% inhibitions at 50 µg/ml and an IC_{50} value of 20.85 µg/ml. Nitric oxide, though essential for physiological signaling, becomes a contributor to oxidative stress when overproduced, reacting with superoxide radicals to form reactive peroxynitrite ions. Thus, the capacity of plant extracts to scavenge nitric oxide radicals is an important indicator of their antioxidant potential [23]. The observed NO scavenging activity in *Musa paradisiaca* extract can be attributed to the presence of bioactive compounds such as flavonoids, tannins, and phenolic acids. These phytochemicals are known to inhibit nitrite formation by competing with oxygen to react with nitric oxide. Sharma *et al*. [20] reported substantial antioxidant potential in various banana plant parts, especially the methanolic and ethanolic extracts of pulp and peel, correlating with high flavonoid and phenolic content. Though NO assay was not directly evaluated in their study, their findings support the idea that such antioxidant activity is linked to phytochemical richness. The total antioxidant capacity of the methanolic extract of *Musa paradisiaca* was evaluated using the phosphomolybdenum method, and the results are presented in Table 4. The extract exhibited a concentration-dependent increase in antioxidant activity, with maximum inhibition of 69.08% observed at 1000 µg/ml. Although slightly lower than the standard ascorbic acid (70.10% inhibition at 50 µg/ml), the extract's performance was notably comparable across the tested concentrations, suggesting that *M. paradisiaca* possesses appreciable total antioxidant potential. These findings align with earlier work by Prieto *et al*.[18] who used the same method to quantify total antioxidant activity in various banana plant parts, expressing the results as ascorbic acid equivalents (AAE). They reported that methanolic stem peel extract (MSPE), pulp extract (MSPU), and stem extract (MSSE) showed total antioxidant capacities of 675.56 ± 5.23, 573.70 ± 314, and 416.85 ± 5.50 mg/g AAE, respectively. Though the present study did not quantify in AAE, the percentage inhibition values suggest that the methanolic extract of *M. paradisiaca* latex may fall within a similar activity range, particularly closer to MSSE in antioxidant strength. Supporting this, Gonzalez-Montelongo *et al*. [24] found that the ethanolic extract of the Pachainadan variety exhibited high total antioxidant capacity (5.85 mM/g), whereas the Nendran variety showed the least activity. The observed variation in antioxidant capacity between banana cultivars and plant parts is consistent with the moderate but significant antioxidant activity demonstrated in this study, likely influenced by differences in polyphenol and flavonoid content.

CONCLUSION

The methanolic extract of *Musa paradisiaca* demonstrated effective antioxidant activity across all tested *in vitro* assays. In the DPPH assay, the extract exhibited moderate free radical scavenging with an IC_{50} value of 349.3 µg/ml, while the ABTS assay showed a much stronger activity with 99.32% inhibition at 1000 µg/ml and a significantly lower IC_{50} value of 36.73 µg/ml, indicating its higher efficacy in scavenging ABTS radicals. The nitric oxide scavenging activity was also notable, reaching 73.42% inhibition at the highest concentration, though with a relatively higher IC_{50} value of 417.04 µg/ml. Total antioxidant capacity increased steadily with concentration, reaching 69.08% at 1000 µg/ml, and closely matched the activity of standard ascorbic acid. Collectively, the results confirm that *M. paradisiaca* possesses concentration-dependent antioxidant properties, with the strongest performance observed in the ABTS assay. While its IC_{50} values were higher than ascorbic acid in all assays, the extract still showed promising antioxidant potential. These findings support the traditional use of *M. paradisiaca* and suggest its suitability for further phytochemical and pharmacological exploration as a natural antioxidant source.

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Table 1: DPPH radical scavenging of *Musa paradisiaca* latex

DPPH radical scavenging of *Musa paradisiaca*						
Concentration	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml	1000µg/ml	IC_{50}µg/ml
Methanolic Latex extract of *M.paradisiaca*	55.19±1.48	62.15±0.74	63.12±1.84	71.7±1.82	77.42±1.10	349.3
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC_{50}µg/ml
Ascorbic acid (% inhibition)	26.39±1.32	41.69±1.03	49.90±0.70	58.81±1.48	68.48±0.54	30.93

Table 2: ABTS Radical Scavenging Activity of *Musa paradisiaca* latex

ABTS Assay of *Musa paradisiaca*						
Concentration	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml	1000µg/ml	IC_{50} µg/ml
Methanolic latex extract (% of inhibition)	80.28±0.6	93.71±1.16	97.42±1.43	98.7±1.72	99.32±0.86	36.73
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC_{50} µg/ml
Ascorbic acid (% of inhibition)	97±1.0	98±0.44	98.42±1.06	99.85±0.55	99.63±0.72	3.57

Table 3: Nitric Oxide Assay of *Musa paradisiaca* latex

Nitric Oxide Assay of *M.paradisiaca*						
Concentration	62.5µg/ml	125µg/ml	250µg/ml	500µg/ml	1000µg/ml	IC_{50}µg/ml
Methanolic latex Extract of *M.paradisiaca*	10.40±2.08	29.01±1.36	64.21±1.65	64.68±1.12	73.42±1.81	417.04
Concentration	10µg/ml	20µg/ml	30µg/ml	40µg/ml	50µg/ml	IC_{50}µg/ml
Ascorbic acid % inhibition	36.25±1.81	50.90±1.9	60.73±1.2	71.07±1.0	83.45±1.80	20.85

Table : 4 Total Antioxidant Assay of *Musa paradisiaca* latex

Total antioxidant assay of *M. Paradisiaca*					
Concentration	62.5 µg/ml	125 µg/ml	250 µg/ml	500 µg/ml	1000 µg/ml
Methanolic latex extract (% of inhibition)	44.66±2.54	56.89±1.36	57.55±1.88	59.86±095	69.08±056
Concentration	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml
Ascorbic acid (% of inhibition)	44.35±1.25	49.89±1.4	56.74±0.8	57.81± 1.6	70.10±.68

REVIEW ARTICLE

Nutritional Status and Muscle Strength among Hospitalised Pediatric Population

K.Janani[1], A.J. Hemamalini[2]* and Soma Basu[3]

[1]Postgraduate Student, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India.
[2]Professor and Head, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India.
[3]Ph.D. Research Scholar, Department of Clinical Nutrition, Sri Ramachandra Faculty of Allied Health Sciences, Sri Ramachandra Institute of Higher Education and Research (Deemed to be University), Chennai, Tamil Nadu, India.

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***Address for Correspondence**
A.J. Hemamalini
Professor and Head,
Department of Clinical Nutrition,
Sri Ramachandra Faculty of Allied Health Sciences,
Sri Ramachandra Institute of Higher Education and Research
(Deemed to be University), Chennai, Tamil Nadu, India.
E.Mail: hemamalini.aj@sriramachandra.edu.in

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ABSTRACT

Handgrip Strength (HGS) is an important indicator of muscle strength and nutritional status, particularly in hospitalized pediatric populations. It has been recognized as a potential tool for diagnosing malnutrition, as low HGS is associated with various negative health outcomes, including prolonged hospital stays and poor recovery. The SQUEGG Smart Dynamometer, a Bluetooth-enabled device, is commonly used to measure HGS and track muscle strength in clinical settings. This review article deals with role of nutritional status and anthropometric datas, as the indicators for muscle strength among hospitalised pediatric population. A systematic review of the literature was conducted using databases such as Medline, Pub Med and Google Scholar. Research articles focusing on pediatric malnutrition, HGS, and muscle strength assessment were included. The STAMP screening tool and SQUEGG dynamometer were highlighted as primary methods for nutritional screening and HGS assessment in hospitalized children. Studies have showed significant correlations between HGS and anthropometric factors such as age, gender, height, and weight. Children with low HGS upon hospital admission were more likely to experience malnutrition and longer hospital stays. These findings suggest

that HGS can be a valuable indicator of malnutrition, providing early insight into a child's nutritional and functional status. HGS, measured through tools like the SQUEGG ball, is a reliable and non- invasive method for assessing muscle strength and nutritional status in pediatric patients. Incorporating HGS assessments in routine clinical practice can improve the detection of malnutrition and optimize care for hospitalized children, potentially leading to better health outcomes and shorter recovery times. Further studies are needed to explore the role of micronutrients in muscle function.

Keywords: Handgrip Strength, Pediatric Malnutrition, SQUEGG Smart Dynamometer, Muscle Strength, Nutritional Status, Hospitalized Children, STAMP Screening Tool, Grip Strength, Nutritional Screening, Muscle Function, Pediatric Nutrition, Malnutrition Diagnosis, Pediatric Hospital Care.

INTRODUCTION

HGS is a numerical indicator of overall muscular strength and function, recently indicated as a criterion for the diagnosis of pediatric malnutrition according to the American Society for Parenteral and Enteral Nutrition and the Academy of Nutrition and Dietetics(ASPEN- AND).HGS(Handgrip grip Strength) is an important indicator of muscle strength and nutritional status in hospitalized children. It is often used as an indicator of general muscle strength in children, and low HGS is associated with a number of negative health outcomes[1]. This is a measure of muscle strength that uses a SQUEGG ball to measure the force/tension produced by the muscles in the arm. A Bluetooth enabled dynamometer can help to assess, track and improve your grip strength[2]. The SQUEGG Smart Dynamometer and Hand Grip Trainer , known as the SQUEGG by The BioSparrow in plantation FL is a handheld digital dynamometer that can connect via Bluetooth to a mobile device(such as a phone or tablet) to show and save handgrip strength data in a cloud system. Food and Drug Administration class II medical device, which is exempt from 510(K) clearance, is egg- shaped with four finger indentations for the second through fifth digits and an outer silicone shell to avoid moisture buildup. The static force that a person's hand produces when it is squeezed around a dynamometer is measured during grip strength test. Newtons, Pounds,or kilograms are used to indicate the production [2]. Hand exercise tools called "Squeeze balls" are filled with beads, sand, or silicon. Silicon-filled products typically employ two latex shells to prevent oil leakage, but latex can trigger allergies. Hand grip strength is a reliable measure of nutritional status[2]. Grip strength is a measure of overall health and can be used to assess frailty and recovery progress.[3]. A weak grip indicates a higher risk of health problems in children, leading to longer hospital stays and poorer overall health outcomes[4]. Low handgrip strength in hospitalized children is a significant predictor of poor health outcomes, extended hospital stays, and increased healthcare expenses.. The nutritional status is assessed using the STAMP screening tool.

Early diagnosis of malnutrition and muscle strength assessment improve recovery, shorten hospital stays, and enhance health outcomes in children[5]. Nutritional deficiencies pose a health risk, but the difficulty in assessing pediatric malnutrition stems from the intricate nature of the problem[5]. Poverty and lack of access to food and healthcare are major contributors to malnutrition and disease, leading to deaths worldwide. Assessing a child's nutrition in a hospital setting is difficult because of the many factors involved and the potential negative effects of hospital environments[4]. Strong hand grip is essential for children's dexterity and everyday activities. Engaging in activities like climbing, writing, and playing with clay helps develop hand and finger muscles, improving dexterity and making tasks like coloring easier[6]. Adriana's group reviewed and selected eight articles from the 270 collection for their study. The study identified five nutrition assessment tools, with STAMP being the most promising option, exhibiting high sensitivity, perfect inter-rater agreement, and consistency with the reference test [5]. Vitamin D influences muscle strength and performance by binding to the Vitamin D receptor. This substance affects muscle cell growth and development. Vitamin D influences muscle growth by activating MAPK and other pathways, leading to increased myotube formation and enhanced expression of myosin heavy chains[7].This review article deals with role

of nutritional status and anthropometric datas, as the indicators for muscle strength among hospitalised pediatric population.

METHODOLOGY

A review was carried out in Medline (National Library of Medicine United States), LILACS (Latin American and Caribbean Health Sciences), and PubMed (US National Library of Medicine National Institutes of Health). in the SCIELO (Scientific Electronic Library Online), via CAPES portal (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior),bases Scopus and Web of Science and Google Scholar. The terms utilized according to the Descriptors in Health Sciences (DeCS)/Medical Subject Headings (MeSH) list were "malnutrition", "screening", and "pediatrics", in addition to the equivalent terms in Portuguese. Types of research included are cross sectional studies and longitudinal study. Total 9 from 2013-2024 were reviewed in order to write this review paper.

RESULTS

A study conducted by J.W. Ploegmakers *et al*. in 2013 found a strong correlation between grip strength and height, weight, and gender in children, as shown in a cross-sectional study of 2241 children and adolescents aged 4 to 15 years of both genders. The study states that As people get older, their strength when using both hands increases, and boys up to the age of 12 show improvement. Males, in contrast, show much greater level of control at ages 4, 5, 6, 8, 9, and 14 in comparison to females. Between 4 and 13 years old, boys had a strength gap of 0 to 14%, and this increased to 26% at 14 years old. Age, height, weight, and gender had a strong connection to grip strength[8]. In a different clinical study titled Handgrip Strength and Nutrition Status in Hospitalized Pediatric Patients by Catarina Silva MSc *et.al*.,(2014) Citations: 23, it was reported that a total of eighty-nine hospitalized patients aged six years and older were part of the longitudinal research. The assessment of nutritional status included measuring body mass index (BMI) z-Score and hand grip strength (HGS) upon admission and discharge. Findings: At the beginning of hospitalization, 30.3% of patients were in critical condition in the complete sample, while 64% experienced a decrease in handgrip strength upon admission. This research concluded that hand grip strength upon admission was linked to malnutrition defined by BMI z-score. In this multivariate analysis, gender, age, height, and BMI z-Score accounted for 67.1% of HGS at hospital admission and suggested that Low HGS could signal malnutrition in pediatric patients, despite the need for interpretation of HGS data. Patient's gender, age, and height[9]. A study conducted by Gabriela Duarte Luz , Danielly Steffen Pereira *et,al* on association of handgrip strength with nutritional status and clinical outcomes in hopsitalized pediatric patients,50 patients were assessed, with a median age of 10.9 years and a male proportion of 55.6%. Reduced HGS was observed in 17.8% patients. Patients with lower HGS levels exhibited a lower H/A z-score (negative compared to positive, p = 0.012)), and a higher frequency of reduced MAMC ((8% vs 13%, p = 0.007)). Reduced HGS was not associated with malnutrition (OR = 0.63; 95%CI 0.23–1.77), prolonged hospital stay (OR = 1.89; 95%CI 0.72–4.92) or readmission to hospital 3 months after hospital discharge (OR = 1.82; 95%CI 0.67–4.93), in a model adjusted for the clinical condition. No association was found between reduced HGS and malnutrition or clinical outcomes. The lower H/A Z-score and MAMC/A percentile values indicate its association with a less favorable nutritional status in hospitalized pediatric patients[1].

DISCUSSION

According to Catarina Silva's research, analyzing HGS data should consider the patient's height, age, and gender, as lower HGS could suggest under nutrition in pediatric hospital patients. Examining the influence of anthropometrics, illness seriousness, and other patient characteristics on hand grip strength (HGS) in hospitalized pediatric patients. The correlation between low BMI z scores and HGS upon admission was significant. Nutritional status also have an impact on muscle strength .various factors such age, gender, height, weight also had a strong connect with muscle strength and nutritional status in children. Handgrip strength is a crucial measure of muscle function and nutritional health, particularly relevant for hospitalized children. Muscle strength can be assessed non- invasively and

affordably, allowing for early detection of malnutrition, even before physical changes become noticeable[9]. The SQUEGG Smart Dynamometer, a Bluetooth-connected device, is a new technology for measuring and monitoring HGS in children. Mobile devices enable healthcare providers to track grip strength, store data, and evaluate progress remotely. This digital dynamometer enhances the precision of HGS testing, making it particularly valuable in pediatric medicine[2]. In clinical practice, HGS measurement can be paired with nutritional screening tools like the STAMP method, which has proven to be highly sensitive in identifying malnutrition in hospitalized children. STAMP provides a multi-faceted assessment of a child's nutritional status, complemented by HGS data for a complete picture of their overall health[5]. Proper nutrition, specifically protein, leucine, n-3 fatty acids, and vitamin D, is vital for achieving optimal muscle strength and recovery. Vitamin D contributes to muscle cell development and growth, promoting overall muscle health[7]. By integrating HGS testing with nutritional assessments, healthcare providers can better diagnose and manage pediatric malnutrition, leading to improved clinical outcomes, quicker recovery, and reduced hospital stays.HGS plays a crucial role in identifying malnutrition and assessing muscle function in children, enabling timely interventions and improved treatment outcomes[2].

CONCLUSION

This study examines the use of the STAMP Screening tool and SQUEGG BALL to assess nutritional status and muscle strength in hospitalized children. Despite no significant differences in hospital stays or readmission rates, HGS remains a promising indicator of nutritional status. The research confirms the value of nutritional screening tools in pediatric care. Future research should explore the effects of micronutrient supplementation, especially vitamin D, calcium, and iron, on muscle function and strength, potentially leading to targeted therapies to improve muscle health in hospitalized children.

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RESEARCH ARTICLE

Antibiofilm Potential of Plant Derived Nano Emulsions against Oral Pathogens: A Nanotherapeutic Approach

S. Likhitha[1], Ch. Pravallika[1], B. Nirmala Jyothi[1] and S. Anju[2]*

[1]M.Sc Student, Department of Microbiology, Bhavan's Vivekananda College of Science Humanities and Commerce, Sainikpuri, (Affiliated to Osmania University, Hyderabad), Telangana, India.
[2]Assistant Professor, Department of Microbiology, Bhavan's Vivekananda College of Science Humanities and Commerce, Sainikpuri, (Affiliated to Osmania University, Hyderabad), Telangana, India.

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***Address for Correspondence**
S. Anju
Assistant Professor,
Department of Microbiology,
Bhavan's Vivekananda College of Science Humanities and Commerce, Sainikpuri,
(Affiliated to Osmania University, Hyderabad), Telangana, India.
E.Mail: anjunair227@gmail.com

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ABSTRACT

Oral biofilms are central to the onset and persistence of numerous dental disorders, demanding innovative and effective antimicrobial strategies. This study investigates the antibiofilm potential of nano emulsions formulated using plant-derived natural extracts. A tube-based biofilm formation assay confirmed strong bacterial adherence, as evidenced by intense crystal violet retention. Antimicrobial susceptibility testing via the Kirby-Bauer disc diffusion method revealed notable resistance patterns among oral pathogens to commonly used antibiotics, reinforcing the need for alternative therapeutic approaches. Natural extracts displayed dual effects on biofilm modulation: cloves and Tulasi effectively inhibited biofilm formation, whereas sucrose, glucose, jaggery, and certain commercial products such as toothpaste and chewing gum enhanced bacterial adhesion. The developed nano emulsion, composed of neem oil, green tea, and turmeric, significantly reduced biofilm biomass by 65–85%. Among the components, neem oil exhibited the highest disruption capacity due to its potent antibacterial properties, while green tea was most effective in preventing biofilm formation owing to its polyphenol content. Turmeric contributed to antimicrobial action but was comparatively less effective. These results highlight the promising role of plant-based nano emulsions as nanotherapeutic agents in managing oral biofilms and enhancing oral health.

Keywords: Nano Emulsion, Oral Biofilms, Plant Extracts, Antibiofilm Activity, Natural Antimicrobials

INTRODUCTION

Biofilms are structured microbial communities enclosed in a self-produced polymeric matrix that adhere to surfaces, contributing significantly to chronic infections and antibiotic resistance [1]. The ability of bacteria to form biofilms presents a major clinical challenge, particularly in the context of medical devices and persistent infections [2]. Traditional antibiotics often fail to penetrate the biofilm matrix effectively, leading to the persistence and recurrence of infections [3]. In recent years, the exploration of natural compounds derived from plants has gained momentum due to their antimicrobial, anti-inflammatory, and antibiofilm properties [4]. Herbal remedies, such as those derived from clove (*Syzygium aromaticum*), turmeric (*Curcuma longa*), and green tea (*Camellia sinensis*), have shown potential as alternative or adjunct therapies against biofilm-forming pathogens [5]. Clove extract is rich in eugenol, known for its antimicrobial and anti-inflammatory actions [6]. Turmeric contains curcumin, a compound with demonstrated antibiofilm and antioxidant effects [7], while green tea polyphenols, particularly epigallocatechin gallate (EGCG), inhibit quorum sensing and reduce bacterial adherence [8]. Nano emulsion technology enhances the delivery and bioavailability of these plant-based compounds. By encapsulating hydrophobic phytochemicals within nanoscale emulsions, it is possible to achieve targeted action with improved stability and penetration into biofilms [9]. Therefore, the development of a herbal nano emulsion oral spray using these traditional extracts may offer a novel, biocompatible approach to disrupt biofilm formation and reduce microbial load, especially in the oral cavity where biofilms contribute to dental plaque and periodontitis [10].

MATERIALS AND METHODS

Dietary Sugars

- **Glucose** – Primary energy source for biofilm-forming bacteria.
- **Sucrose** – Facilitates extracellular polymeric substance (EPS) production in biofilms.
- **Jaggery** – Natural sugar alternative with potential antimicrobial effects.

Oral Hygiene Agents

Herbal-based Mouthwash Formulated with turmeric, neem oil, and cloves, known for their antimicrobial properties.
Commercial Toothpaste Extract Extracted from Colgate toothpaste for comparative analysis.

Plant-Based Extracts

- Neem (Azadirachta indica) – Anti-biofilm and antibacterial effects.
- Tulsi (Ocimum sanctum) – Natural antimicrobial activity.
- Clove (Syzygium aromaticum) – Contains eugenol, an antibacterial agent.

Synthetic and Commercial Extracts Charcoal extract, Polo ring, Bubble gum for biofilm modulation.

Sample Collection

Selection of Subjects: Healthy individuals aged 18–40 with no recent antibiotic use.
Collection Method: Sterile cotton swabs were used to collect samples from buccal cavity, teeth, and tongue. Samples were placed in sterile PBS to maintain microbial viability and transported on ice to the lab.

Sample Processing and Bacterial Isolation

10 µL of bacterial suspension from the swab tube was spread onto Nutrient Agar plates. Plates were incubated at 37°C for 24 hours. Colony morphology (size, shape, pigmentation) was observed.

Gram Staining for Bacterial Identification

Bacterial colonies were smeared on a glass slide and air-dried.The smear was stained
with crystal violet, iodine, ethanol, and safranin for bacterial differentiation.Examined

under a light microscope to classify bacteria as Gram-positive or Gram-negative.

Biofilm Formation Assay

Tube Method: In the Tube Method for biofilm formation, 10 mL of nutrient broth is inoculated with the bacterial strain of interest and incubated at 37°C for 24 to 48 hours. After the incubation period, planktonic (free-floating) cells are discarded, leaving behind the biofilm that has formed on the walls of the test tube. The biofilm is then stained with crystal violet, which binds to the bacterial cells and extracellular matrix, providing a visual indication of biofilm formation. The intensity of the violet stain is assessed, which correlates to the amount of biofilm present; stronger staining indicates a higher degree of biofilm formation. This method allows for the semi-quantitative evaluation of bacterial biofilm development[11]

Microtiter Plate Method: In the Microtiter Plate Method for biofilm formation, bacterial cultures are first grown and the suspension is adjusted to a standardized concentration. Next, 100 µL of the bacterial suspension is dispensed into each well of a 96-well plate. Test extracts are then added to the wells, and the plate is incubated at 37°C for 24 hours to allow biofilm formation. After incubation, biofilm formation is assessed by crystal violet staining, which binds to the biofilm and allows for its quantification. The optical density (OD) of the stained biofilm is measured, and based on the OD values, biofilm formation is categorized as follows: $OD < 0.120$ indicates a non-biofilm former; $0.120 \leq OD < 0.240$ represents a weak biofilm former; $0.240 \leq OD < 0.480$ indicates a moderate biofilm former; and $OD \geq 0.480$ represents a strong biofilm former. This method provides a quantitative assessment of bacterial biofilm formation and can be used to evaluate the effects of various test extracts on biofilm production.[12].

Preparation of Herbal Nano emulsion Oral Spray: The preparation of a herbal nano emulsion oral spray begins with the extraction of natural components. Clove, turmeric, and green tea extracts are prepared using both aqueous and ethanol extraction methods to obtain the active compounds from these plants. After the extraction, the oil phase is prepared by mixing neem oil, Tween 80, and glycerol. This oil phase is then combined with the aqueous phase containing the extracted plant components. The mixture is subjected to sonication, a process that utilizes sound waves to break down the particles, ensuring uniform dispersion and formation of a stable nano emulsion. The resulting herbal nano emulsion is then ready for use in the oral spray formulation, designed to deliver the therapeutic properties of these natural extracts in a finely dispersed form[13] [14].

RESULTS AND DISCUSSION

Isolation and Characterization of Oral Biofilm-Forming Bacteria

To evaluate the efficacy of natural extracts and nano emulsions against oral biofilms, biofilm samples were collected from multiple volunteers using sterile swabs. These swabs were transported to the laboratory within one hour to maintain microbial viability and were streaked on selective and non-selective media such as Luria Bertani Agar, Blood Agar, and MacConkey Agar. Plates were incubated at 37°C for 24 hours. Following incubation, colonies exhibiting distinct morphologies were isolated and sub-cultured to obtain pure cultures. Colony characteristics such as colour, opacity, elevation, and margin were recorded (Fig1). Preliminary bacterial identification was supported by Gram staining and basic biochemical tests.

Isolation of Biofilm-Forming Bacteria

Pure isolates were subjected to biofilm screening using the tube method. Each isolate was inoculated into 5 mL of nutrient broth and incubated at 37°C for 24 hours. The broth was decanted, and the tubes were gently washed with phosphate-buffered saline (PBS), stained with 0.1% crystal violet, and visually inspected for biofilm formation. A visible purple ring at the air-liquid interface indicated positive biofilm formation. Isolates showing strong adherence were selected for further testing. (Fig 2)

Screening of Natural and Dietary Extracts

The biofilms were quantified by measuring their biomass using absorbance at 630nm.The inhibition percentage was calculated by comparing treated samples to untreated controls. The bar chart illustrates the biofilm inhibition percentage for each isolate. Neem leaves extract (1%) exhibited high biofilm inhibition, with percentages close to 100% in most cases. The neem leaves extract effectively inhibits biofilm formation in oral biofilm isolates. These findings support the potential antibiofilm properties of neem for oral health applications. The biofilm inhibition percentage for 1%Tulasi extract was determined. Tulasi leaves extract demonstrated high biofilm inhibition, with most isolates showing inhibition above 90%. The Tulasi leaves extract effectively inhibits biofilm formation in oral biofilm isolates. The data supports the potential antibiofilm properties of Tulasi, indicating its possible role in oral health treatments. The biofilm inhibition percentage for 1%cloves extract was determined. Cloves extract demonstrated strong biofilm inhibition, with most isolates showing inhibition close to or above 90%.Thecloves extract effectively inhibits biofilm formation in oral biofilm isolates. The results highlight the potential antibiofilm properties of cloves, supporting its use in oral health applications.(Fig 3)

Synthetic and Commercial Extracts

The biofilm inhibition potential of 1% plant-derived nanoemulsions—charcoal extract, Polo Ring extract, chewing gum extract, and Colgate extract—was assessed against oral biofilm isolates and is illustrated in the accompanying bar graph (Fig 4). All extracts demonstrated high levels of biofilm inhibition, predominantly within the 85–100% range. Charcoal extract showed strong antibiofilm activity with most values between 85–100% and minor variations across isolates, suggesting its effectiveness as a potential oral therapeutic. Polo Ring extract, while showing significant inhibition, also exhibited slightly higher biofilm growth in a few isolates, yet overall maintained strong antibiofilm efficacy. Chewing gum extract consistently inhibited biofilm formation, with inhibition percentages primarily between 85–95%, again showing minimal variation. Similarly, Colgate extract displayed inhibition levels comparable to charcoal, with a majority of values near or at 100%, reinforcing its potential as an effective antibiofilm agent. Overall, the majority of inhibition values across all extracts exceeded 90%, with distinct peaks at 100%, indicating strong and consistent antibiofilm activity. The inhibition trends for Polo Ring and chewing gum extracts were notably similar, with both showing a few instances below 90%, but these were infrequent. These findings support the promise of these plant-derived nanoemulsions as potent candidates for nanotherapeutic interventions in oral health.

Promotion of Biofilm by Dietary Sugar Extracts

There is a strong positive correlation between glucose concentration and biofilm growth. Higher glucose levels lead to higher biofilm formation. The regression line follows an upward trend, suggesting glucose directly supports biofilm development. Biofilm formation is more scattered compared to glucose. While a general upward trend exists, there are outliers where biofilm formation is high even at lower sucrose concentrations. This suggests that sucrose may not be the sole factor influencing biofilm formation. The strongest positive correlation is observed here. The data points are tightly clustered along the regression line, indicating that jaggery has a significant impact on biofilm formation. This suggests that jaggery may be a more potent biofilm inducer compared to glucose or sucrose Promotion of Biofilm by Dietary Sugar Extracts (Fig: 5). Biofilm inhibition by Different Extracts (Table 1)

Development of Nano emulsion

present study highlights the potent antibiofilm activity of plant-derived nanoemulsions formulated with turmeric, clove, green tea extracts, and neem oil. The small droplet sizes of the nanoemulsion (ranging from 1.29 to 45.10 nm) significantly enhanced the penetration of bioactive compounds into the biofilm matrix, enabling effective disruption of bacterial biofilms. In the first phase of the study, biofilm disruption percentages ranged from 66.68% to 98.26%, indicating moderate to high efficacy, with variability likely due to differences in initial nanoemulsion formulation or biofilm robustness. Subsequent optimization of the formulation resulted in a marked improvement, with disruption percentages consistently ranging from 96.82% to 100%, demonstrating the reliability and potency of the final nanoemulsion. Scanning Electron Microscopy (SEM) analysis further confirmed these findings, revealing substantial structural disruption in treated Streptococcus mutans biofilms. Untreated samples retained dense, multilayered EPS

matrices with intact cells, whereas treated samples exhibited cell shrinkage, lysis, and a disrupted biofilm architecture. Among the nanoemulsion components, neem oil showed the highest antimicrobial effect, followed by clove and turmeric. The synergistic action of these natural extracts, coupled with the nanoscale formulation, underscores the formulation's potential for oral biofilm management. Scanning Electron Microscopy- visualization of nano emulsion droplets (Figure: 6)

CONCLUSION

Based on the comparative analysis of natural extracts and formulations, Neem and Tulsi extracts emerged as the most effective agents in inhibiting oral biofilm formation, consistently demonstrating inhibition percentages ranging from 98% to 100% across multiple isolates. While Neem extract displayed slight variability, it recorded the highest number of complete inhibitions (100%), confirming its strong potential as a lead candidate in biofilm control strategies. Clove extract, though effective, exhibited broader variability in inhibition (75%–100%), indicating the need for concentration optimization or synergistic formulations. In contrast, Jaggery, glucose, and sucrose were found to promote biofilm formation, with jaggery showing the strongest enhancement, likely due to its complex sugar matrix promoting microbial adhesion and EPS synthesis. Importantly, the nanoemulsion formulation, incorporating active botanicals like turmeric, clove, green tea, and neem oil, demonstrated 90%–100% inhibition efficacy, benefiting from enhanced bioavailability and biofilm penetration due to its nano-sized droplets. Collectively, these findings underscore the superior efficacy of neem-based nanoemulsions and natural plant extracts as promising, eco-friendly alternatives for managing oral biofilms.

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Table 1: Biofilm inhibition by Different Extract

Extract Type (1%)	Biofilm Inhibition Range (%)	General Trend
Neem Leaves	~95-100%	Strongest inhibition, highly consistent
Tulasi Leaves	~90-100%	High inhibition, minor variability
Cloves	~90-100%	Strong inhibition, minimal variation
Glucose	~90-100%	Effective across all isolates
Sucrose	~85-100%	Slightly lower inhibition in some isolates
Jaggery	~80-100%	Moderate to high inhibition, some variability
Charcoal	~85-100%	High inhibition, consistent results
Polo Ring	~85-100%	Effective across all isolates
Chewing Gum	~85-95%	Slightly lower inhibition compared to others
Colgate	~85-100%	High inhibition, similar to charcoal extract

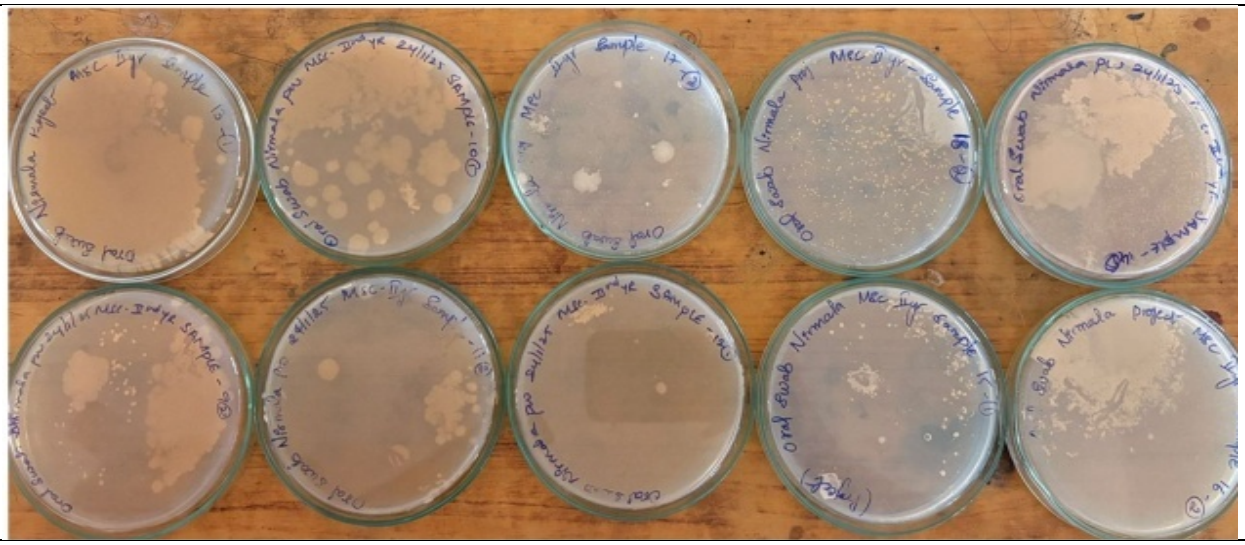


Fig 1: Isolated colonies on nutrient agar



Fig:2 Biofilm forming bacteria by tube method presence of ring formation indicates bacteria is present.

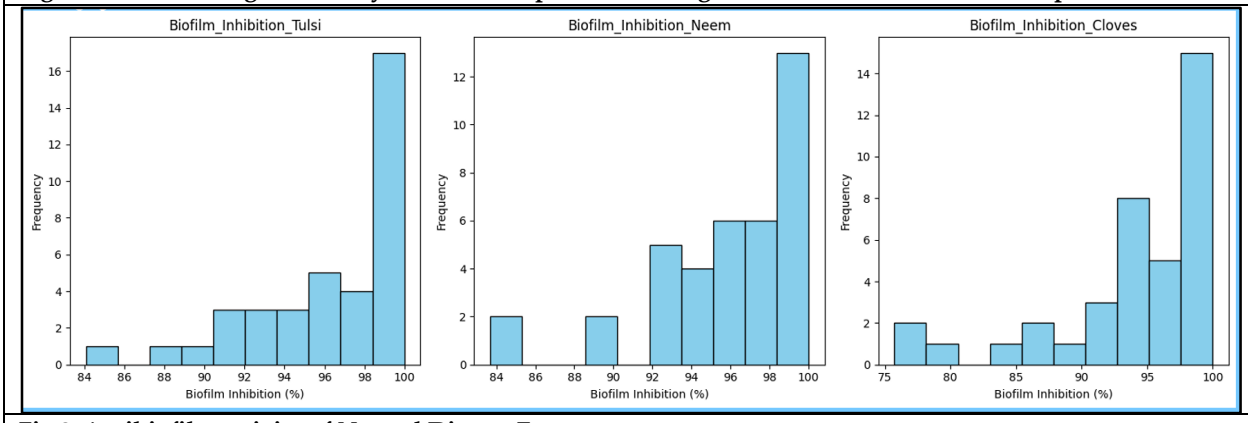


Fig 3: Antibiofilm activity of Natural Dietary Extracts

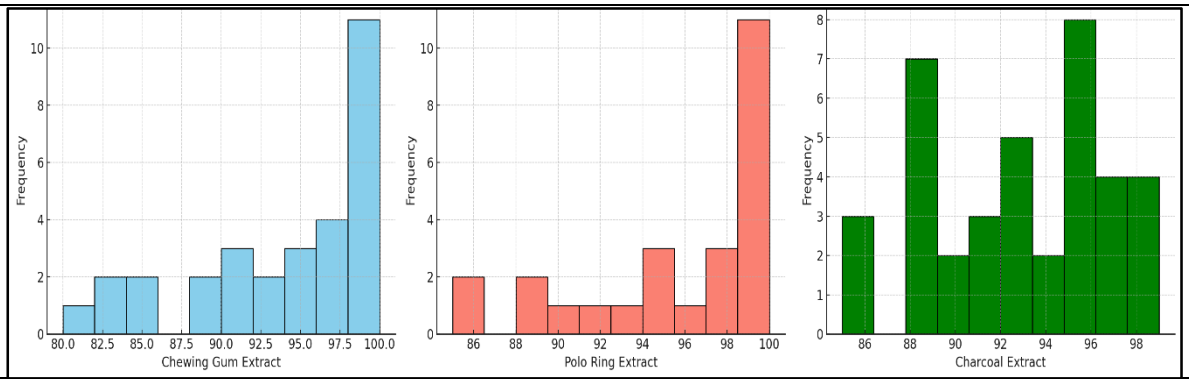


Fig 4: Percentage biofilm Inhibition of Synthetic and Commercial Extracts

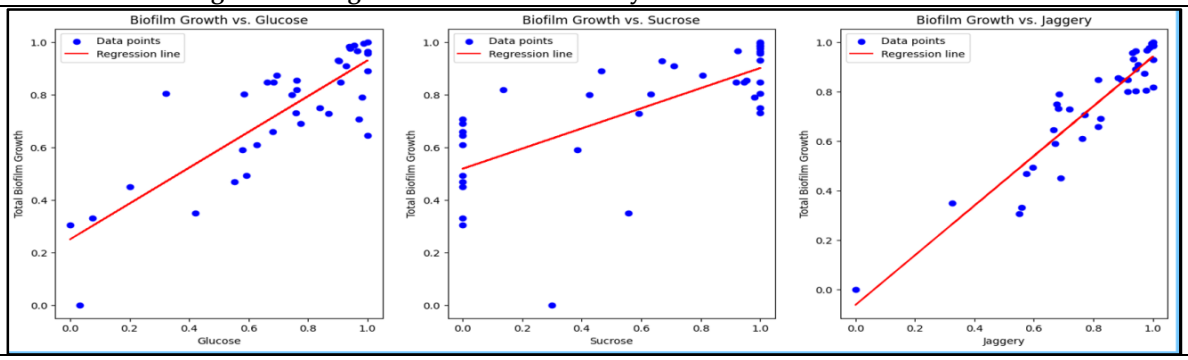


Fig 5: Promotion of Biofilm by Dietary Sugar Extracts

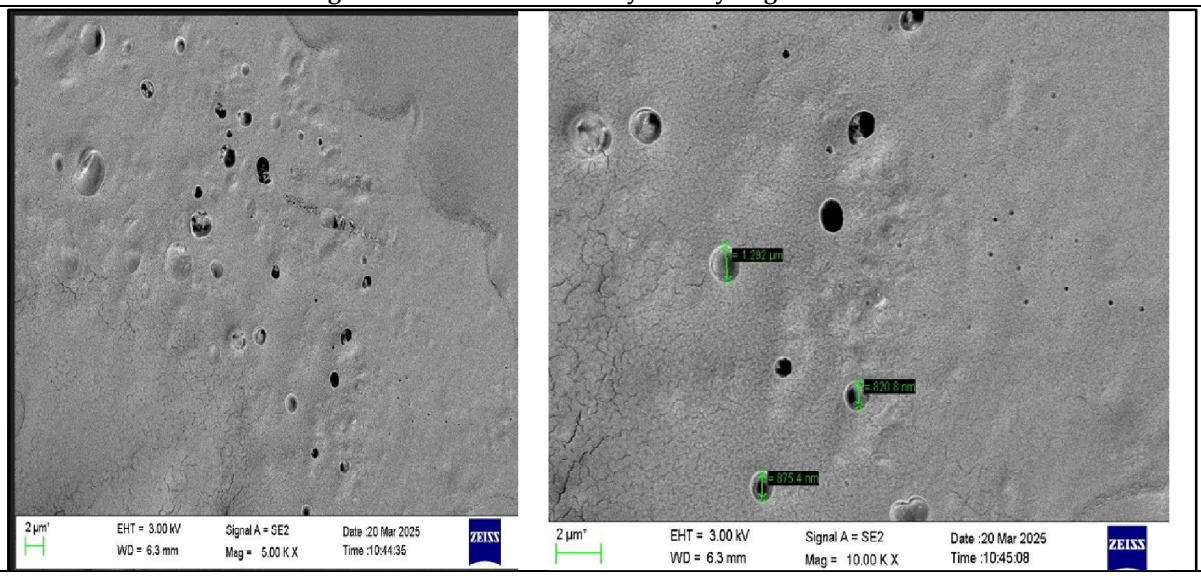


Figure 6: Scanning Electron Microscopy- visualization of nano emulsion droplets

RESEARCH ARTICLE

Development and Nutritional Evaluation of Roasted Composite Flour Incorporated with Lotus Seed Flour

S. Kowsalya[1]*, S. Angeline Esther Preethi[2] and K. M. Buvaneswari[3]

[1]Ph.D. Scholar, Department of Foods and Nutrition, Rathnavel Subramaniam College of Arts and Science (Autonomous), Sulur, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Associate Professor and Head, Department of Foods and Nutrition, Rathnavel Subramaniam College of Arts and Science (Autonomous), Sulur, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[3]Assistant Professor, Department of Nutrition and Dietetics, Thassim Beevi Abdul Kader College for Women, Kilakarai, Ramanathapuram, (Affiliated to Alagappa University, Karaikudi), Tamil Nadu, India.

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***Address for Correspondence**
S. Kowsalya
Ph.D. Scholar,
Department of Foods and Nutrition,
Rathnavel Subramaniam College of Arts and Science (Autonomous), Sulur,
(Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
E.Mail: kowsikrish74@gmail.com

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ABSTRACT

Composite flours are gaining recognition due to their potential to enhance the nutritional and useful attributes of meals merchandise. This observe advanced a roasted composite flour the usage of lotus seed flour and millet flour in varying ratios (5%, 10%, and 15%) and assessed its nutritional composition. Lotus seeds, acknowledged for his or her high protein, fiber, and antioxidant content material, along with bioactive compounds together with flavonoids and polyphenols, provide useful and therapeutic advantages. The nutritional evaluation revealed excessive carbohydrate and moderate protein content material, large nutritional fiber, and a wealthy mineral profile, making the composite flour suitable for fitness-orientated food formulations.

Keywords: Composite flour, Lotus seed flour, Millet flour, Nutritional composition, Bioactive compounds, Dietary fiber, Antioxidants, Functional foods

INTRODUCTION

Malnutrition and nutrient inadequacy continue to be most important public health issues globally, specifically in growing countries, in which the reliance on staple cereal-based diets frequently outcomes in unbalanced nutrient

intake. In reaction to these challenges, composite flours—combinations of flours from cereals, legumes, tubers, oilseeds, and purposeful substances—have emerged as a promising dietary intervention. These formulations are strategically developed to decorate each the dietary profile and functional houses of staple ingredients, supplying a greater balanced source of energy, protein, dietary fiber, vitamins, and minerals (Ayo *et al*., 2014; Adebowale *et al*., 2012). Among the numerous novel ingredients, lotus seed flour (from *Nelumbo nucifera*) has received large medical and industrial hobby because of its wealthy composition of essential vitamins and bioactive compounds. Traditionally used in Ayurvedic and Chinese medicine, Lotus seeds are assessed for their terrible proteins, complex carbohydrates, low fat and giant mineral content materials including calcium, phosphorus and magnesium (Balasubramian *et al*, 2012). In addition, lotus seeds are rich in polyphenolic compounds, including flavonoids, alkaloids and tannins, such as antioxidants, anti -inflammatory, hypoglycemic and neuropotive results (Jiang *et al*, 2013; Luo *et al*., 2021)These attributes make lotus seed flour a really perfect useful factor for developing composite flours targeting fitness-conscious and prone populations, inclusive of children, pregnant ladies, and individuals with metabolic issues. Roasting, a commonplace thermal processing approach, is regularly carried out in flour production to beautify palatability, nutrient digestibility, and shelf existence. It also plays a vital role in modifying the functional properties of flour such as water and oil absorption capability, pasting conduct, and protein solubility (Kumar *et al*., 2019). Importantly, roasting has been shown to improve the bioavailability of certain micronutrients via lowering anti-nutritional elements like phytates and tannins, as a consequence helping higher nutrient absorption (Zhang *et al*., 2020). When combined with lotus seed flour, roasted composite flour has the capacity to function a nutritionally enriched useful meals base, appropriate for various dietary packages including weaning meals, health beverages, and diabetic-pleasant products. Recent studies have shown the value of at least seeds and millet flour. For example, studies by Sharma *et al*. (2018) and Olapade and Aver (2012) emphasized the ability of such mixtures to improve protein efficiency, increase dietary fiber content and increase antioxidant capacity. Mirets, which form the base of the total flour in this study, are known for its dry resistance, low glycemic index and micronutrients such as iron and zinc (Goddess *et al*., 2014). The strategic combination of millet and lotus frame provides a double advantage: extended macronutrical materials and better mineral bioavaling, which is especially important in the context of the prevention of non-infectious disease and the deficiency of micronutrients (Kumar *et al*, 2021). Despite these benefits, there is limited scientific literature that focuses specifically on the nutritional characterization of fried total flour by incorporating Lotus Frømel. The study addresses as systematically approaches composition (moisture, carbohydrates, proteins, fat, fiber and ash) and mineral content (calcium, iron, phosphorus, sodium, potassium and vitamin C) have been evaluated by 5%, 10%and 15%15%by evaluating 5%, 10%and 15%. Through this study, the purpose of the study is to determine the ability of these yogas to make meaningful contributions to dietary improvement and serve as the basis for health -oriented, nutritious food products. This study focuses on growing roasted composite flour by way of incorporating lotus seed flour at various degrees into millet-primarily based formulations. The objectives encompass reading the proximate and mineral composition of each mixture and comparing their nutritional capacity for practical food programs. By utilising nutrient-wealthy lotus seeds and millets, the research promotes sustainable nutrients, agro-biodiversity, and value addition at the same time as helping the conversion of underused assets into health-selling food merchandise.

METHODOLOGY

Area of the study: The study was chosen on the basis of strategic high quality raw material access, efficient processing infrastructure Accessibility and local consumer preference for millet -based products. In the area's strong agricultural foundation, especially millet cultivation and availability of lotus stem seeds, ensured a stable supply of fresh, nutritious material. In addition, the presence of small and medium -sized food processing units is equipped with hygiene, food quality milling and semination equipment, which facilitates clean and effective preparation of total flour. Consumers with traditional millet -based diets continued product development in known and increasing health awareness. Local research institutes also provided technical assistance and laboratory facilities, which led to a complete nutritional analysis.

Sample Selection: Sample choice turned into based totally on dietary capacity, availability, and suitability for composite flour manufacturing. Millet flour and lotus stem seeds had been prioritized due to their complementary nutrient profiles. Millet, recognised for its richness in nutritional fiber, protein, and micronutrients, was procured from nearby markets and agricultural studies facilities to make sure variety and fine. Lotus stem seeds, valued for their protein, critical fatty acids, and bioactive compounds, were obtained from certified assets and processed into excellent powder. This planned ingredient selection contributed to the development of a nutritionally superior and functionally various composite flour.

Processing of Food Ingredients

Lotus Stem Seed Processing: The lotus stem seeds have been thoroughly wiped clean to do away with dirt, debris, and different impurities. They have been then dried in a hot air oven at 50–60°C to lessen moisture and enlarge shelf lifestyles. After drying, the seeds had been floor right into a nice powder the use of a meals-grade milling unit. The powder turned into finally handed through a high-quality mesh sieve to ensure a uniform particle size, facilitating homogenous blending with millet flour.

Millet Flour Preparation: Millet grains were first cleaned to dispose of dust, stones, and different foreign materials. Following cleaning, the grains had been dehulled to eliminate the outer husk, enhancing digestibility and texture. The dehulled grains have been milled into pleasant flour the usage of a commercial grinder. The flour become then sieved to gain a regular particle length for smooth blending into the composite flour components.

Composite Flour Formulation

The lotus seed powder turned into incorporated into millet flour at 3 tiers—five%, 10%, and 15%—to produce different composite flour versions. Blending became performed the usage of a dry blending method to ensure uniform distribution of lotus seed powder. The mixed flour samples were packed in airtight, food-grade containers to protect them from moisture and oxidative degradation. The samples were saved in cool, dry conditions until in addition use in dietary analysis and product improvement.

Nutrient Analysis of composite flour

The composite flour advanced in this study become analyzed for its proximate composition the use of popular strategies. Moisture content material turned into decided the usage of the oven drying approach (AOAC, 2016) through heating the sample at one hundred and five°C till a consistent weight become executed. Carbohydrate content material was expected the use of the Anthrone approach, primarily based on the colorimetric response of hydrolyzed sugars with Anthrone reagent. Protein was quantified the usage of both Lowry's technique, regarding Folin–Ciocalteu reagent, or the Kjeldahl approach, which measures nitrogen content material. Fat content material changed into assessed the use of the Soxhlet extraction approach with petroleum ether as the solvent. Ash content, indicating total mineral presence, turned into measured through incinerating the pattern in a muffle furnace at 550°C. Crude fiber turned into determined thru sequential acid and alkali digestion, setting apart indigestible residue. Dietary fiber turned into expected the use of the enzymatic gravimetric technique, which eliminates digestible components enzymatically, leaving fiber to be measured gravimetrically.

Mineral Analysis of composite flour

Mineral content evaluation become achieved the usage of Atomic Absorption Spectrometry (AAS) following acid digestion of the sample. The crucial minerals analyzed protected calcium, phosphorus, iron, zinc, copper, and magnesium. The samples had been digested with concentrated acids, and the mineral concentrations were quantified using AAS by using the use of measuring the absorption of unique wavelengths similar to every mineral. This approach ensured specific and correct mineral quantification within the composite flour.

RESULT AND DISCUSSION

Nutritional Composition of Composite Flours

Composite flours are formulated by blending flours derived from cereals, legumes, tubers, and pseudocereals to improve the nutritional quality and functional attributes of food products. These blends are increasingly used to combat nutritional deficiencies and enhance dietary diversity, especially in developing regions. Several studies have emphasized the nutritional and health-promoting potential of such formulations (Ayo *et al*., 2014; Adebowale *et al*., 2012; Sharma *et al*., 2018).Table 1 shows that macronutrient content of composite flour

Carbohydrate: The overall flour developed in this study performed a carbohydrate material of 69.7 grams per 100 grams per 100 grams. That is consistent with previous reports from Sharma *et al*. (2018), which found carbohydrates that they are in grain-cinema mixtures from 63.33% to 68.79%. High carbohydrate materials suitable for these flour for energy-erosion food applications, especially in areas where calorie management is a concern.

Protein: Protein content was found to be 10.35 grams per 100 grams. This corresponds to the findings from Ayo *et al*. (2014) and Singh *et al*. (2020), which noted that the inclusion of legumes and pseudosrel improved protein quality and total flour content, often between 9.62% and 11.93%. This makes them valuable when it comes to addressing protein-energy subsequent and improving amino acid profiles in a vegetarian diet.

Fat: 2.65 grams measured to 100 grams, in this formulation, the fat content was lower than any legumes-rich flour, but still contributes to the required fatty acid profile. Previous research (Adebowale *et al*., 2012) has shown that fat content in total flour can be more than 2.0% to 13% depending on the presence of oil -rich seeds. Fat supports cellular functions, absorption of vitamins and hormone control.

Dietary fiber: Dietary fiber material was recorded as 4.15 grams per 100 grams. This is slightly higher than the values reported by Olapade and Aver (2012), which visited the fiber level between 2.33% and 4.03% in grain level mixtures. Fiber is crucial to digestive health, blood sugar control and cholesterol control, and its presence increases the functional and satisfaction value of foods.

Moisture : The moisture content was 3.5%, which falls within the safe area reported by Fammy *et al* (5.70%to 8.57%).Low moisture levels are critical for prolonging shelf life and preventing microbial spoilage, especially under ambient storage conditions.

Ash: At 2.4%, the ash material reflects the total mineral content of flour. It is more than 1.23% -1.54% range documented by Oakpala and Egwu (2015), suggesting a rich mineral appearance due to incorporating Lotus seed powder and millet, both known for their dense micronisty profiles. The composition of total flour nutrition highlights their ability to increase the nutritional profile of different food applications. With high carbohydrate content for energy, medium protein level for muscle maintenance, and a good fiber content for digestive health, these give them a dough a nutritionally favorable profile. Low moisture material ensures better storage stability, while ash material reflects the presence of essential minerals. Ensuring proper handling and storage will help maintain general quality and nutritional value for extended use.

Micronutrient content of composite flour incorporated with lotus seed powder Table 2

Composite flour is formulated via blending various flours derived from cereals, legumes, tubers, and pseudocereals to beautify its dietary great. This segment presents a detailed evaluation of its mineral and diet C content material, highlighting their useful importance and nutritional significance. The evaluation turned into performed following standardized methods, which includes the FSSAI Manual of Methods, ensuring accuracy and reliability.

Calcium: Calcium content of total flour was found to be 97.5 mg per 100 grams. Calcium plays an important role in maintaining bone health, muscle contractions, nerve signaling and blood clots. Adequate calcium intake is necessary to prevent osteoporosis and ensure proper skeletal growth. Inclusion of calcium -rich ingredients such as legumes and pseudosrel in total flour increases the nutritional value, which is suitable for strengthening staple food (Gupta *et al*., 2021).

Phosphorus: The phosphorus content was measured at 283.3 mg per 100 grams. Phosphorus and teeth are important for minerals, energy metabolism and cell signaling. It creates a main component in ATP (adenosinrifosphate), body energy. A balanced intake of phosphorus with calcium supports general skeletal health and metabolic processes (Mahmood *et al*., 2020).

Iron: The iron content was recorded as 5.21 mg per 100 grams, which was determined using the FSSAI manual for methods. Iron is important for oxygen transport in the blood, as it creates an important part of hemoglobin. Lack of iron can lead to anemia, fatigue and impaired cognitive function. The inclusion of iron -rich sources in total flour improves nutritional quality and contributes to the prevention of anemia (Kumar and Sharma, 2019).

Potassium: Potassium level in total flour was measured at 177.65 mg per 100 grams. Potassium is an important electrolyte that controls fluid balance, muscle contraction and nerve transmission. It also helps maintain healthy blood pressure levels and reduce the risk of heart disease. Potassium supports a dietary and kidney function (Singh *et al*., 2022) to fight the negative effects of sodium.

Sodium: Sodium content was found to be 4.1 grams per 100 grams. Sodium is necessary to maintain fluid balance, nerve function and muscle contraction. However, excessive sodium intake can contribute to high blood pressure and heart disease. Medium sodium content in total flour ensures that it can be included in a balanced diet by recommending the daily sodium intake (World Health Organization, 2021).

Vitamin C: The total flour was 1.54 mg of vitamin C per 100 grams. Vitamin C is an essential water-soluble vitamin known for its antioxidant properties, the support and role of the immune system in collagenynthesis. It increases the absorption of iron from plant -based foods and contributes to wound healing and skin health. Although the total flour is not a primary source of vitamin C, its presence combines the general nutritional value (Patel *et al*., 2020) of the appearance. Nutrition evaluation of general flour reveals its rich mineral profile, which contributes to bone health, energy metabolism, oxygen transport and heart function. The presence of vitamin C, although it is antioxidant properties and supports immune function. The production of overall flour with different nutrients-ingredients makes a valuable alternative to improve dietary intake and address the deficiencies of micronutrients.

CONCLUSION

Lotus seeds and millet-based total mel minerals and vitamin C profiles reveal a nutritious formulation, especially in calcium, phosphorus, iron and potassium. While monitoring the sodium content right, the general composition suggests that the flour can contribute significantly to the intake of micronutrients. Supported by previous research, these findings confirm the utility of overall flour to increase the quality of diet and address nutritional deficiency. Such yogas have the ability to improve food safety, especially in a sensitive population of malnutrition and micronutrition intervals.

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Table I-Macronutrient content of composite flour incorporated with lotus seed powder

S.No	Nutrient	Amount per 100gm	Methods
1	Carbohydrate	69.7gm	FSSAI Manual of Methods
2	Protein	10.35gm	
3	Fat	2.65gm	
4	Fiber	4.15gm	
5	Moisture	3.5%	
6	Ash	2.4%	

Table 2: Micronutrient content of composite flour incorporated with lotus seed powder

S.No	Nutrient	Amount per 100gm	Methods
1	Calcium	97.5mg	FSSAI Manual of Methods
2	Phosphorous	283.3mg	
3	Iron	5.21mg	
4	Potassium	177.65mg	
5	Sodium	4.1gm	
6	Vitamin C	1.54mg	

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RESEARCH ARTICLE

Effect of Copper Oxide and Iron Oxide Nanoparticles on Morphological Characteristics of *Triticum aestivum* (Wheat)

Roshni Lalwani[1]*, Rita N Kumar[2] and Nirmal Kumar J.I[3]

[1]Research Scholar, Department of Biological and Environmental Science, Natubhai V. Patel College of Pure and Applied Sciences, (Affiliated to The Charutar Vidya Mandal University), Anand, Gujarat, India.
[2]Ex-HOD, Department of Biological and Environmental Science, Natubhai V. Patel College of Pure and Applied Sciences, (Affiliated to The Charutar Vidya Mandal University), Anand, Gujarat, India.
[3]Ex-Professor and Head, Department of Environment Science and Technology, Institute of Science and Technology for Advanced Studies and Research, (Affiliated to The Charutar Vidya Mandal University), Anand, Gujarat, India.

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***Address for Correspondence**
Roshni Lalwani
Research Scholar,
Department of Biological and Environmental Science,
Natubhai V. Patel College of Pure and Applied Sciences,
(Affiliated to The Charutar Vidya Mandal University),
Anand, Gujarat, India.
E.Mail: lalwaniroshni17@gmail.com

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ABSTRACT

The development of materials with enhanced or entirely new qualities is made possible by nanotechnology. Nanotechnology offers better growth, faster germination rates, and increased tolerance to environmental stressors, it has the potential to fundamentally alter the way seeds germinate. The present study focuses on the synthesis and characterization of copper oxide and iron oxide nanoparticles using the sol-gel and chemical precipitation methods. The size, composition, and surface morphology of these nanoparticles were assessed using characterization techniques such as energy dispersive X-ray microanalysis (EDAX) and transmission electron microscopy (TEM). Additionally, the impact of these nanoparticles on the physiological characteristics of *Triticum aestivum* seeds was noted. The results indicated that synthesized nanoparticles were copper oxide and iron oxide and were of size 20-50 nm having squarish to spherical morphology for copper oxide nanoparticles and hexagonal to spherical morphology for iron oxide nanoparticles. The *Triticum aestivum* seeds exhibited that iron oxide and copper oxide nanoparticles considerably improved physiological characteristics at lower concentrations, however they have phytotoxic effects at greater doses, which impede the growth of seedlings and decrease seed germination.

Keywords: Copper oxide, characterization, Iron oxide, *Triticum aestivum*

INTRODUCTION

Over past ten years the widespread usage of nanoparticles (NPs), or particles smaller than 100 nm, in a variety of sectors of the national economy, such as the food industry, water purification, and medicine. However, there hasn't been enough research done on the application of nanoparticles in agriculture. In an effort to boost plant production and lower the environmental risk associated with fertilizer use, the combination of nanoparticles with fertilizers is made so as to produce Nano fertilizers. Among varieties of nanoparticles the Metallic nanoparticles offer a wide range of applications and have much potential in nanotechnology. Consequently, it is essential to understand these nanoparticles environmental fate and long-term impacts. Plants can absorb metal nanoparticles in different ways. [1] [2][3]. Numerous studies have examined the effects of different nanostructures on various crops in recent years, with a focus on the early stages of development, seed germination, and seedling growth. Depending on the kind, size, concentration, exposure time, and growing circumstances of the nanoparticles, priming them (also known as Nano-priming) may have a positive, negative, or no effect on the germination process. However, only few investigations have been conducted to understand the effect of Copper oxide and Iron oxide nanoparticles at the physiological levels in major food crop like wheat. Copper oxide nanoparticles (CuO NPs) are widely used in commercial applications, including heat transfer fluids, catalysts, semiconductors, photovoltaic cells, and sensitive gas sensor [4]. They have also been used in agricultural applications such as fertilizers and pesticides [5], largely due to its relatively slow dissolution and anti-microbial properties.Iron oxide nanoparticles (IONPs) have also gained attention in agriculture due to their unique properties, such as high surface area, magnetic behavior, and biocompatibility. Their potential applications in agriculture can benefit crop production, pest control, and help in soil improvement. Wheat (*Triticum aestivum*) crop belongs to the family *Graminae* (Poaceae) and is an annual, large shrub plant commonly used as food. Wheat is a globally consumed cereal crop, which is a staple food in most part of Asia, Africa, and other parts of the world. It is the most valuable food and strategic cereal crop in Egypt and ranks third globally, after corn and rice, as the most productive grain [6]. It is a major source of energy, protein and dietary fibre in human nutrition [7].Due to its ability to thrive in a variety of environmental circumstances, wheat can be grown extensively and stored for a long time. Being the most important staple food crop of India, a wide number of agricultural research programs are involved in increasing the growth and yield of this particular crop.

MATERIALS AND METHODS

Synthesis of Copper oxide and Iron oxide Nanoparticles

The figure 1 represents the flow chart for synthesis of copper oxide and iron oxide nanoparticles. The copper oxide nanoparticles were synthesized by Sol- gel method [8] and Iron oxide nanoparticles by precipitation method [9].

Characterization of Synthesized Nanoparticles

A variety of advanced scientific tools, like energy dispersive X-ray microanalysis (EDAX) and transmission electron microscopy (TEM). A transmission electron microscope (TEM), model Philips Tecnai 20, Holland, with an accelerating voltage of 100 kV, was used to evaluate the particles' size and shape. For TEM examination, the material was placed on copper grids coated with carbon. The samples were made by dispersing the particles in 2-propanol. EDAX was used at a voltage of 30 kV and a magnification of up to 2, 50,000X for elemental analysis. The Sophisticated Instrumentation Centre for Applied Research and Technology (SICART) was the center for all characterization activities.

Preparation of seeds and seed priming in Nano solutions

For the study purpose two nanoparticles i.e. copper oxide and iron oxide nanoparticles were taken into consideration. Different concentration of nanoparticles were dissolved in double distilled water, further these solutions of different concentration were sonicated thoroughly for even mixing. Thus different concentration of Nano solutions were prepared.

For the study purpose *Tritium aestivum (Wheat)* seeds were selected, these seeds were thoroughly washed with water, then after these seeds were suspended in 2.5% sodium hypochlorite solution for surface sterilization, washed with distilled water and primed in a suspension of different concentration of prepared Nano solutions for 12 hours. Then after the primed seeds were kept on double folds of filter paper in a Petri plate and then transferred to germination sheets as shown in figure 2 Each treatment was replicated three times, with ten seeds in each concentration [10].

Measurement of Physiological Parameters

Different parameters like germination percentage, seedling length, seedling vigor index and mean root length and shoot length were recorded on different period of growth. The study was done for the period of week.

Germination Percentage

By creating ideal circumstances for every experimental set, seed germination was observed. Every experimental set's germination was noted, and the total germination was computed and reported as a percentage [11].

Germination percentage *(%) =Number of Germinated seeds/Total number of seeds×100*

Seedling Length (cm)

The length of roots and shoots of the germinated seeds were measured for every experimental set. The average shoot length was measured from the base of the primary leaf to the base of the hypocotyl and was stated in centimeters. The mean root length was measured from the primary root's tip to the hypocotyl base and was recorded in centimeters. The seedling length was computed and represented in centimeters by summing the root and shoot lengths [12].

$$\boldsymbol{Seedlinglength}\ (\boldsymbol{cm}) = Shootlength + Rootlength$$

Seed Vigor Index

The seed vigor index was calculated and given as a number using the formula proposed by [13].

SVI= Germination (%) X Seedling Length

RESULTS AND DICUSSION

Characterization of Nanoparticles

Energy dispersive X-ray microanalysis (EDAX)

The elemental composition of the nanoparticles can be determined through EDAX analysis. The Copper oxide nanoparticle has 73.94 % Copper and 26.06 % oxygen by weight, which indicates the successful synthesis of copper oxide nanoparticles, similar composition was observed by Amna *et al*., 2013 [14], correspondingly the Iron oxide nanoparticle has 54.53 % Iron and 45.47 % oxygen as shown in figure 3. [15]

TEM (transmission electron microscopy) analysis

TEM analysis pictures of Copper oxide and Iron oxide nanoparticles were generated at room temperature indicating that the copper oxide Nano catalyst were of agglomerated spherical to Squarish shape with an average size about 20-50 nm. On contrary Iron oxide Nano catalyst exhibited Hexagonal to spherical shape (Figure 4) [16]. The particle size of both Nano catalyst was found to be consistent with the particle size estimated using XRD examination.

Measurement of Physiological parameters

Effect on seed germination

The germination percentage of the decreased with an increase in the concentration of copper oxide and iron oxide nanoparticles. At lower concentrations of 50ppm and 100ppm the germination percentage was maximum for copper oxide and iron oxide nanoparticles, but as the concentration increases beyond 100 ppm the germination percentage shows a gradual decrease. At 50 ppm concentration 95% germination was recorded at 100 ppm concentration it was 98%which was maximum, then after at 150 ppm and 200 ppm concentration gradual decrease of 88% and 85% was

observed for copper oxide nanoparticles. For Iron oxide nanoparticles at 50 ppm 86% germination followed by 90% at 100 ppm, 83%at 150 ppm and 73% at 200 ppm concentration was observed. The results revealed that an increasing concentration of nanoparticles results in decreases germination percentage. The least of all was observed in control. Enhanced seed germination in lower concentration of NPs may be attributed with the findings of [17]as stated that NPs have tendency to penetrate plant seed coats and enhance seed germination and growth.

Effect on seed Vigor Index

The seed vigor index of *Triticum aestivum* decreased with increasing copper oxide and iron oxide nanoparticle concentrations. The seed vigor index at 50 ppm, 100 ppm, 150 ppm and 200 ppm concentration for copper oxide nanoparticles were 906.3, 991.76,794.64 and 700.4 for copper oxide and for iron oxide nanoparticle at 50 ppm, 100 ppm, 150 ppm and 200 ppm concentration it were 738.74, 837.9, 767.75 and 681.72 respectively. However, it was found that Seed vigor index was reduced at higher concentration.

Effect in seedling length

Seedling length is an important indicator of plant growth. It is an essential measure in assessing a plant's growth, health, and potential for successful establishment. At lower concentration of 50 ppm the seedling length was 9.54 cm and 8.59cm for copper oxide and iron oxide nanoparticles respectively and that at 100 ppm concentration it was maximum which was around 10.12cm and 9.31cm for copper oxide and iron oxide nanoparticles respectively. As concentration increased the seedling length decreased this is supported by fact that the seedlings may grow more quickly at lower concentrations of copper oxide and iron oxide nanoparticles because of the nutritional advantages and stress-reduction strategies, which will result in longer seedlings. However, at higher concentrations, the toxicity and harmful effects of these nanoparticles can overwhelm the plant's protective mechanisms, resulting in stunted growth, oxidative stress, and reduced seedling length.

Mean Shoot length and root length

The wheat seeds were germinated on germination sheets and kept for a period of 15 days Root and shoot length were measured up to 15th day by using ruler. The increasing trend was observed as the number of days increased. The Shoot length of copper oxide treated seeds increased from 5.78 cm to 14.22cm from day 5 to day 15 at 50 ppm concentration, whereas root length increased 3.76 cm to 10.78 cm at 50 ppm concentration. At 100 ppm concentration the shoot length increased from 5.92 cm to 14.56 cm, whereas root length increased from 4.2 cm to 11 cm from day 5 to day 15. At 150 ppm concentration the shoot length increased from 5.51 cm to 14.1 cm, whereas root length increased from 3.52 cm to 10.53 cm from day 5 to day 15. The least growth was observed at 200 ppm concentration which was only 5.06 cm to 13.88 cm and root length increased from 3.18cm to 10.21 cm from day 5 to day 15.For Iron oxide nanoparticles at 50 ppm concentration the shoot length increased from 5.27 cm to 13.52 cm from day 5 to day15 and root length increased from 3.32 cm to 10.36 cm from day 5 to day 15. At 100 ppm concentration the shoot length increased from 5.43 cm to 13.8 cm from day 5 to day 15 whereas root length increased from 3.88 cm to 10.61 cm from day 5 to day 15. At 150 ppm concentration the shoot length increased from 5.1 cm to 13.35 cm from day 5 to day 15and root length increased from 4.15 cm and 10.13 cm from day 5 to day 15. The least growth was observed at 200 ppm concentration which was only 4.79 cm to 13.17 cm of shoot length from day 5 to day15 and the root length observed was around 3.95 cm to 9.71 cm from day 5 to day 15, this may be due to combination of improved nutritional availability, hormonal stimulation, increased stress tolerance, and the plant's normal development cycle causes wheat seeds treated with copper oxide and iron oxide nanoparticles to gradually lengthen their roots and shoots. The apparent increase in length is the result of several variables working together to support continuous growth as the days pass.

CONCLUSION

The Study indicates successful synthesis of copper oxide and Iron oxide nanoparticles. The Characterization technique helped in confirming the shape, size and composition of Nanoparticles. The results proved successful

synthesis of both the Nano catalyst. Furthermore the influence of synthesized NPs on different physiological parameters like seed germination, seedling length, seed Vigour index and mean Root and shoot length was studied on *Triticum aestivum.* The results indicated that at lower concentrations of upto 100 ppm the copper oxide and iron oxide nanoparticles significantly enhanced physiological properties. However, at higher concentrations, both the nanoparticles exhibit phytotoxic effects, reducing seed germination and inhibiting seedling growth.

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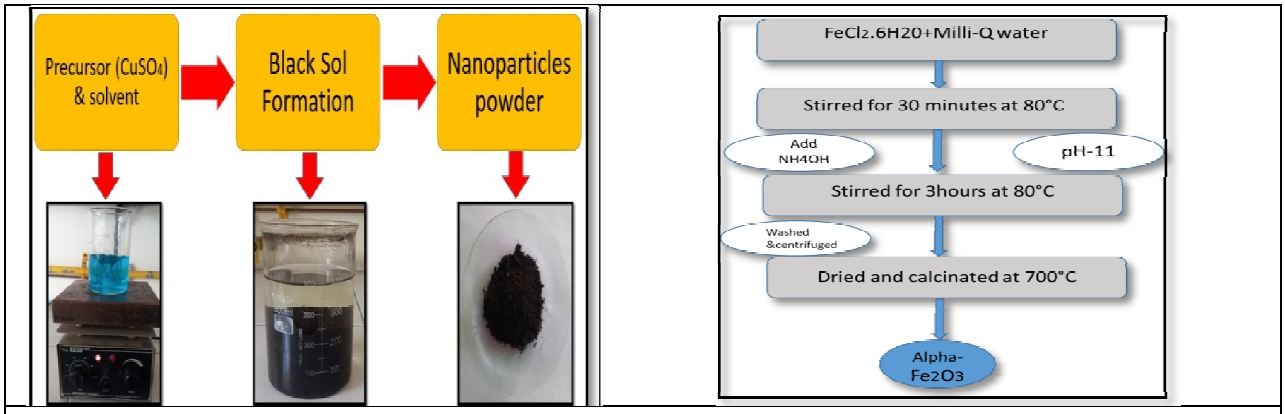


Figure 1: Synthesis of Copper oxide and Iron oxide nanoparticles

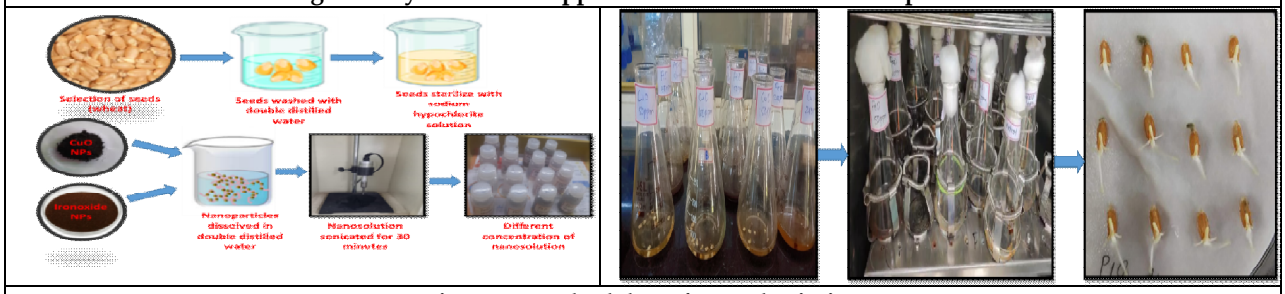


Figure 2: Methodology for seed priming

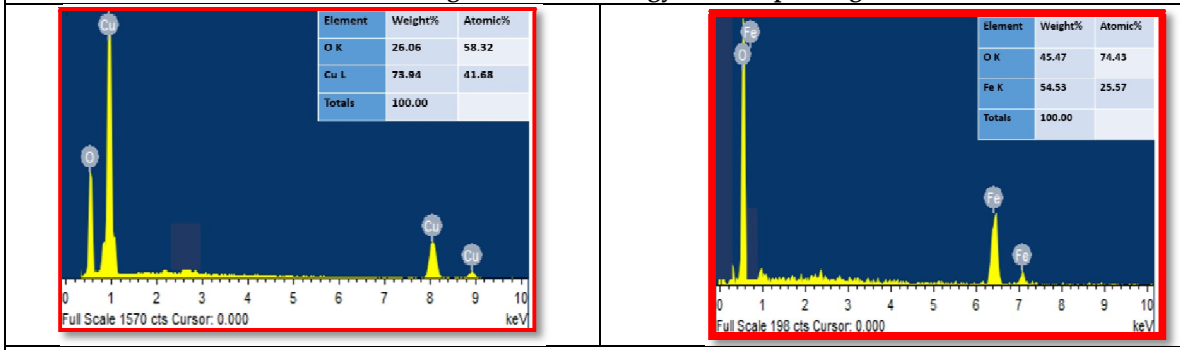


Figure 3: EDAX Spectra of synthesized copper oxide and iron oxide nanoparticles

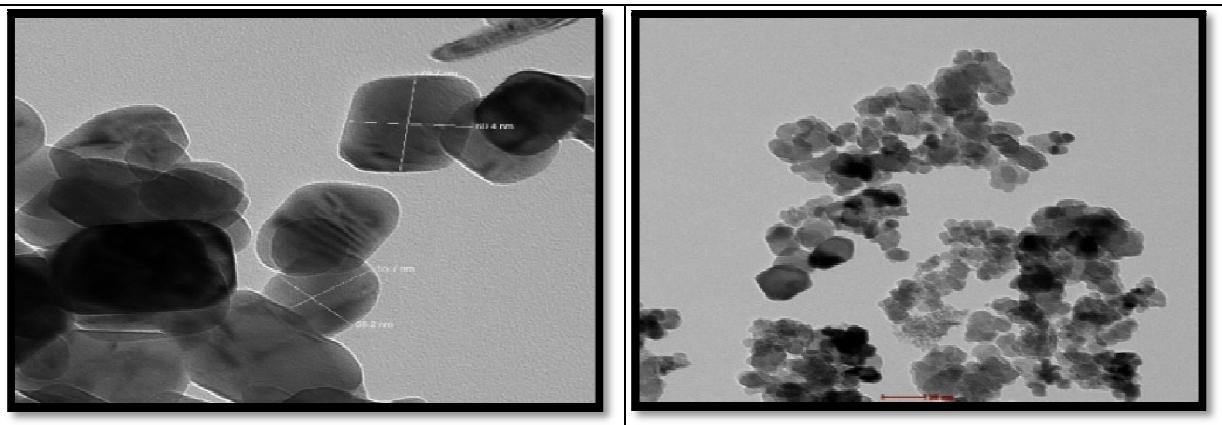
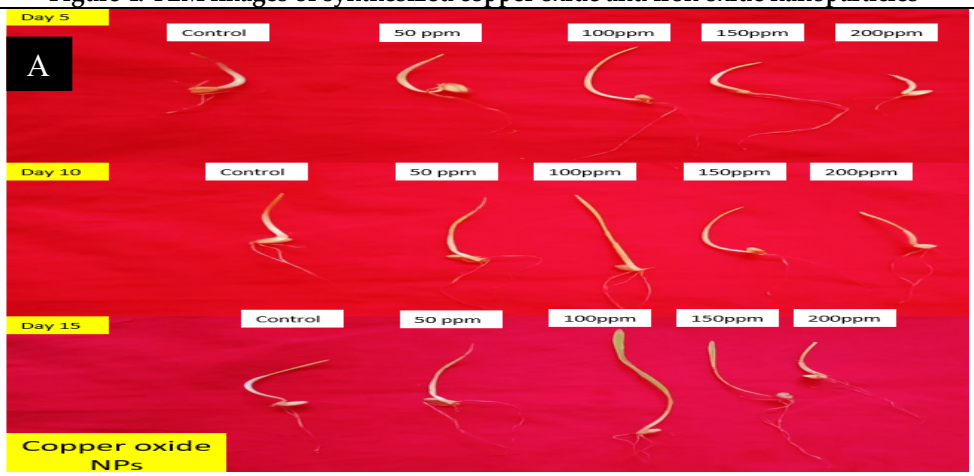
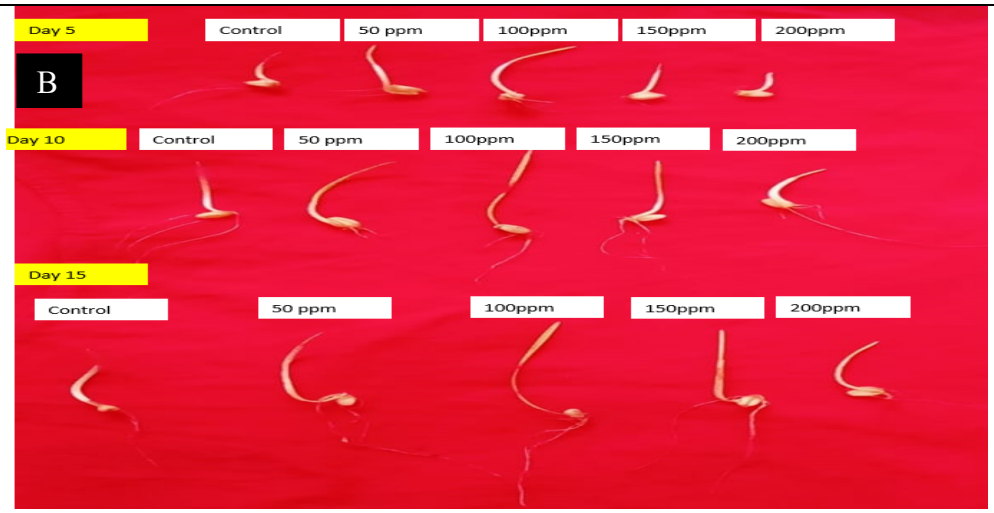


Figure 4: TEM images of synthesized copper oxide and iron oxide nanoparticles





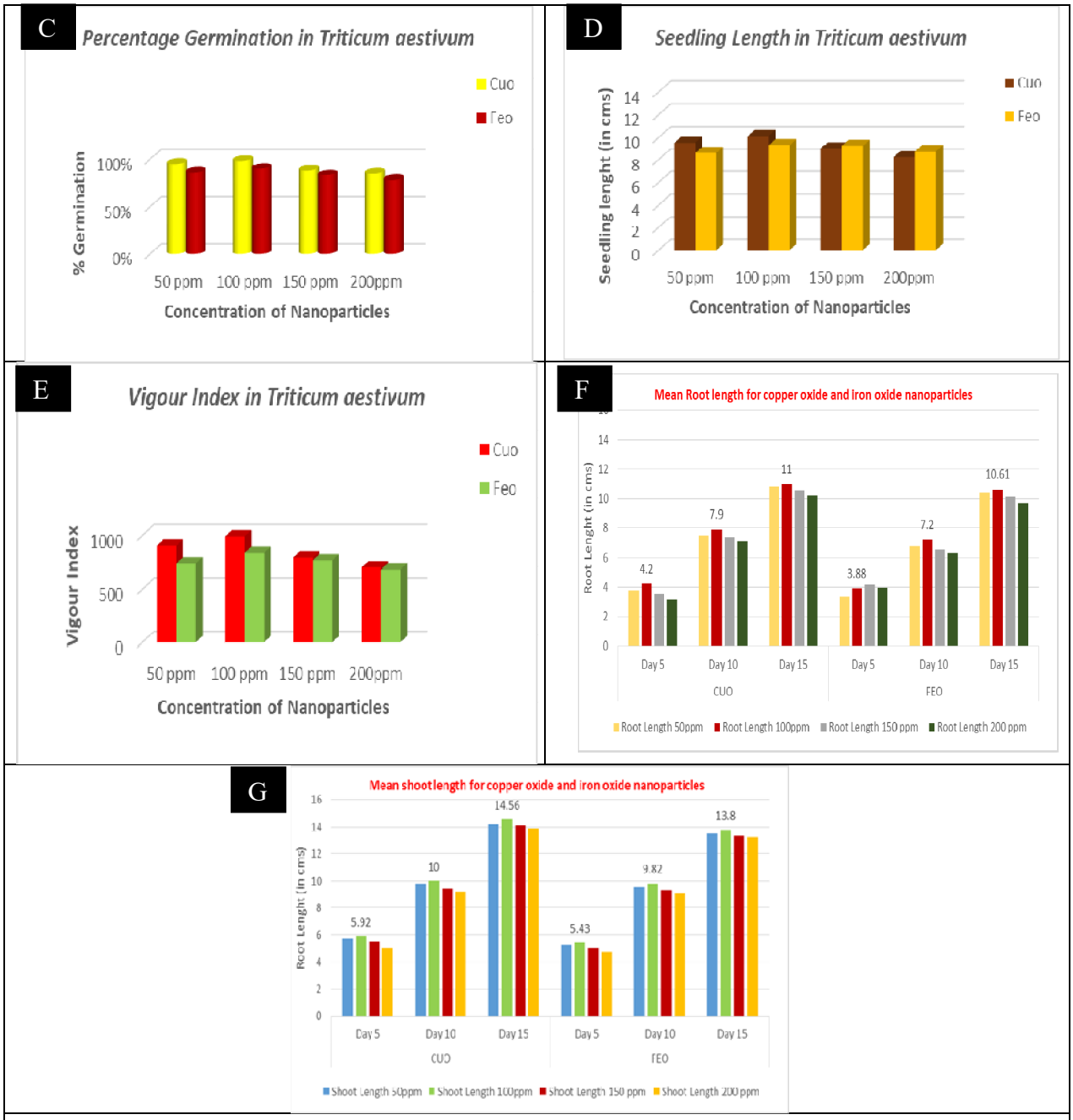


Figure 5: [A] Growth of wheat seeds day 5 to day 15 using copper oxide NPs [B] Growth of wheat seeds day 5 to day 15 using Iron oxide NPs [C] Percentage Germination in *Triticum aestivum* [D] Seedling Length in *Triticum aestivum* [E] Vigor Index in *Triticum aestivum* [F] Mean Root length of copper oxide and Iron oxide nanoparticles [G] Mean Shoot length of copper oxide and Iron oxide nanoparticles

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RESEARCH ARTICLE

Qualitative and Bacterial Analysis of Milk Samples from Different Sources

Haripriya Das[1] and Mintu Ann Varghese[2]*

[1]Advanced Learner, Department and Zoology, Catholicate College, Pathanamthitta, (Affiliated to Mahatma Gandhi University, Kottayam), Kerala, India.
[2]Assistant Professor (On Contract), Department and Zoology, Catholicate College, Pathanamthitta, (Affiliated to Mahatma Gandhi University, Kottayam), Kerala, India.

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***Address for Correspondence**
Mintu Ann Varghese
Assistant Professor (On Contract),
Department and Zoology,
Catholicate College, Pathanamthitta,
(Affiliated to Mahatma Gandhi University, Kottayam),
Kerala, India.
E.Mail: mintuvar@gmail.com

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ABSTRACT

The present study focuses on the qualitative and bacterial analysis of raw milk from three different sources. Samples were named as S1 for cow milk, S2 for goat milk and S3 for pasteurised packet milk. S1 and S2 were collected from nearby houses whereas S3 from a retail market in Pathanamthitta district. Qualitative analysis was determined by organoleptic test, clot on boiling test, acidity test, starch test, shake test and flow test. Bacterial analysis was done using Bergey's Manual of Determinative Bacteriology (2000). The study revealed that the cow milk contains harmful bacteria whereas goat milk and pasteurised packet milk contains useful bacteria. The cow milk maybe contaminated due to milking, handling or storage or due to environment. This work can be further studied by collecting more samples or by comparing samples from different area, can be conducted as a survey on consumers choice on pasteurised or unpasteurised or different brands.

Keywords: Raw milk, Pasteurised packet milk, Qualitative analysis, Bacterial analysis

INTRODUCTION

Milk is one of the most important and widely consumed by people of all ages. It is a rich source of essential nutrients that contribute to overall health and well-being. It is obtained primarily from mammals such as cows, goats, and buffaloes, with cow's milk being the most popular and widely available. Milk is a compensatory part of daily diet

especially for the expectant mothers as well as growing children (Rajagopal *et al*., 2005; Javaid *et al*., 2009; Olatunji *et al*., 2012). Milk is known for being an excellent source of calcium, which is essential for strong bones and teeth. It also contains protein, vitamins such as vitamin D, B12, and riboflavin, and minerals like phosphorus and potassium. Milk and dairy products are among the basic foodstuffs, which are necessary for the nutrition of children of growing age. Milk has protective properties against obesity, hypertension, certain cancer types, osteoporosis, and dental health (Tufekci *et al*., 2019). In 2018, global milk production reached 843 billion litres, with an estimated value of USD 307 billion and is projected to grow by 22% by 2027(OECD-FAO, Agricultural Outlook 2018-2027*).* Approximately 80% of yearly milk production comes from cows, with the rest from other dairy animals like buffaloes, goats, camels, and sheep, according to the Food and Agriculture Organization (FAO, The Global Diary Sector: Facts, 2019). Milk quality is important to ensure consumer health and safety. Microorganisms, including bacteria, yeast, and mold can contaminate milk at various stages, from production to consumption. Physiological analysis is important tool to monitor the quality of milk and dairy products (Woldemariam and Asres, 2017). However, raw milk is a very sensitive food that is subject to rapid change if it is not handled properly and hygienically. Due to its high nutritional value, neutral pH and high-water activity, raw milk is a good medium for the growth of microorganisms (Planinic *et al*., 2022). Thus, the present study was carried out with the objective to understand the quality and bacterial analysis of milk samples namely S1 (cow milk), S2 (goat milk) and S3 (pasteurised milk) from different sources in Pathanamthitta district.

MATERIALS AND METHODS

A total of 3 samples were collected from 3 different sources from Pathanamthitta district. The collected milk samples were fresh. Samples were named as S1 (cow milk), S2 (goat milk) and S3 (pasteurised milk). The raw cow milk and the raw goat milk was collected in a sterile glass bottle and the pasteurised packet milk was collected in a sterile container. The samples were immediately transported to the laboratory for further analysis. Various qualitative analysis was determined. Organoleptic test, clot on boiling test, acidity test, starch test, shake test and flow test was done (Nath and Aravindkumar, 2021). Bacterial analysis is done by the enumeration of bacteria by serial dilution of sample followed by standard plate method. The plates were incubated for 48 hours at 28±2° C for bacterial colony isolation and purified using nutrient agar medium. The bacterial genus was identified by various morphological and biochemical methods using Bergey's Manual of Determinative bacteriology (2000).

RESULT AND DISCUSSION

The milk samples were studies for qualitative analysis and represented in Table 1. The organoleptic test shows that all samples show excellent quality. Clot test displays no precipitation in three samples. All the three samples provide no starch formation. Flow test revealed excellent flow in S2 and S3 while good flow in S1. Foam formation was absent in all samples and all the samples were acidic. All the three samples show excellent quality could be due to the absence of adulterants. Sial *et al*. (2018) studied organoleptic analysis of milk samples collected from milk shops, tea shops and households. Figure1 represents Total heterotrophic bacteria found in all the samples. An overall abundance of bacteria is observed in pasteurized milk (41×10^{-5} cfu/ml). This predicts that a significant number of bacteria is found in it. This can lead to premature spoilage of milk, which is commonly found closer to the expiration date. Spoilage could perhaps be due to changes in temperature, improper pasteurization or post contamination due to unsanitary practices. Defective pasteurization, adulteration of pasteurized milk with raw milk and unsanitary handling are all contributory factors to early milk spoilage. Also, it could indicate the presence of good bacteria present in it. Cow milk showed comparatively high bacterial growth than goat milk indicated contamination while handling it. The international standard for acceptable quality raw milk is not to exceed a total bacterial count of 1×10^5 cfu/ml (Reta and Addis, 2015). Raw milk may be contaminated from the environment, unsterile utensils, unhygienic storage tanks, dirty condition of milking hands, diseased animals (Sharma *et al*., 2023). Table 2, represents the biochemical characteristic and identification test of bacteria. Four bacterial isolates from cow milk sample, two isolates from goat milk sample and four isolates from pasteurised milk samples were studied. Colony morphology,

gram staining and biochemical analysis were carried out to identify bacteria. *Staphylococcus* sp. is isolated from both cow milk and goat milk sample. There is a substantial occurrence of pathogenic coagulase-positive *Staphylococcus aureus* in milk and dairy products found from informal sectors in developing nations (Singh and Prakash, 2010). Enhancing milk quality and ensuring its safety for consumption by people requires implementing improved methods, strict adherence to good manufacturing standards and rigorous hygiene measures (Remaz and Elhag, 2015; Nyokabi *et al*., 2021). *S. aureus* is more common in dairy cows' mammary gland infections because it can live in the keratin layers of the teat canal (Qayyum *et al*., 2016). *Lactococcus*, *Streptococcus* and *Lactobacillus* are lactic acid bacteria. Lactic acid bacteria (LAB) belong to a group of gram positive bacteria that produce lactic acid as their main fermentation product into the culture medium and generally recognized as safe (Konings, 2000). El-Shafei *et al*. (2002) also reported the preponderance of higher *Lactobacillus* spp. LAB species are extremely valuable in food industry as beneficial organisms. It was demonstrated that lactic acid given by lactic acid bacteria not only keeps foods and beverages in the state of perfect preservation but can also boost the growth of healthy microbiota throughout the intestine beside of reducing the pathogens. One of the most important contributions of these microorganisms is the extended shelf life of the fermented products (Wassie and Wassie, 2016). In conclusion, the three milk samples indicated that all the three are quite good for consumption. The identified bacterial genera were commonly present in milk samples. Most of them improves the health of intestine. However, further study needs to be conducted by analysing the percentage composition of various components found in the milk samples will help consumers choose the brands with the best quality milk

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Table 1. Qualitative analysis of milk samples

Serial No.	Name of the test	Cow milk (S1)	Goat milk (S2)	Pasteurised milk (S3)
1.	Organoleptic test	Excellent	Excellent	Excellent
2.	Clot on boiling test	No precipitation formed	No precipitation formed	No precipitation formed
3.	Starch test	No starch formed	No starch formed	No starch formed
4.	Flow test	Excellent	Excellent	Excellent
5.	Shake test	No foam formed	No foam formed	No foam formed
6.	Acidity test	Acidic	Acidic	Acidic

Table 2. Morphological and Biochemical characteristics of isolated bacteria

Source	Sample No.	Colony morphology	Gram stain	Biochemical test Result	Genus
Cow milk	S1-1	White colour, circular, mucoid, convex	Gram positive cocci in pairs	Non motile, non spore forming, catalase negative	*Streptococcus* sp.
	S1-2	Cream, circular	Gram positive cocci in clusters	Catalase negative, Citrate negative, oxidase negative	*Lactococcus* sp.
	S1-3	White colour, circular	Gram positive rods	Catalase negative, Citrate negative, oxidase negative	*Lactobacillus* sp.
	S1-4	Greyish white, circular, translucent, small mucoid, convex	Gram positive cocci in clusters	Tube coagulase Positive, Mannitol Fermented, non-motile	*Staphylococcus* sp.
Goat milk	S2-1	Yellow, opaque, raised, circular small, moist	Gram positive cocci in cluster	Tube coagulase negative, mannitol non fermented, Indole Negative, non-motile	*Staphylococcus* sp.
	S2-2	Fuzzy white, circular, rough	Gram positive long rod	Mannitol Non-fermented, non-motile, urease negative, citrate negative	*Lactobacillus* sp.
Pasteurised packet milk	S3-1	Small, white, circular	Gram positive cocci	Tube coagulase negative, mannitol non-fermented	Unidentified
	S3-2	Creamy white, small, translucent, mucoid, circular	Gram positive bacilli	Indole negative, mannitol fermented, non-motile, urease negative	*Bacillus* sp.

	S3-3	White, opaque, raised	Gram positive cocci in pairs	Catalase negative, citrate negative, oxidase negative	*Lactococcus* sp.
	S3-4	Yellow, circular, small	Gram positive bacilli	Catalase negative, Citrate negative, oxidase negative	*Lactobacillus* sp.



Fig 1. Total Heterotrophic Bacteria in three milk samples

CASE STUDY ARTICLE

Reinstating Fertility through Siddha: A Case Report on Anovulation

Vinodini Ramamoorthy[1]*, Shunmugaram Shenbagaraj[2], Saraswathi Balasubramanian[3] and Vinu Bharathi Balasubramaniam[1]

[1]Research Associate (Siddha), Department of Clinical Research, Siddha Regional Research Institute, Kuyavarpalayam, Puducherry, Central Council for Research in Siddha, Chennai, India.
[2]Research Officer (Siddha) S-II, Department of Clinical Research, Siddha Regional Research Institute, Kuyavarpalayam, Puducherry, Central Council for Research in Siddha, Chennai, India.
[3]Research Associate (Siddha), Department of Clinical Research, Central Council for Research in Siddha, Chennai, India.

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***Address for Correspondence**
Vinodini Ramamoorthy
Research Associate (Siddha),
Department of Clinical Research,
Siddha Regional Research Institute,
Kuyavarpalayam, Puducherry,
Central Council for Research in Siddha, Chennai, India.
E.Mail: vinodini02@gmail.com

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ABSTRACT

Infertility is a disease that affects one in six adults globally representing it as a serious health concern. The causes may be the male, female or combinatorial factors and the female factor contributes 37%. A 21 years old female with complaints of irregular menstrual cycle with the history of an ovulatory cycle and right ovarian cyst approached Siddha clinic for further management. In this case altered *Vaḷikuṟṟam* due to increased *Aiyam* and *aḻal*, might be the causative factor of immature follicle development and ovarian cyst, so the treatment was started with the help of Siddha diagnostic methods. After 6 months of treatment patient got conceived. Hence the prescribed Siddha drugs significantly improved the altered *Vaḷi* and *Aiyam*, as well as regulated the hormonal parameters and ovulation, resulting in normal conception.

Keywords: Anovulatory cycle, Infertility, Female Infertility, *Penmaladu*, Siddha medicine

INTRODUCTION

Infertility is global health concern that affects millions of peoples in reproductive age. It is defined by failure to conceive after 12 months or more of frequent, unprotected sexual contact. The World Health Organization reported that one in six adults or 17.5% of the global population suffer with infertility that indicates the critical need to expand

access to high-quality, cost-effective fertility treatment [1]. Also World Health Organization reported that 11.8% of Indian women in the reproductive age group have been diagnosed with primary infertility [2]. The etiology of infertility have been classified as male, female or combinatorial factors and the statistics revealed that head wise contribution were 8%, 37% and 35% respectively. Disorders of fallopian tube (tubal block, salpingitis, hydrosalpinx etc.), uterus (Fibroid uterus, polyps, unusual shape of uterus, cervical stenosis, scar tissues etc.), ovaries (Polycystic ovaries, tumors, tubo-ovarian abscess, ovarian torsion etc.), endocrine system (PCOS, Hypothyroidism, anovulation, hyperprolactinemia, precocious puberty etc.,) were the major female factors causing infertility and out of these, ovulatory factor marks upto 25% [3,4,5]. The infertile women in developing countries where suffered with negatively impacted lifestyle and it was a great challenge for them to take part in social activities [6]. They were mostly experienced with overt ostracism, domestic abuse, divorce, emotional stress, anxiety, depression, and low self-esteem. The availability, access and quality of infertility treatment were diversified and have been unattainable by most of the people in many countries. Even the government health policies and strategies were not prioritized the infertility therapeutic management and the increased out of pocket expenses for assisted reproductive technology, that will lead to individuals financial crises [3]. The diagnostic and therapeutic approach for female infertility was precisely mentioned in Siddha system of medicine and distinctive body constituent based management are also mentioned. In '*Agathiyar Karba Kol*' and '*Gnanavettiyan 1500*', female infertility was termed as *Peṇmalaṭu* or *Karpa roham* and derangement in *aḻal*, *vaḷi*, infection of reproductive organs, pox virus infections, irregular sexual practice, centripetal obesity, scar tissues such as adhesions, alkaline nature of cervix were mentioned as causative factors.

This present case report postulates the cost effective and non-invasive Siddha management for Primary infertility with anovulatory cycle and ovarian cyst. This case report has been prepared in accordance with CARE guideline.

PATIENT INFORMATION

A 21 years old female housewife and her 32 years spouse visited private Siddha clinic OPD with the complaints of inability to conceive for the past 2 years. The seminal parameters of the spouse were found to be in normal range. She had a history of irregular scanty menstrual flow for 2 to 3 days with an interval of 60 to 90 days. She underwent Hormone Replacement Therapy (HRT) for one year and observed rapid weight gain during the treatment course. So the couple approached Siddha management due to the poor prognosis and increased out-of-pocket expenses of modern medicine.

CLINICAL FINDINGS

The patient had a normal body built with a BMI of 24.3. On physical examination, the vitals were found to be normal and no abnormalities were noted in abdominal palpation. The Ultra-Sonogram pelvis revealed that right ovarian complex cyst measuring 33 x 26 mm and follicular study report showed no dominant follicle on Day 12 in ovaries. No abnormalities were detected is Hysterosalpingography. There was no evidence of Acanthosis nigrican's, hirsutism and acne. The patient also examined by Siddha diagnostic methodology *eṇvakait tērvu* and *tēka ilakkaṇam* was tabulated in table 2. The past history was evident that she was not suffering with T2DM, SHT and any other systemic illnesses.

DIAGNOSTIC ASSESSMENT

On detailed clinical evaluation of the signs and symptoms, the patient was diagnosed as *Peṇmalaṭu* (primary infertility) associated with anovulatory cycle. The ovulation process was also assessed by follicular study in 3rd and 7th month.

THERAPEUTIC INTERVENTION

The patient had *Aḻalvaḷi* pattern of *Nāṭi Naṭai* (Pulse pattern) and *Neykkuṟi* (oil drop urine test) so the treatment modalities was started to normalize the deranged *Mukkuṟṟam*. The patient was treated for seven months and the medicines used in the due course were tabulated in Table 1. She was advised to take the internal medicine (d) alone in empty stomach on early morning during menstruation as per procedure. Throughout the course of treatment period the patient was instructed to consume black urad dal porridge, fenugreek seed porridge, sesame balls, flax

seeds, lotus stem decoction. As well as to abstained from tamarind, *Sesbania grandiflora* leaves, bitter guard, pumpkin and junk foods.

Follow-Up and Outcome

The patient recuperate to regular menstrual cycle with normal blood flow after 60 days of treatment and her BMI reduced from 24.3 to 21.8. On the seventh month of treatment course she attained a healthy conception and confirmed by obstetric USG. The patient was followed up throughout the Antenatal period and she delivered a full term healthy male baby by normal vaginal delivery. There was no adverse drug reactions reported in treatment course. The outcome assessments with prognosis were detailed in table 2.

DISCUSSION

Infertility is a major life crisis with a multitude of sociocultural, emotional, physical, and financial issues. The patient was diagnosed as primary infertility associated with anovulatory cycle; the same was described as *Peṇmalaṭu* in Siddha medicine. Increased *aiyam* and *aḻal kuṟṟam* attenuate the *Vaḷi kuṟṟam*, which may be the disease's causative factor and lead to diminutive ovarian follicle. The patient was advised to take an *Eṇṇey kuḷiyal* (oil bath) on day 1 in order to normalize the deranged *Aḻal kuṟṟam*. *Agasthiyar kuzhambu* was subsequently administered, along with tender *Ficus religiosa* leaves ground up and mixed with cow milk, three consecutive days (days 2 - 4) to normalize the *Vaḷi kuṟṟam*. Since the Siddha basic principles state that drugs with *kaippu*, *kārppu* and *tuvarppu* tastes (bitter, hot and astringent) will both increase diminished *Vaḷi* and decrease aggravated *Aiyam* and *Aḻal* [7]. So the drug possessed those taste was chosen to administered as an internal medication. Due to their taste, hot potency and post-digestive biotransformation, *Vishnukranthi* and *Notchi* were advocated. *S. Angayarkanni et al.,* evaluated the Anti-hyperandrogenemic activity of *Evolvulus alsinoides* at a dose of 500 mg/ kg, gonadotropin hormone-releasing hormone (10 µg/ kg) used as a standard. The study results showed that *Evolvulus alsinoides* have a capability to alleviate the effects of polycystic ovaries and also reduces the progesterone, testosterone, ALT and AST levels [8]. Also, *Vishnukranthi* (*Evolvulus alsinoides*) have 16 bioactive compounds, most of them have the properties have endocrine modulator and also has antidepressant, anti-mutagenic, gastro-protective and hepato-protective [9,10]. These activities may regulate the endocrinal secretion and regularize the menstrual cycle. The leaves, root, bark and seed of the plant *Notchi (Vitex negundo)* are diversely used in folklore practice for female reproductive issues. Research revealed that phytoestrogens, vitexin of *Vitex negundo* were useful in Hormone replacement therapy due to its high estrogenic like activity. The extract of *V.negundo* regulates the LH, prolactin, testosterone and serum insulin level which may leads to proper ovulation [11]. Siddha text mentioned that administration of *Malaivembathy thylam* (*Kalingathi thylam*) in an empty stomach during the menstrual phase will help to eliminate the *Malaṭṭu kirumi* from uterus and ovaries [12]. Also, *Malaivembu* have hot potency with *kaippu* taste and *kārppu* taste as a post-digestive biotransformation [13]. Those tastes have the potency to increase *Vaḷi (kīḻ nōkkuṅkāl)* which may normalize the menstrual flow and helps to remove the accumulated debris of uterus. Anovulation, abnormal prostaglandin metabolism and Endocrine disorders are some causative factors for improper endometrial shedding that may also leads to endometrial hyperplasia, Dysfunctional Uterine Bleeding, etc [14]. *Cederblad et al.,* revealed that the fibrinolytic activity of endometrium in normal women was higher in luteal and menstrual phase than in follicular phase that was found to be a normal physiology in menstruation [15]. *Nithya rani et al.,* evaluated the fibrinolytic activity of *Melia dubia* latex protease and the result showed that it has fibrinolytic activity with clot hydrolysis and wound healing [16]. So, the ingestion of *Malaivembathy thylam* in menstrual phase may expel the endometrial debris and enhance the shedding, involution and healing of endometrium during menstrual phase. As a result, the prescribed Siddha drugs significantly improved the altered *Vaḷi* and *Aiyam*, as well as regulated the hormonal parameters and ovulation, resulting in the patient became pregnant shortly after six months of treatment and gave birth to a healthy, full-term boy.

CONCLUSION

This case study provides glimpse into the meticulous method for effective management of *Peṇmalaṭu* (primary infertility) associated with anovulation and ovarian cyst using Siddha therapeutic management. The outcome of this present case report will pave a way to further studies with sizable sample size.

Patient Perspective

The patient thanked for their healthy conception without much expenses which they can't afford. And the couple assured that they will refer patients with same issues for cost - effective Siddha management.

Informed Consent

The patient was obtained written informed consent for publishing their clinical details in journal.

Source of Funding

None

Conflict of Interest

The author declared no conflict of interest

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Table 1: Line of treatment

Timeline	Line of treatment	Medicine name	Dose/ Interval	Adjuvant and duration
Day 1	Oil bath	*Arakku thylam*	30 ml	-
Day 2,3,4	Internal medicine	a. *Agasthiyar kuzhambu*	130 mg	Tender leaves of *Ficus religiosa* grinded with cow's milk
Day 5		b. *Vishnukranthi chooranam*	1 gm / BD	Warm water
		c. *Notchi chooranam*	1 gm/ BD	Warm water, at day 10 to day 15 of each cycle
		d. *Malaivembathy thylam*	10 ml / OD	First three days of menstruation at early morning with *Nīrākāram* (fermented rice water)

Table 2: Outcome Assessment

S.No	Assessment		Findings		
			Before treatment	**3rd month**	**7th month**
1	***Eṇvakait tērvu***	***Nāṭi***	*Aḻal vaḷi*	*Vaḷi aḻal*	*Vaḷi aḻal*
		Nā	Coated	Normal	Normal
		Niṟam	Wheatish	Wheatish	Wheatish
		Moḻi	Low pitched	Low pitched	Normal pitched
		Viḻi	Normal	Normal	Normal
		Sparicam	Warmth	Warmth	Warmth
		Malam	Constipated	Constipated	Normal
		Mūttiram	Normal	Normal	Normal
		Neykkuṟi	*Aiyavaḷi*	*Aiya aḻal*	*Vaḷi aḻal*
2	**Follicular study (Day 12)**		NDF on both ovaries with Right ovarian complex cyst (33 x 26.6 mm)	Multiple small immature peripheral follicle in both ovaries	Urine Pregnancy Test - Positive

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RESEARCH ARTICLE

Bioactive Profiling and Antimicrobial Evaluation of *Solanum torvum* and *Solanum xanthocarpum* using GC-MS and *In vitro* Assays

M. Prabha[1]*, M. Brintha[2], S. Mariammal Priyadharshini[3], G. Sarosmi[3] and N. Abinaya[3]

[1]Assistant Professor, Department of Botany, St. Mary's College (Autonomous), Thoothukudi, (Affiliated to Manonmanium Sundaranar University, Tirunelveli), Tamil Nadu, India.
[2]Assistant Professor, Department of Plant Science, Manonmanium Sundaranar University, Tirunelveli, Tamil Nadu, India.
[3]M.Sc Student, Department of Botany, St. Mary's College (Autonomous), Thoothukudi, (Affiliated to Manonmanium Sundaranar University, Tirunelveli), Tamil Nadu, India.

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***Address for Correspondence**
M. Prabha
Assistant Professor,
Department of Botany,
St. Mary's College (Autonomous), Thoothukudi,
(Affiliated to Manonmanium Sundaranar University, Tirunelveli),
Tamil Nadu, India.
E.Mail: prabhamerfin@gmail.com

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ABSTRACT

This study investigates the phytochemical composition, antibacterial activity, and GC-MS profile of *Solanum torvum* and *Solanum xanthocarpum* fruit extracts. Using solvents including methanol, ethyl acetate, chloroform, petroleum ether, and water, extracts were prepared and screened for key phytochemicals. Methanolic extracts revealed the highest flavonoid (9.99 mg GAE/g) and terpenoid content (64.1 mg GAE/g). Antibacterial assays using the disc diffusion method indicated strong inhibition against *Staphylococcus aureus* (36.2 mm by *S. torvum*) and *Pseudomonas aeruginosa* (30.4 mm by *S. xanthocarpum*). GC-MS analysis identified bioactive compounds such as phytol, neophytadiene, linoleic acid methyl ester, and squalene, which are known for antimicrobial and antioxidant properties. These findings validate the medicinal potential of these plants and support further pharmacological exploration.

Keywords: *Solanum torvum, Solanum xanthocarpum*, Phytochemicals, Antibacterial, GC-MS, Secondary metabolites

INTRODUCTION

Medicinal plants are essential to both traditional and modern medicine, with over 80% of the global population relying on plant-based remedies. *Solanum torvum* (turkey berry) and *Solanum xanthocarpum* (yellow-fruit nightshade), [1] members of the Solanaceae family, have a long history of use in Ayurveda for treating infections, inflammation, and respiratory disorders. Despite their widespread traditional use, there is limited scientific documentation of their phytochemical profile and antibacterial potential. The increasing prevalence of antibiotic resistance necessitates new sources of antimicrobials. This study aims to evaluate the phytochemical constituents, antibacterial activity, and GC-MS profiles of *S. torvum* and *S. xanthocarpum* fruit extracts to support their therapeutic relevance [9].

MATERIALS AND METHODS

Fruits were collected from Thoothukudi, Tamil Nadu (Dec 2024). Dried powders were extracted using Soxhlet apparatus with methanol, ethyl acetate, petroleum ether, chloroform, and water. Qualitative screening was performed using standard methods to detect flavonoids, terpenoids, alkaloids, tannins, saponins, and phenols. Quantitative flavonoid content was measured by aluminum chloride method; terpenoids were estimated via spectrophotometry at 538 nm. The disc diffusion method was used against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Each extract (5 mg/mL) was applied to sterile discs (6 mm) on Mueller–Hinton agar. Amikacin served as the control [7]. GC-MS analysis was conducted using a Perkin-Elmer Clarus 500 system with Elite-5MS column. Compounds were identified using the NIST library.

RESULTS AND DISCUSSION

All extracts tested positive for key phytoconstituents. Methanol extracts exhibited the highest concentrations, notably 9.99 mg GAE/g flavonoids in *S. torvum* (Table 1) and 64.1 mg GAE/g terpenoids in *S. xanthocarpum*.(Table 2) These metabolites are linked with antioxidant and antimicrobial effects[1]. Significant antibacterial activity was demonstrated by the plant extracts. Notably, the ethyl acetate extract of *Solanum torvum* exhibited a pronounced inhibition zone of 36.2 mm against *Staphylococcus aureus*, (Table 3)[1] while the methanolic extract of *S. xanthocarpum* produced a 30.4 mm inhibition zone against *Pseudomonas aeruginosa*.[3,4](Table 4) These findings indicate a strong correlation between antimicrobial efficacy and the concentration of flavonoids and terpenoids present in the extracts [6]. The mode of action is likely attributable to the ability of phytochemicals to disrupt microbial membranes, inhibit protein synthesis, or interfere with nucleic acid function, as suggested in earlier literature [10]. GC-MS analysis revealed several bioactive compounds with known pharmacological relevance. In *S. torvum*,[2] major constituents included phytol, 11,14,17-eicosatrienoic acid methyl ester, and squalene. (Table 5). In contrast, *S. xanthocarpum* yielded neophytadiene, n-hexadecanoic acid, and linoleic acid methyl ester(Table 6). These compounds have been previously reported to exhibit antimicrobial, antioxidant, and anti-inflammatory properties, which may underlie the observed antibacterial activity[7]. These compounds are reported to exhibit antibacterial, anti-inflammatory, and cytotoxic properties, confirming the plants' pharmacological relevance [8],[9].

CONCLUSION

Both *Solanum torvum* and *Solanum xanthocarpum* demonstrate rich phytochemical profiles and potent antibacterial effects, particularly in methanol and ethyl acetate extracts. GC-MS analysis further supports the presence of therapeutically important compounds. These findings highlight the potential of these species as candidates for natural drug development.

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Conflict of Interest

The authors declare no conflict of interest.

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Table 1: Quantitative analysis of Total Flavonoid Content in different fruit extract of *S. torvum* and *S. Xanthocarpum*

S. No	Name of the Fruit Extracts	*Solanum torvum* mg (GAE)/g	*Solanum xanthocarpum* mg (GAE)/g
1	Petroleum Ether	1.162	0.76
2	Methanol	9.99	9.67
3	Chloroform	0.442	0.674
4	Ethyl Acetate	6.88	5.29
5	Aqueous	4.37	3.16

Table 2: Quantitative analysis of Total Terpenoid Content in different fruit extract of *S. torvum* and *S. xanthocarpum*

S. No	Name of the Fruit Extracts	*Solanum torvum* mg (GAE)/g	*Solanum torvum* mg (GAE)/g
1	Petroleum Ether	13.9	52.9
2	Methanol	52.32	64.1
3	Chloroform	32.4	35.4
4	Ethyl Acetate	40.7	27.3
5	Aqueous	21.9	19.2

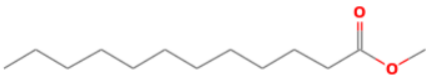
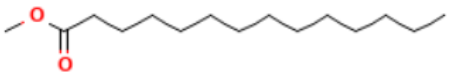
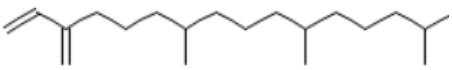
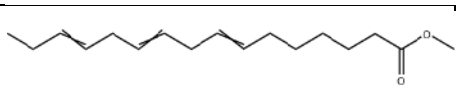
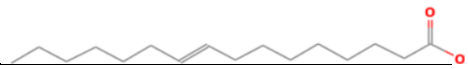
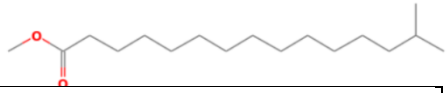
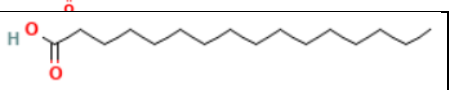
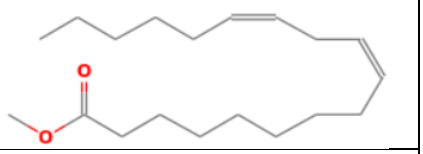
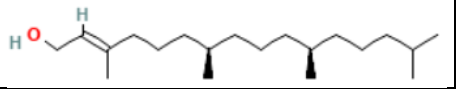
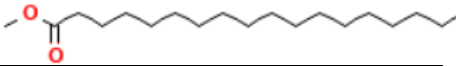
Table3: Anti-bacterial activity of different extract of *Solanum torvum* fruits

S. No	Microorganism	Control (Amikacin)	Zone of inhibition (mm)				
			Methanol	Aqueous	Ethyl Acetate	Petroleum Ether	Chloroform
1.	*Staphylococcus Sps*	28.4	28.2	11.5	36.2	13.4	27.4
2.	*Pseudomonas Sps*	30.3	30.32	-	18.6	12.2	26.32

Table 4: Anti-bacterial activity of different extract of *Solanum xanthocarpum* fruits

S. No	Microorganism	Control (Amikacin)	Zone of inhibition (mm)				
			Methanol	Aqueous	Ethyl Acetate	Petroleum Ether	Chloroform
1.	*Staphylococcus Sps*	30.4	30.2	-	12.08	7.05	13.06
2.	*Pseudomonas Sps*	13.48	30.4	-	27.4	24.05	20.6

Table 5:Compounds identified from Methanolic fruit extract of *Solanum torvum*

Peak No	Compound Name	Area of %	Retention Time (Min.)	Molecular Weight (G/mol.)	Molecular formula	Structure
1	Dodecanoic acid, methylester	0.72	16.734	214	C13H26O2	
2	Methyltetradecanoate	1.08	19.236	242	C15H30O2	
3	Neophytadiene	1.55	20.505	278	C20H38	
4	7,10,13-Hexadecatrienoicacid,methylester	1.14	21.212	264	C17H28O2	
5	Methylhexadec-9-enoate	5.19	21.441	268	C17H32O2	
6	Pentadecanoicacid,14-methyl-,methylester	16.47	21.511	270	C17H34O2	
7	n-Hexadecanoic acid	4.81	22.091	256	C16H32O2	
8	9,12-Octadecadienoicacid(Z,Z), methylester	15.49	23.253	294	C19H34O2	
9	Phytol	10.22	23.438	296	C20H40O	
10	Methylstearate	7.31	23.546	298	C19H38O2	

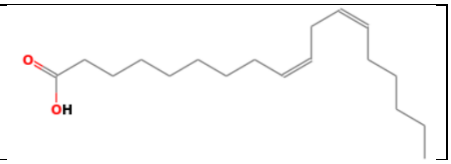
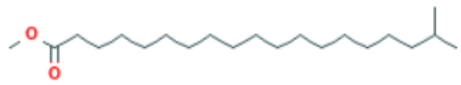
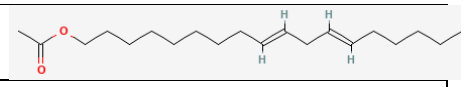
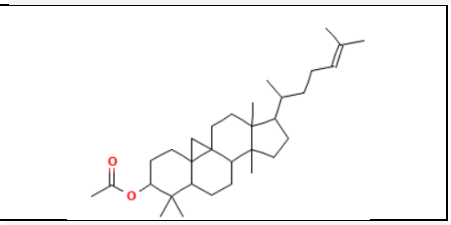
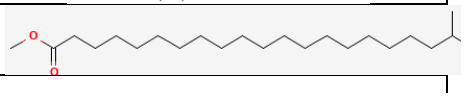
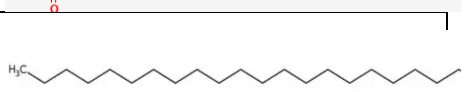
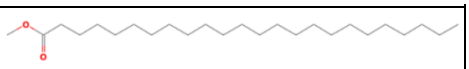
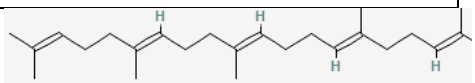
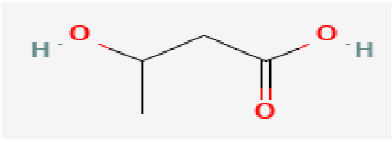
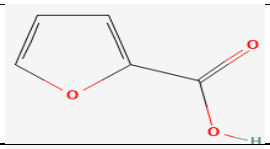
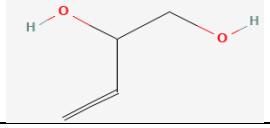
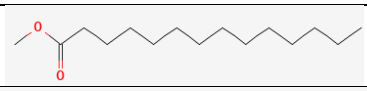
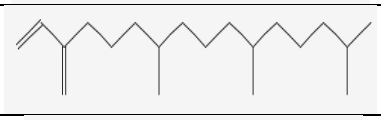
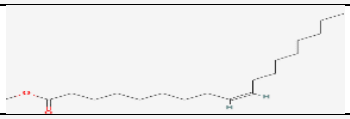
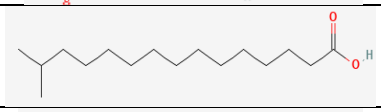
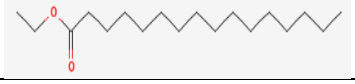
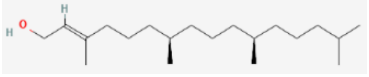
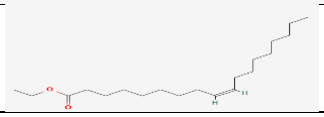
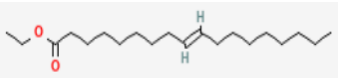
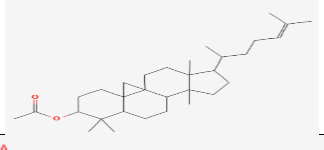
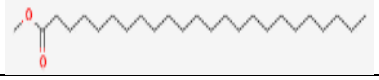
11	9,12-Octadecadienoicacid(Z,Z)-	2.06	23.790	280	C18H32O2	
12	Methyl18-methylnonadecanoate	1.25	25.409	326	C21H42O2	
13	Linoleylacetate	0.77	25.931	308	C20H36O2	
14	9,19-Cyclolanost-24-en-3-ol,acetate,(3.beta.)-	8.42	26.755	468	C32H52O2	
15	Methyl20-methyl-heneicosanoate	1.04	27.134	354	C23H46O2	
16	Tricosanoic acid, methylester	0.64	27.948	368	C24H48O2	
17	Tetracosanoic acid, methylester	0.97	28.731	382	C25H50O2	
18	Squalene	1.11	29.389	410	C30H50	

Table 6:Compounds identified from Methanolic fruit extract of *Solanum xanthocarpum*

Peak No	Compound Name	Area of %	Retention Time (Min.)	Molecular Weight (G/mol.)	Molecular formula	Structure
1	Butanoicacid,4-hydroxy-	2.96	6.434	104	C4H8O3	
2	2,5-Furandione,dihydro-3-methylene-	3.32	8.719	112	C5H4O3	
3	3-Butene-1,2-diol	4.32	10.473	142	C8H18N2	

4	Methyltetradecanoate	1.36	19.235	242	C15H30O2	
5	Neophytadiene	3.02	20.505	278	C20H38	
7	Methylhexadec-9-enoate	0.96	21.432	296	C19H36O2	
8	Pentadecanoicacid,14-methyl-,methylester	18.18	21.502	290	C17H34O2	
9	n-Hexadecanoic acid	12.89	22.096	256	C16H32O2	
10	Hexadecanoic acid, ethylester	7.73	22.192	284	C18H36O2	
11	9,12-Octadecadienoic acid(Z,Z)-, methylester	1.77	23.231	294	C19H34O2	
12	Phytol	11.30	23.429	296	C20H40O	
13	Methylstearate	8.30	23.539	298	C19H38O2	
14	EthylOleate	1.63	23.928	310	C20H38O2	
15	Octadecanoic acid, ethylester	2.18	24.166	312	C20H40O2	
16	Methyl13,16-docosadienoate	0.97	25.608	350	C23H42O2	
17	9,19-Cyclolanost-24-en-3-ol,acetate,(3.beta.)-	4.12	26.745	468	C32H52O2	
18	Tetracosanoic acid, methylester	1.45	28.729	382	C25H50O2	

RESEARCH ARTICLE

Floristic Survey of Understory Plant Species (Herbs and Shrubs) in Kurseong Subdivision of Darjeeling District, West Bengal, India

Shyam Sundar Manna*

Faculty, Department of Botany, Panskura Banamali College (Autonomous), Panskura R.S, Purba Medinipur, (Affiliated to Vidyasagar University, Midnapore), West Bengal, India.

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***Address for Correspondence**
Shyam Sundar Manna
Faculty,
Department of Botany,
Panskura Banamali College (Autonomous),
Panskura R.S, Purba Medinipur,
(Affiliated to Vidyasagar University, Midnapore),
West Bengal, India.
E.Mail: ssmanna09@gmail.com

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ABSTRACT

Understory plant species, especially herbs and shrubs, have been overlooked by researchers for a long time. They have a great role in nutrient cycling and controlling the microclimate of the region. Therefore, a survey was conducted in the Kurseong subdivision of Darjeeling district of West Bengal, India. It is the part of the Eastern Himalaya which is also a biodiversity hotspot site of the country. The present study reported thirty plant species comprising four shrubs and twenty-six herbs. These species belong to sixteen families. Asteraceae is the dominant family with eight species, followed by Poaceae with four species. Further, according to distribution status, 24 are native and the rest of the 6 species are introduced. The alarming rate of anthropogenic disturbances has destroyed the rich biodiversity of the region, recommending immediate conservation.

Keywords: Understory species, Kurseong, Darjeeling, Herbs, Shrubs, Native, Introduced.

INTRODUCTION

Darjeeling is a rich biodiversity site in West Bengal, India. The district is situated in the lap of the Eastern Himalaya (Chauhan and Sharma, 1996). It represents one of the richest Phytogeographical provinces in the country (Chatterjee,1962). However, Himalaya is the one of the four Biodiversity hotspot site of India. The area is made up of sedimentary and metamorphic rock with slopes from south to north. According to the researchers, the district is highly prone to landslides. The last few decades have damaged thousands of houses and lost more than hundreds of lives through it (Nad, 2015). Darjeeling is famous as a plant lover's paradise. The forest area is dominated by Oaks,

Maples, Pines, Rhododendron and Cinchona trees. The vegetation varies with the variation of altitude from temperate to evergreen forest. Kurseong subdivision of the Darjeeling district has a variety of understory Herbs, shrubs and fern species. These floor plants are the indicator species of greater soil fertility (Supriyati and Ariyanti, 2024). They can impact the regeneration process of trees. Thereby influenced the plant succession process (Thrippleton *et al*., 2016). The present study area comprises vast tea gardens with Cinchona and Orange planted sites. These canopy species directly enhance atmospheric deposition, microclimate and light transmission in the region. It leads to controlled Biodiversity and nativeness of ground vegetation (Augusto *et al*., 2003). The understory vegetation has been overlooked for a long time. However, very few studies on floor vegetation are conducted by the researcher. The main objective of the present study is to document the understory vegetation (Herbs and Shrubs) and their distribution status in Kurseong subdivision area of Darjeeling district.

MATERIALS AND METHODS

Darjeeling Himalaya is formed from a comparatively new rock structure in the Himalaya. It is the foothills district of West Bengal, India. The river Tista divided the district into two parts. The eastern part comprises Chola range and Kalimpong. However, the Singalila and the Darjeeling-Kurseong range are situated in the Western part. Darjeeling district is formed of four subdivisions: Darjeeling Sadar, Mirik, Kurseong and Siliguri (Figure-1). Kurseong subdivision comprises different forest ranges, like Bagdogra Forest range, Dalkajhar forest etc. The hillslopes of the region face a sufficient amount of sunlight whichfavour the growth of forest floor plant species. The present study was conducted in Kurseong subdivision (Figure-1). The field survey was done in different seasons from June 2024 to May 2025. The photograph of the live specimen in flowering condition was taken with the help of GPS map camera. The different sites like Sittong, Latpanchar, Ahaldara view point, Namthing pokhri etc were chosen for the survey. The information regarding local names, habits, and habitat was noted down during the survey. Scientific names and families are recorded with the help of regional flora. The distribution status of different plant species is listed with the help of the Royal Botanic Gardens online database. These statuses have been classified into two categories; Native and Introduced species. Native species originated and occur naturally in a particular geographical region. However, introduced species are transported and established in new regions beyond their native place.

RESULT AND DISCUSSION

The present study recorded thirty understory plant species with their family, habit and geographical distribution status (Table-1). It comprises twenty-six herbs and four shrubs. The plant species belongs to sixteen families. Asteraceae is the dominant family, comprising eight species. Further, Poaceae became the second-largest family with four species. Four families are bispecific and the rest ten families are monospecific (Figure-2). Out of thirty species, six are recorded as introduced and the remaining are native in the study region. Photograph of live specimens are store at digital data base of Panskura Banamali College (Autonomous) for future study (Figure-3-11).These understory species not only have ecological significance, like controlling soil erosion, nutrient recycling and maintaining the health of the local vegetation, but also have crucial medicinal values. This study is comparable with the previous studies reporting 49 herbs species belongs to 21 families from a foothill forest of Cooch Behar district, West Bengal in the Eastern Himalaya (Shukla and Chakravarty, 2014). Further, Haq *et al*. (2024) reported a total of 60 distinct species (9 shrubs and 51 herbs) belonging to 52 genera from the Zabarwan Mountain Range, Indian Western Himalaya. Hence, the present study reported fewer numbers of understory plant species than earlier studies (Sangry *et al*., 2024; Dar and Parthasarathy, 2025; Shameem *et al*., 2010). Still, it has greater significance as it enriched the region-specific flora database. However, the dominance of the Asteraceae family on understory plant species and the maximum contribution of Poaceae in the shady slopes Vegetation is supported by another study of Han *et al*. (2023) in other parts of the Himalaya. Out of the total recorded understory species, 80% are native in the study region. This means the geographic area has fulfilled the main ecological requirements like light, moisture, temperature and soil condition for the growth of native floor species. The native understory species are a vital component of the ecosystem which enriches the biodiversity of the region. Few understory species have been observed as introduced plant

species. They have been introduced by human activity, either intentionally or unintentionally. Hence, the geographical region has dispersal ability, habitat suitability and lots of disturbances.

CONCLUSION

Understory plant species are the dynamic layer of any vegetation that controls soil erosion, regimes water and improves the fertility of soil. The driving mechanisms of understory plant species have important implications for the conservation of biodiversity. It also helps to maintain the function of ecosystem during global climate change. The present report draws the attention of new researchers to focus on this field. Further, the introduced species can potentially outcompete with the native species. Hence, it is important to prepare a checklist of regional native plant species, otherwise the introduced species may alter its ecosystem dynamics. The study also assumed that the anthropogenic disturbances through increased destination priority by tourists and regular new household settlements in the area are continuous threads to the local flora. Hence, immediate steps need to be taken to protect overlooked understory plant Biodiversity.

ACKNOWLEDGEMENTS

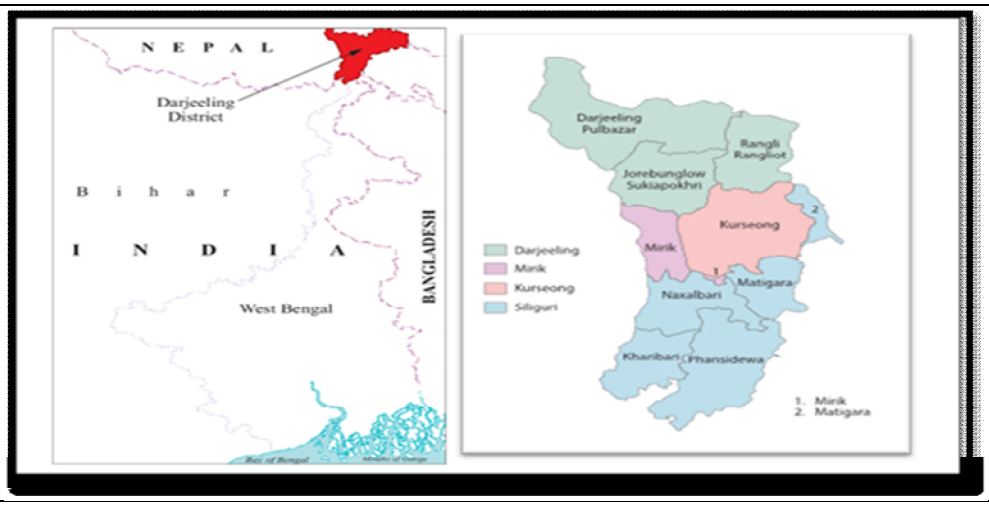
I am very much thanks full to my beloved students of session 2023-2024 of our department. They were help me during my field survey at Kurseong subdivision of Darjeeling district, West Bengal, India.

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Table-1. Showing Scientific name, Family, Habit and Distribution Status of the species.

Sl. No	Scientific Name	Family	Habit	Distribution Status
1.	*Achyranthes bidentata Blume*	Amaranthaceae	Shrubs	Native
2.	*Acmella paniculata (Wall. ex DC.) R.K.Jansen*	Asteraceae	Herbs	Native
3.	*Ageratina adenophora (Spreng.) King and H. Rob.*	Asteraceae	Shrubs	Introduced
4.	*Ageratum conyzoides L.*	Asteraceae	Herbs	Introduced
5.	*Amomum subulatum Roxb.*	Zingiberaceae	Herbs	Native
6.	*Anaphalis margaritacea (L.) Benth. and Hook.f.*	Asteraceae	Herbs	Native
7.	*Bergenia ciliata (Haw.) Sternb.*	Saxifragaceae	Herbs	Native
8.	*Bidens pilosa L.*	Asteraceae	Herbs	Introduced
9.	*Calceolaria tripartita Ruiz and Pav.*	Calceolariaceae	Herbs	Introduced
10.	*Centella asiatica (L.) Urb.*	Apiaceae	Herbs	Native
11.	*Erigeron canadensis L.*	Asteraceae	Herbs	Introduced
12.	*Curcuma aromatica Salisb.*	Zingiberaceae	Herbs	Native
13.	*Cynodon dactylon (L.) Pers.*	Poaceae	Herbs	Native
14.	*Didymocarpus aromaticus D.Don*	Gesneriaceae	Herbs	Native
15.	*Erigeron emodi L.*	Asteraceae	Herbs	Native
16.	*Fragaria nubicola (Lindl. ex Hook.f.) Lacaita*	Rosaceae	Herbs	Native
17.	*Hemiphragma heterophyllum Wall.*	Plantaginaceae	Herbs	Native
18.	*Hypoestes triflora (Forssk.) Roem. and Schult.*	Acanthaceae	Herbs	Native
19.	*Imperata cylindrica (L.) Raeusch.*	Poaceae	Herbs	Introduced
20.	*Craniotome furcata (Link) Kuntze*	Lamiaceae	Shrubs	Native
21.	*Koenigia mollis (D. Don) T.M. Schust. and Reveal*	Polygonaceae	Herbs	Native
22.	*Oxalis corniculata L.*	Oxalidaceae	Herbs	Native
23.	*Perilla frutescens (L.) Britton*	Lamiaceae	Herbs	Native
24.	*Persicaria capitata (Buch.-Ham. ex D.Don) H.Gross*	Polygonaceae	Herbs	Native
25.	*Primula malacoides Franch.*	Primulaceae	Herbs	Native
26.	*Pseudognaphalium affine (D.Don) Anderb.*	Asteraceae	Herbs	Native
27.	*Pupalia lappacea (L.) Juss.*	Amaranthaceae	Shrubs	Native
28.	*Sida acuta Burm.f.*	Malvaceae	Herbs	Native
29.	*Thysanolaena latifolia (Roxb. ex Hornem.) Honda*	Poaceae	Herbs	Native
30.	*Oplismenus undulatifolius (Ard.) P.Beauv.*	Poaceae	Herbs	Native



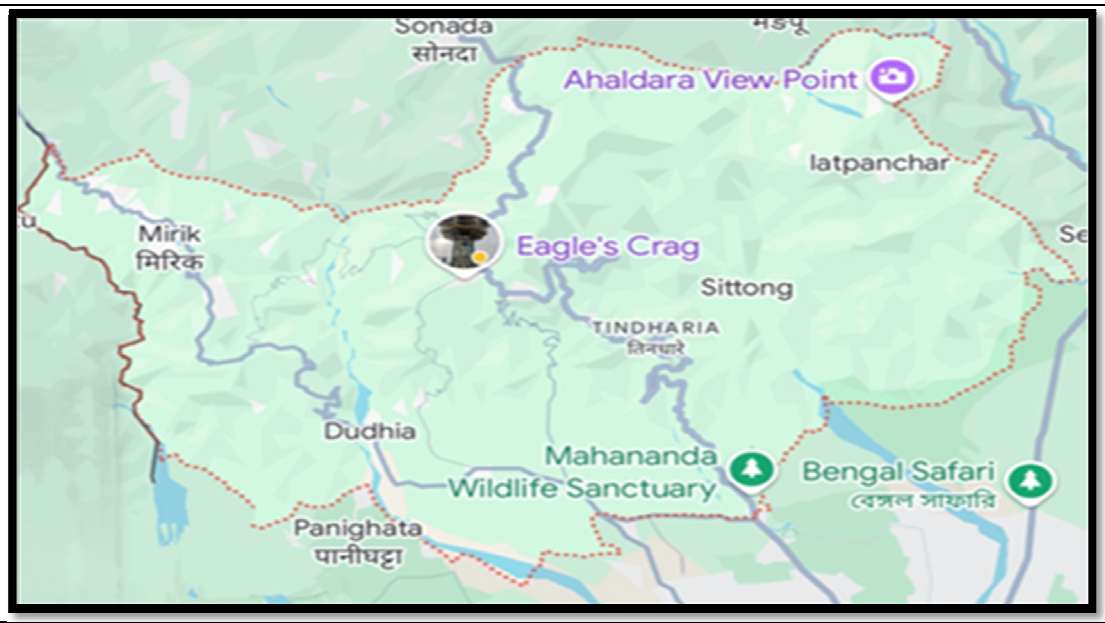


Figure.1: Map showing the location of Kurseong sub-divisions of Darjeeling district, West Bengal, India.

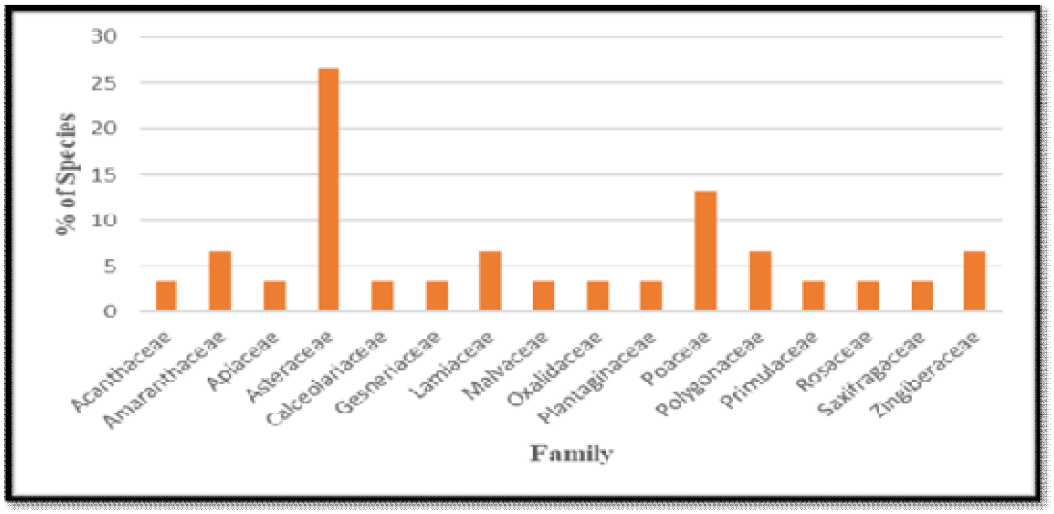


Figure. 2: Showing percentages of species in different families



Figure. 3: ***Hypoestes triflora (Forssk.) Roem. and Schult., Craniotome furcata (Link) Kuntze, Erigeron canadensis L., Ageratina adenophora (Spreng.) King and H. Rob., Persicaria capitata (Buch.-Ham. ex D.Don) H.Gross, Pseudognaphalium affine (D.Don) Anderb., Amomum subulatum Roxb., Calceolaria tripartita Ruiz and Pav., Achyranthes bidentata Blume.***

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RESEARCH ARTICLE

Evaluation of Antioxidant and Antidiabetic Properties of Polyphenol Extract from Tender Coconut Shell

Anie T. Uthup[1] and T R Satyakeerthy[2]*

[1]Research Scholar, Department of Chemistry, JJT University, Jhunjhunu, Rajasthan, India.
[2]Deputy Director, IGNOU Regional Centre, Trivandrum, Kerala, India.

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***Address for Correspondence**
T R Satyakeerthy
Deputy Director,
IGNOU Regional Centre,
Trivandrum, Kerala, India.
E.Mail: trsatyakeerthy@ignou.ac.in

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ABSTRACT

Coconut trees are widely grown in several tropical and sub-tropical countries and is consumed in various forms. Tender coconuts are nutritious and have several health benefits but, the waste produced in the form of tender coconut shell is underutilised. To make better use of the tender coconut shell the polyphenolic extract from tender coconut shell has been studied for its antioxidant and antidiabetic property. Antioxidant activity in terms of IC_{50} value has been 163.07 µl and antidiabetic activity inhibiting α-glucosidase has an IC_{50} value of 321.08 µl for the polyphenolic extract indicating intense activity.

Keywords: Polyphenols, Tender Coconut Shell, antioxidant, antidiabetic, inhibition.

INTRODUCTION

Consumption of fruits and vegetables has lots of health benefits as they contain secondary metabolites like polyphenols which are natural antioxidants. These polyphenols have been widely studied for their strong radical scavenging and antioxidant activity as they contribute to prevent disease such as diabetes, hyper tension, coronary diseases and cancers [2].Oxidative stress is found to be a cause for the dysfunction of the pancreatic β-cells which are responsible for secretion of insulin. Thus, polyphenols play an important role as potent antioxidants and antidiabetic contributing to the prevention of type 2 diabetes.In addition, there are several studies which reveal the role of polyphenols as antidiabetics and antioxidants in various food stuffs. Coconut trees *Cocos nucifera.* (L) commonly called coconut palm is a member of the Arecaceae family and is also the only living member of the genus Cocos. Coconut trees are widely grown in the tropical and subtropical regions providing multiple use to the mankind[3]. Coconut, like other fruits, it has three layers: the exocarp, mesocarp and endocarp. The exocarp is the glossy outer skin, usually yellow-green to yellow-brown in colour. The mesocarp is composed of a fibre, called coir, which has

many traditional and commercial uses. Both the exocarp and the mesocarp make up the "husk" of the coconut, while the endocarp makes up the hard coconut "shell". Coconut and its products have been an important part of the diet among Indian population from time immemorial and consumption of tender coconut water as healthy drink is known across several countries. Unfortunately, after consumption of the tender coconut water and the testa inside, the tender coconut shell is discarded causing pollution. It has been previously reported that several parts of the ripe coconut including coconut oil, virgin coconut oil and the tender coconut water to contain polyphenols which have antioxidant and anticancerous properties [1]. Also, in our previous study we have reported the presence of polyphenols in the tender coconut shell. Thus, to make better use of the polyphenols present in the tender coconut shell and to explore its potential applications, the present study has been formulated to evaluate the antioxidant and antidiabetic properties of the polyphenols extracted.

MATERIALS AND METHODS

Plant Material and Extraction

The plant material consists of two fresh tender coconuts of 4 – 5 months maturity cleaned, de-husked and dewatered to obtain the tender coconut shell. These tender coconut shells were cleaned properly with water, broken into small pieces, and are immersed in 750 ml distilled water in a 1L glass beaker. The extract thus collected after 48 hours containing the maximum amount of polyphenols is the polyphenol extract.

Determination of Antioxidant property

The free radical scavenging activity of the concentrated polyphenols or the polyphenol extract was determined by DPPH (2,2-diphenyl-1-picrylhydrazyl) assay, according to the method of Brand-William et al.,[4] and Kevin P. Anthony and Mahmoud A. Saleh[5] with slight modifications. For DPPH assay ascorbic acid was used as reference standard. Ascorbic acid stock solution was prepared in distilled water (1 mg/ ml; w/v). A 60µM solution of DPPH in methanol was freshly prepared and a 200µl of this solution was mixed with 50µl of test sample at various volume (100, 200, 300, 400 and 500 µl). The plates were kept in the dark for 15 minutes at room temperature and the decrease in absorbance was measured at 515 nm. Control was prepared with DPPH solution only, without any extract or ascorbic acid. 95% methanol was used as blank. Each sample, as well as each control was analysed in triplicates.
Radical scavenging activity was calculated by the following formula

$$\text{Percent inhibition} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

Determination of Antidiabetic property

The antidiabetic activity of the polyphenol extract was assessed using the α-glucosidase inhibition assay according to the method described by Shai et al.,(2011) [6] with slight modification. 400 µL of α-glucosidase (0.067 U/mL) was pre incubated with different volume of the polyphenol extract for 30 min. Then 200 µL of 3.0 mM 4-nitrophenyl-α-D-glucopyranoside (pNPG) used as substrate dissolved in 0.1M sodium phosphate buffer (pH 6.9) was then added to start the reaction. The reaction mixture was incubated at 37°C for 30 min and stopped by adding 2 mL of 0.1 M Na2CO3. The α-glucosidase activity was determined by measuring the yellow-coloured para- nitro phenol released from pNPG at 400 nm. The results were expressed as percentage of inhibition. The Same procedure was done with Acarbose (1mg/ml stock) which was used as the standard.
The percentage of inhibition was calculated using the formula

$$\text{Percentage inhibition} = \frac{\text{Absorbance of Control - Absorbance of Sample}}{\text{Absorbance of Control}} \times 100$$

RESULTS AND DISCUSSION

The antioxidant activity of the polyphenol extract is summarised in Table 1. Half-maximal inhibitory concentration (IC_{50}) is the concentration needed to reduce half of the free radical concentration. In this study it has been seen that the IC_{50} value of the reference standard (ascorbic acid) has been 28.19µg/ml whereas the polyphenol extract has an IC_{50} value of 163.07 µl. Thus, the result clearly indicates that the polyphenol extract form tender coconut shell has profound antioxidant activity. The free radical DPPH, which is widely used to evaluate the ability of compounds to operate as free-radical scavengers and hydrogen suppliers, is a rapid, simple, and inexpensive method for testing antioxidant capabilities. The DPPH test relies on the elimination of DPPH, a stabilized free radical. DPPH is indeed a dark-coloured crystalline compound made up of free-radical particles that are stable. It is a well-known radical and a popular antioxidant test. In nature, antioxidant compounds are commonly available in com plex botanical mixtures. Interactions of hundreds or even thousands of antioxidant compounds attribute unique chemical- and biological-related features to each plant [7].In vitro, several extractions of plants have been shown to neutralize DPPH radical scavenging activity [8]. Furthermore, DPPH free radicals have been found to be scavenged by several tea extracts consisting of a variety of polyphenols [9]. The specific method of free-radical scavenging activity of the extract is unknown. Further, the extract may contain different types of polyphenols and the particular polyphenol responsible for the free radical scavenging activity has to be explored. The antidiabetic activity determined using the α-glucosidase inhibition assay of the polyphenol extract is summarised in Table 2. From the results it is seen that the α-glucosidase inhibitory activity in terms of the IC_{50} value of standard Acarbose has been found to be 16.72 µg/ml whereas, the polyphenol extract has an IC_{50} value of 321.08 µl. Therefore, the polyphenol extract is a potential inhibitor of α-glucosidase. Phytochemicals with strong antioxidant properties have been reported to be good inhibitors of α-glucosidase, and regulators of hyperglycaemia and other diabetic complications arising from oxidative stress [10]. Generally, the drugs acting asα-glucosidase inhibitors are considered as oral hypoglycaemic agents owing to their role of inhibiting the degradation of di-saccharides to monosaccharides, thereby maintains the bloodsugar level normal (UK prospective diabetes study) [11]. It has been reported that polyphenols exerted excellent antioxidant activities, which repaired pancreatic β-cells, enhanced insulin sensitivity, and decreased blood glucose in diabetic mice [12]. There are several studies on different parts of coconut exhibiting the antidiabetic property [13],[14],[15]. However, antidiabetic property exhibited by polyphenol extract from tender coconut kernel is first of its kind and further studies on animal models and cell cultures are still needed to ascertain these properties.

CONCLUSION

Generally, many kinds of polyphenols show both the antioxidant activity and the antidiabetic activity. The antioxidative properties and antidiabetic efficacy of polyphenols are independent of each other and their interrelationship is still unclear. Understanding the interaction of the antioxidant activities and antidiabetic effects of polyphenols will need to be clarified in the future studies. However, when we take polyphenols from a food, its effect may not be same as the effect of the chemical alone. Antioxidative and antidiabetic activity may change by processing methods, such as heating etc and is different in different parts of the fruit, seed, leaf etc. In the present study the polyphenols from tender coconut shell have profound antioxidant and antidiabetic property which suggests that it can find applications against many degenerative diseases.

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Table 1. Antioxidant activity of polyphenol extract

Substrate Material	**DPPH (IC_{50}) value**
Ascorbic acid (reference standard)	28.19 µg/ml
Polyphenol extract	163.07µl

Table 2. Antidiabetic property of polyphenol extract

Substrate Material	**α-glucosidase inhibition (IC_{50}) value**
Acarbose (standard)	16.72 µg/ml
Polyphenol extract	321.08 µl.

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REVIEW ARTICLE

Investigating the Biology of *Macrophomina phaseolina* (Tassi) Goid and its Management: A Review

Jeya Rani M[1], Kalaichelvi K[2] and Murali Sankar P[3]*

[1]Associate Professor, Department of Agronomy, Pushkaram College of Agriculture Sciences, Thiruvarankulam, Pudukkottai, (Affiliated to TamilNadu Agricultural University, Coimbatore), Tamil Nadu, India.
[2]Assistant Professor, Department of Agronomy, ICAR-Krishi Vigyan Kendra, Virdhachalam, (Affiliated to TamilNadu Agricultural University, Coimbatore), Tamil Nadu, India.
[3]Assistant Professor, Department of Plant Pathology, Pushkaram College of Agriculture Sciences, Thiruvarankulam, Pudukkottai, (Affiliated to TamilNadu Agricultural University, Coimbatore), Tamil Nadu, India.

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***Address for Correspondence**
Murali Sankar P
Assistant Professor,
Department of Plant Pathology,
Pushkaram College of Agriculture Sciences,
Thiruvarankulam, Pudukkottai,
(Affiliated to TamilNadu Agricultural University, Coimbatore),
Tamil Nadu, India.
E.Mail: muralisankar58@gmail.com

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ABSTRACT

M. phaseolina is a polyphagous pathogen belonging to the Botryosphaeriaceae family, which is both soil and seed-borne. It is responsible for causing dry root rot and blight in over 500 crop species across more than 100 families, particularly in tropical and subtropical regions with semi-arid climates. This pathogen exhibits two distinct phases: a saprophytic phase (*Rhizoctonia bataticola*) and a pathogenic phase (*M. phaseolina*). Officially recognized as the correct taxonomic name since 1947, *M. phaseolina* (Tassi) Goid has a significant impact on a variety of crops, including soybeans, common beans, mung beans, peanuts, sesame, cowpeas, chickpeas, cluster beans, sorghum, corn, and cotton. The identification of *Macrophomina* is characterized by the formation of brown or black structures that eventually darken. Microsclerotia develop within the vascular tissue, imparting a greyish-black hue to the stem. Management strategies for this pathogen encompass soil solarization, the use of inorganic salts, bioagents, resistant varieties, and chemical control. The application of biocontrol agents such as *Trichoderma* and *Pseudomonas* spp. as seed treatments, soil applications, and foliar sprays has proven effective in combating this pathogen and mitigating yield losses. Additionally, combining phytoextracts with chemical fungicides has been shown

to reduce disease incidence. An integrated disease management approach is often employed, emphasizing sustainability.

Keywords: Agriculture, Dry root rot, Microsclerotia, Saprophytic, Vascular tissue.

INTRODUCTION

M. phaseolina is a polyphagous pathogen that is both soil and seed-borne, responsible for causing dry root rot and blights in 500 crop species across more than 100 families. Known as charcoal rot, dry root rot belongs to the Botryosphaeriaceae family within the Dothideomycetes class, which is recognized as the most diverse class of Ascomycetes [1]. This fungus has been identified in regions across North and South America, Asia, Africa, and Europe. It holds significant economic importance in subtropical and tropical countries characterized by semi-arid climates [2]. *M. phaseolina* exhibits heterogeneous host specificity, infecting both monocotyledonous and dicotyledonous plants, and its distribution in soil is not uniform. The pathogen is responsible for both pre-emergence and post-emergence mortality in plants. Notable symptoms observed after emergence include spindle-shaped lesions characterized by a dark border and a light grey center, which may develop microsclerotia and occasionally pycnidia [3]. Sclerotia can persist in the soil for extended durations. The pathogen has been identified as seed-borne [4] in infected seeds and can be transmitted through seedlings [5]. *M. phaseolina* is also an occasional human pathogen that leads to various fungal infections and has the capacity to neutralize plants and animals through preventive antifungal treatments [6]. The organism exhibits two distinct phases: a saprophytic phase characterized by *Rhizoctonia bataticola*, which produces microsclerotia and mycelia, and a pathogenic phase represented by *M. phaseolina*, which also generates microsclerotia, mycelia, and pycnidia [7]. Documented evidence indicates the presence of both phenotypic and genotypic variation within the pathogen population in the same geographical area [8]. The existence of heterokaryotic mycelium shows significant variability. These complexities pose challenges in the development of effective and sustainable disease management strategies [9, 10]. Additionally, this review emphasizes the classification of fungi, identification of the pathogen, symptomatology, epidemiology, disease cycle, host range, and management strategies pertaining to *M. phaseolina*.

Fungal classification

The genus *Macrophomina* was initially described by Petrak in 1923, based on dried specimens of *Sesamum orientale* collected by G.M. Ryes in the Philippines in 1921 [11]. The pycnidial form of the fungus was first identified as *Macrophomina phaseolina* by Tassi in 1901 and later as *Macrophomina phaseoli* by Maublanc in 1905 [12]. Literature also documents additional symptoms associated with *Macrophomina phaseolina*, including references to *Sclerotium bataticola* by Taubenh in 1913, *Macrophomina cajani* by P. Sydow and Butler in 1916, *Macrophoma chorchori* by Sawada in 1916, *Macrophoma sesami* by Sewada in 1922, and *Tiarosporella phaseolina* by Tassi in 1981 [13]. In India, the fungus was first isolated from cowpea in 1912 by Shaw and was referred to as *Sclerotium bataticola* by Sheikh and Ghaffar in 1979. In 1981, Van der Aa reclassified the species as *Tiarosporella phaseolina* (Tassi) Van der Aa. Currently, *M. phaseolina* (Tassi) Goid, established in 1947, is recognized as the valid taxonomic name, with the sclerotial phase identified as *Rhizoctonia bataticola* by Holliday and Punithalingam in 1970. *Macrophomina* is a monotypic genus, comprising solely the species '*phaseolina*' [14].

Yield loss

This disease is prevalent in agricultural areas, leading to considerable reductions in crop yield. The extent of yield loss can range from 5% to 50%, and in optimal conditions for susceptible varieties, it may reach as high as 100% [15]. In Tamil Nadu, it has been documented to cause a yield reduction of 28.6% in black gram, with disease prevalence between 67.7% and 81.2% [16]. A survey on the dry root rot disease affecting rice fallow black gram in Tamil Nadu indicated that the incidence of the disease varied from 16% to 33%. It was noted that sandy loam and sandy clay soils exhibited a higher incidence of dry root rot compared to clay soils [17].

Symptomatology

Seedlings can become infected when the soil is excessively dry and the temperature exceeds 35ºC for duration of 2-3 weeks [18]. Symptoms may manifest as brown or dark spots on the cotyledons following their development. In certain instances, the edges of the cotyledons may turn brown or black and fall off prematurely. Symptoms begin to appear from the unifoliate leaf stage in hypertrophic seedlings [19]. These symptoms present as circular or oblong reddish-brown lesions that may darken to brown or black after several days. The lesions can also spread down the stem. Seeds may perish if the soil remains excessively hot and dry [20]. The initial indication of the fungus typically manifests on the plant's surface within 1 to 4 weeks prior to reaching its full growth stage. It inflicts damage on the roots, stems, pods, and seeds from ground level upwards [21]. The lesions are generally light brown or gray in colour and are not commonly observed on the stem. Microsclerotia develop within the vascular tissue and substance, imparting a grayish-black hue to the stem. These discolorations first appear at the nodes as large, randomly distributed black dots [22]. In cases of nursery infections, a twin stem anomaly is often present. The early yellowing of the roots and lower stem is usually followed by a reddish-brown discoloration as the fungus advances along the stem. Infected pods and grains are covered with black bodies that may be either locally or widely dispersed [23]. The fungus can produce various symptoms depending on its method of penetration into the pod and grain, and it may also result in the seeds exhibiting thick black spots [24]. Upon the death of the plant, numerous minute micro sclerotic lesions resembling pinheads become visible. These lesions can be accurately identified by removing the epidermis from the lower stem and roots of the plant. The infected crop exhibits early yellowing in distinct patches. Under severe conditions, the pathogen may affect a significant area of the field. Typically, within the impacted crop, dead leaves remain attached to the petioles for several days following the plant's demise. The affected plant can be easily extracted from the soil, and both root hairs and lateral roots will decay, accompanied by peeling bark at the collar region [25].

Identification of the pathogen

Macrophomina is characterized by the development of brown or black coloration, which eventually darkens further. In culture plates, one can observe numerous aerial mycelium with sclerotia embedded within the hyphae. Initially, the septate hyphae are hyaline, transitioning to a honey or black colour [26]. On the underside of the culture plate, a significant presence of dark brown to black sclerotia is visible. The vegetative mycelium forms monilid or barrel-shaped cells, with septum formation occurring near the branching of the mycelium. Branching typically occurs at acute angles, but can also occur at right angles to the parent hypha [27]. Aggregated hyphae lead to the formation of microsclerotia, consisting of 50 to 200 individual cells that are produced by melanin pigment. The microsclerotia of Macrophomina are black and range in size from 50 to 150 micrometers. *M. phaseolina* generates globose or flattened pycnidia, measuring between 100 to 200 micrometers in diameter [28]. The inner layer is hyaline, while the outermost layer of cells appears dark brown to black. The conidiophores are septate and simple, and the conidia are hyaline and obovoid with a truncate base. The gelatinization of the outer layer of the conidial wall leads to the development of apical and cap-shaped conidial appendages [29].

Fungus

The genus "*Rhizoctonia*" is an anamorphic fungus belonging to the Cantharella group and the Ceratobasidisaceae family. This genus does not produce spores; instead, it is characterized by the presence of hyphae and sclerotia (*hyphal propagules*) during its asexual phase [30]. While certain species function as saprotrophs, others act as facultative plant pathogens, leading to significant crop diseases. The term "*Rhizoctonia*" was first introduced in 1815, meaning "root killer" in French, and has since been expanded to encompass over 100 additional names. A comprehensive review and re-evaluation of the species name "rhizoctonia" was published in 1994 in the peer-reviewed journal "Evolutionary Fungi." The findings indicate that the correct taxonomical designation for *Rhizoctonia* is "*Macrophomina phaseolina*" Goid 1947, which is now acknowledged as *Macrophomina phaseolina* [31].

Host range

Macrophomina phaseolina could be a nonspecific parasitic species that has been watched to assault around 500 species in over 100 families around the world. The run of have species and topological conveyance propose that

Macrophomina phaseolina is moderately assorted [32]. This plant pathogen incorporates a wide extend of impacts on an assortment of crops, counting soyabeans, common beans, mungbean, sorghum, corn, cotton, shelled nut, sesame, cowpea, chickpea and cluster bean and cotton. Moreover, it has been watched to influence softwood and other timberland trees, as well as citrus, cocoas, coffee, ziziphus, leucaena, therapeutic plants and unwanted weeds [33].

Disease cycle

M. phaseolina is responsible for causing seedling blight, root rot, and stem rot in a variety of crops, including soybean, common bean, sorghum, maize, cotton, peanut, and cowpea. It also affects softwood forest trees such as *Abies, Pinus,* and *Pseudotsuga*, as well as fruit trees like citrus, coconut, and coffee, along with various weed species [34]. Under unfavorable environmental conditions, the fungus forms sclerotia within the root and stem tissues. In potato dextrose agar (PDA), pycnidia are occasionally produced in host crops; for instance, in cowpea, pycnidia appear at the conclusion of the rainy season. In the soil, microsclerotia can infect seeds or host tissues, serving as the primary inoculums [35]. The infective hyphae penetrate the plant through root epidermal cells or wounds. In the early stages of pathogenesis, the mycelium invades the root epidermis via intercellular spaces and progresses through the cortex of the primary roots, ultimately resulting in the death of the plantlets. During the flowering stage, the fungal hyphae grow intracellularly within the xylem vessels, leading to the appearance of necrotic lesions on the stems, branches, and peduncles of the infected plants. The production of fungal toxins, particularly phaseolin, is significantly increased in the infected plants, contributing to premature death [36]. Following the demise of the plants, the processes of mycelial colonization and sclerotial formation persist until the tissues become desiccated. The mycelium and microsclerotia generated from the infected plant material, which includes plant residues, serve as the primary means of pathogen propagation. The microsclerotia found in the soil, as well as in the roots and stems of the host, represent the principal surviving propagules [37]. Once the roots and plant debris decompose, the microsclerotia are released into the soil. They are typically found in clusters on the soil surface and are predominantly located at a depth of 0-20 cm (Figure 1) [38].

Epidemiology

Temperature

Temperature was the essential restriction figure for the development and pathogenicity of *Rhizactonia bataticola* segregates determined from roots, leaf and seeds of beat like black gram, green gram, cowpea, soybean, and redgram. As the temperature expanded between 10ºC and 30ºC, mycelial development expanded steadily, and there was a continuous diminish at 35ºC [39].

Soil moisture

This infection was increases by the dry soil conditions, especially at blossoming organize, which caused the plants to dry out all of the sudden. Root colonization was higher at 40% to 50% soil dampness compared to 10% to 20% soil dampness. Contamination and side effect advancement was most influenced by higher temperatures (25-40ºC) in overflowed soil as contradicted to dry soil (40-60%) [40].

pH

The pH extend for dry root decay was decided to be between 3.0 and 9.0 with 5.5 being the ideal pH for development and 6.0 being the least pH for development. As the pH expanded, the development rate diminished until it comes to 7.0 [41].

Management strategies

The aim of the control methods is to reduce the number of sclerotia in soil and to minimize the contact of the inoculum and host.

Soil solarization

Soil solarisation effectively diminishes soil-borne pathogens such as *Pythium* spp., *M. phaseolina*, and *Fusarium* spp. This method is cost-effective for organic farmers as it does not require specialized skills or technology, but is reliant

on climatic conditions and necessitates a prolonged duration. The combination of soil amendments and solarisation elevates soil temperatures, thereby inhibiting pathogen growth to a depth of 30 cm [42]. Furthermore, integrating biological methods with soil solarisation has proven to significantly reduce pathogen presence. This technique has been shown to manage diseases caused by various fungal pathogens, including *Rhizoctonia solani, Macrophomina phaseolina, Fusarium* spp., *Pythium* spp., *Phytophthora* spp., *Verticillium* spp., *Bipolaris sorokiniana*, *Plasmodiophora brassicae*, and *Sclerotium rolfsii* [43].

Inorganic salts

Instead of synthetic fungicides, certain inorganic salts are employed to manage *M. phaseolina*. Effective salts include sodium carbonate at a concentration of 1.5%, sodium bicarbonate at 2.0%, and ammonium molybdate at 4.0%, which have demonstrated efficacy against the growth of *M. phaseolina*. Additionally, seed priming with these salts has been shown to diminish seed-borne infections [44]. Augment with organic matter and nutrient availability in silicate treated soil was depicted by physiochemical analysis. These findings suggest the possibility of using potassium silicate as an alternative to fungicides against dry root rot disease [45].

Resistant varieties

A total of 41 genotypes of black gram were evaluated for their resistance to the dry root pathogen *M. phaseolina*. The genotypes CO-5, IPU 07-3, and MASH 1-1 exhibited moderate resistance to the disease and this assessment was conducted using the paper towel method. These genotypes hold potential for the development of resistant varieties in black gram breeding programs [46]. Additionally, soybean cultivars were tested for resistance to charcoal rot caused by *M. phaseolina* under high disease pressure conditions using an artificial cut stem inoculation technique with two aggressive isolates in a poly house screening. The germplasms GNG 2299, H12-24, IPC 2002-31, GNG 1958, IPC 10-134, IPCK 2009-165, PG 0104, GL 2003, IPC 2007-28, H 12-26, BG 0109, and CSJ 515 demonstrated moderate resistance [47].

Using bioagents

Biocontrol agents are the microbial community in the physical environment around the plants that suppresses the growth of the pathogen in the sustained manifestation of interactions. The antagonistic effects of *T. viride* and *T. harzianum* on *M. phaseolina*, which is responsible for charcoal rot in cowpea and mung bean [48]. In the experiment, carriers such as talc and gypsum are utilized alongside *Rhizobium* [49]. The application of *Trichoderma* leads to a 50% reduction in the incidence of *M. phaseolina* in black gram, in conjunction with a *Rhizobium* biofertilizer mixture. Seeds treated with *T. viride* and *Rhizobium* exhibit enhanced nodulation [50]. The dry root rot pathogen has been introduced to the black gram, which has been treated with *P. fluorescence*. The enzymes PAL (phenylalanine ammonia lyase) and PO (polyphenol oxidase) were activated in the plants, and an increase in lignin accumulation was noted. Furthermore, the combination of *T. viride* and neem cake promotes greater shoot length and germination rates, yielding improved outcomes. Additionally, the synergistic effect of *T. viride* and neem cake as a soil amendment contributes to a decrease in disease incidence in the soil [51]. An endophytic isolate of *Klebsiella pneumonia*, designated HR1, was obtained from the root nodules of *Vigna mungo*. This isolate is capable of producing indole-3-acetic acid (IAA), hydrogen cyanide (HCN), and siderophores, which facilitate the solubilization of phosphate and zinc. The soil borne ubiquitious pathogen, *M. phaseolina* is a pot case study, when treated with isolate HR1 increases the shoot and root length, germination percentage compared to greenhouse study. *M. phaseolina* was in least percentage when the isolate of *Klebsiella pneumonia* was applied in the seed bacterization and soil drenching application. Chitinase, phenylalanine ammonia lyase defence enzymes were increased after the application of *Klebsiella pneumonia* [52]. Talc formulation of *T. viride* mixed with zinc and boron suppresses *M. phaseolina* which increases root length, shoot length leads to increase in the yield vigour index [53].

Chemical control

The findings indicate that thiram, vitavax, ceresin, and benlate demonstrate significant efficacy in reducing fungal disease colonization in seeds [54]. Carbendazim at a concentration of 0.2% was particularly effective in mitigating seed-borne diseases in black gram. Furthermore, mancozeb and captan, both at 0.2%, yielded superior results [55]. In

conjunction with carbendazim, captan, propineb, tebuconazole, and the combination of thiram and carbendazim exhibited complete inhibition of *M. phaseolina* growth [56]. Soil drenching with carbendazim at a 0.1% concentration for soybean crops resulted in a minimal incidence of *M. phaseolina*, reducing it by 5.36%, thereby enhancing crop yield. Tebuconazole and trifloxystrobin provided better outcomes compared to other treatments against the pathogen *M. phaseolina* [57].

CONCLUSION

This soil-borne pathogen exhibits heat tolerance and is responsible for diseases in various crops, including black gram, groundnut, soybean, maize, sorghum, and green gram, at any growth stage. Management practices are challenging, and the resulting severe damage to crops can lead to significant yield losses. The application of biocontrol agents such as *Trichoderma* and *Pseudomonas* species, whether as seed treatments, soil applications, or foliar sprays, has proven effective against this pathogen and helps mitigate yield loss. Implementing these practices enhances crop growth and aids in the elimination of inoculum. Additionally, combining phytoextracts with chemical fungicides can reduce disease infection. Furthermore, an integrated disease management approach often emphasizes sustainability.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this paper.

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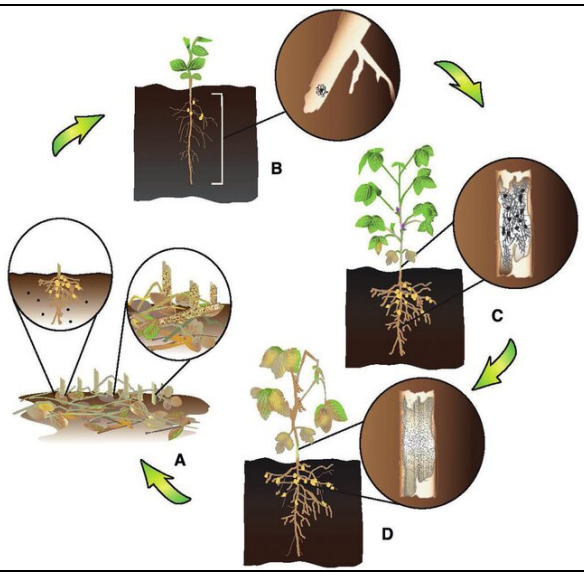


Figure 1. Disease Cycle of *Macrophomina phaseolina* causing Dry Root Rot

a. *M. phaseolina* survives the winter in the form of microsclerotia found within crop residues.
b. Fungi develop within the stem and roots, impairing and disturbing the vascular system.
c. Roots that have become contaminated as a result of contact or close proximity to microsclerotia.
d. The significant presence of microsclerotia on the lower stem and taproot tissue results in a charcoal-like appearance.

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REVIEW ARTICLE

Historical Background Causes. Mode of Transmission, Prevention and Treatment of Plague : A Review

Rajesh Kumar Sharma[1]*, Sachin Kumar[2] and A. Elphine Prabahar[3]

[1]Associate Professor, Department of Pharmacognosy, Teerthanker Mahaveer College of Pharmacy, (Affiliated to Teerthanker Mahaveer University), Moradabad, Guretha, Uttar Pradesh, India.
[2]Associate Professor, Department of Pharmacy, Ganesh Shankar Vidyarthi Memorial Medical College, Kanpur, (Affiliated to Atal Bihari Vajpayee Medical University, Lucknow), Uttar Pradesh, India.
[3]Professor, Department of Pharmaceutical Chemistry, Chettinad School of Pharmaceutical Sciences, Chettinad Hospital and Research Institute, Chettinad Academy of Research and Education (Deemed to be University) Kelambakkam, Tamil Nadu, India.

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***Address for Correspondence**
Rajesh Kumar Sharma
Associate Professor, Department of Pharmacognosy,
Teerthanker Mahaveer College of Pharmacy,
(Affiliated to Teerthanker Mahaveer University),
Moradabad, Guretha, Uttar Pradesh, India.
E.Mail: rajeshsharma7529@gmail.com

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ABSTRACT

The plague is a communicable bacterial disease having a high death rate without treatment. It is known as the Black Death. The Gram negative bacterium *Yersinia pestis* (0.5 to 0.8 pm in diameter and 1 to 3 pm long) that reveals bipolar stain with Giemsa, nonmotile, non-spore-forming coccobacillus is accountable for fatal plague. It is a zoonotic disease well-known in stable foci in the Eurasia, Africa, and Americas. Spread from individual host to a new relies mostly on infected flea bites, bringing typical painful, inflamed lymph nodes referred to as buboes, followed by septicemic spreading of the pathogen. In similarity, droplet inhalation later close interaction with infected mammals encourages primary pneumonic plague. The infrequently stated ingestion of dirty raw meat causes pharyngeal and gastrointestinal plague. Point of attention diagnosis, first antibiotic treatment, and confinement measures contribute to epidemic control despite residual death. Required primary inhibition depend on the active investigation of recognized plague foci and ectoparasite control. Antimicrobial medications given early after the beginning of signs, prevented many deaths, those suggested for treatment and prophylaxis included doxycycline, fluoroquinolones and gentamicin.

Keywords: Plague, *Yersinia pestis*, Black Death, Rat flea

INTRODUCTION

Plague is communicable disease has caused the greatest fear universal, mostly through the three main plague pandemics. The first wave was called the "plague of Justinian" (541 to 750//767) after the emperor of the Roman Empire of the Orient, Justinian [1]. However, the first pandemic has conventionally been believed to be responsible for tens of millions of demises, the exact number of deaths remains unknown, and some studies suggest that the death estimates have been exaggerated and are not supported by the current suggestion [2]. The Second Pandemic recognized as the Black Death owing to the intense cyanosis of the dying, killed roughly 1/4 of the European population and spread to the Middle East and the Far East. The Third Plague Pandemic is began in china in 1850[3]. The present incidences of plague epidemics were of 3248 cases and 584 deaths reported globally between 2010 and 2015[4]. Since 2001 ahead, the WHO has documented 14 chief epidemics, mainly originating from Africa and Asia (https://www.who.int/ news-room/fact-sheets/detail/plague, accessed on 29 April 2025). Consequently, *Y. pestis* is still a public health threat today, producing sporadic infections and rare local epidemics[5-11]. Plague is a severe illness that can result in swollen glands, blood infection, and death. It is caused by bacterium known as *Yersinia pestis* commonly found in small mammals and their fleas**.** Plague also called the Black Death. It is mainly a disease of rodents which are having the infections of bacteria *Y. pestis*. People can spread by the bite of an infected flea. Individuals infected with Y. pestis frequently develop signs after an incubation period of one to seven days Human beings can expose to naturally-occurring plague by

- If plague-infected flea bites the human.
- Touching the infected fluid of the glands.
- Inhalation of small droplets.
- Get exposed to the body of an animal that died from the disease of plague[12].

Prevention of plague from a natural source

- Use gloves and face and eye protection while handling dead bodies.
- Prevent insect bites by using an insect repellent material.
- Remove fleas from residential area.
- Do not touch infected tissues or body fluids from a plague-infected persons or animal.
- Keep a minimum of 3 feet distance between healthy person and patient.
- Maintain hygienic condition.

In the description of sylvatic plague, some mammalian species, especially rodents, are act as primary hosts, and to serve as disease carriers[13, 14].

Causes of plague

It is a serious infectious disease caused due to attack of the bacteria *Y. Pestis*, This bacterium; occur in rates and their fleas. When infected fleas bite animals and humans the transmission occurs, although plague is also transmitted through inhalation, contact, and ingestion of materials which is infected by *Y. Pestis*. Plague has case-fatality ratio of 30%-60% if untreated. As per WHO in the year 2015 there were approximate 320 cases were observed all over the world with approximate 77 deaths[15].

Modes of Transmission of Plague

Bacterium Y. pestis is transmitted to human beings by the bites of a rat fleas or other parasitic insects (those are infected with bacteria *Y. pestis*). The most common form results from the bite of the rat flea (*Xenopsylla cheopis*) or other parasitic insects such as the common louse (*Pediculus humanus*) or the human flea (*Pulex irritants*)

Sometimes infection occurs due to

a. Exposure with infectious tissues or body fluids during handling infected animals.
b. Due to inhalation of droplets of respiratory or other infectious fluid such as laboratory-generated materials containing *Y. pestis*. When infected person feels malaise, shaking chills, headache, myalgia, gastrointestinal symptoms[16, 17].

Plague first infects rodents by their fleas, which can infect human population. It is can be transmitted when infected rodent fleas bite to peoples. It is a zoonotic disease, with can fatal rates of 50-60 percent, if it is not treated[18].

Symptoms of plague

- After infection "Flu-like" symptoms occur.
- Sudden onset of fever.
- Vomiting and nausea.
- Chills
- Weakness
- Hand body-aches.
- Pain
- Advanced stage of infection causes inflammation in lymph nodes, can turn into open sores filled with pus

The incubation period is no more than seven days after a person has been bitten by an infected flea[19, 20].

Main forms of plague

Following are the main forms of plague infection

Bubonic plague It is the most commonly occur and is caused due to the bite of an infected flea (these fleas found on body of rodents). Plague bacillus, *Y. pestis*, enters in human body due to the bite of fleas and reaches to the lymphatic system of the body to the nearest lymph node where it multiplies itself. The lymph node becomes infected, tense and painful condition and is called a "bubo. This type of plague has no inter human transmission.

Septicaemic plague When infection occurs through bloodstream, it is called Septicaemic plague. This type of plague may result from direct contact with infective fluid, bites of flea and from through cracks in the skin. Septicemic plague is fulminant and hazardous in the absence of quick supportive treatment that contains effective antibiotic management (death differs from 30 to 100%, according to the WHO).

Pneumonic plague-or lung-based plague It is the least common form of plague. In this type mainly lungs are infected. Secondary pneumonic spread through droplets to other humans. If pneumonic plague untreated it is 100% fatal. Pneumonic or lung-based plague is the maximum dangerous form and can happen when *Y. pestis* feasts to the lungs[21].

Diagnosis

Diagnosis of plague is confirmed by laboratory testing of samples of fluids from blood or sputum a bubo.

Prevention and control

- Minimize rodent occurrence around home, work place. Make home and outbuildings rodent-proof.
- Wear gloves while handling infected animals to prevent contact between skin and the *Y. pestis.*
- Use repellent material if anyone could be exposed to rodent fleas camping, hiking, or working outdoors.
- Protects pets from flea by using pesticides or products.
- Do not allow pet animal to sleep on the bed.
- Educating public alertness[22].
- Eliminates food and shelter for rodents in and around homes by using insecticides to kill fleas.
- Treatment of pets such as dogs and cats for flea control once a week[23].

The Responsibilities of the Institutions having anti-plague activities

- To determine the area where Plague is in endemic stage.
- To destroy insects around human residential area.
- To eradicate rats in populated areas.
- To immunize human beings at risk.

- To train veterinary staff and medical and public through media.
- Media should play very important role through advertising.

Precautions which protects from droplet

Placement of Patient

Individual room should be allotted to the patient. When separate individual rooms are not available provide partition least two 2 metres between the infected person and other persons. Special type of ventilation should not require, and the door should be open.

Mask

Always wear a mask which protects people from infection.

Patient Transport

Transfer of patient from one place to another should be avoided. If it is must, then dispersal of droplets should be minimize by using mask.

Safety rules for disposal of the bodies of the persons who have died due To Plague

- Post-mortem should be avoided.
- Fallow rules of local authorities which have been undertaken for disposal of dead bodies.
- Funeral ceremonies in the houses of plague patient should be avoided.
- The relatives should not handle dead bodies of plague patient. These dead bodies should be handled by professional with precaution.
- Those peoples who are near to the victim should take chemoprophylaxis.
- Layer of Lime should be kept in the coffins before placing dead bodies in them.
- The dead bodies should be packed in an impervious body bag for transport form one place to the other place.
- In addition to above measures, special precautions as per local culture, religion should be followed by national authorities[24].

Administration of Vaccine

Indications

Vaccination of human beings is applicable only if peoples are exposure to the *Y.pestis*. Thus, vaccination should be useful for peoples in the following high-risk groups:

- Peoples working in laboratory with the presence of *Y. pestis.*
- Professionals like ecologists, mammalogists, and other peoples who have regular contact with wild rodents.

Treatment of Plague

There are some antibiotics and other supportive therapies are used for the treatment of plague if given to the patient properly in time. Patients having Pneumonic plague must be isolated and cared by trained medical staff[25].

Streptomycin

The main treatment is with i.v. or i. m. administration of streptomycin. This antibiotic is useful for the treatment of septicaemic, pneumonic, bubonic types of plague. The drawback of this antibiotic is ototoxicity which is irreversible and unavailability of an oral formulation.

Gentamicin

The main advantage of this antibiotic is it can be taken once a day.

Sulfonamides, Tetracyclines, and Chloramphenicol,

Tetracyclines are bacteriostatic antibiotic with some many side-effects, however they should not be given to woman having pregnancy and to infants. They are used for the treatment of septicaemic plague and bubonic plague and can be taken orally or iv.

Chloramphenicol
Is a bacteriolytic and penetrate cerebrospinal fluid however, it is not used in many cases due to the severe adverse effects like bone marrow suppression and anaemia.

Sulfonamides
Bubonic form of plague can be treated with the use of sulphonamides.

Fluoroquinolones
These are under clinical trial, they are effused only in experimental animals, but there are no studies have been performed in humans[26].

Treatment of plague in pregnant women and in children
Complications of plague in pregnancy can be prevented by correct use of drugs. The aminoglycosides antibiotics normally given to pregnant women and in children, because these are effective and safe for mother, foetus and in children. Because of its safety, i.v or i.m. administration. Gentamicin is also effective.

Modern study on Plague
Ever since the mid of the 20th century several physiological, biochemical and genetic particulars of *Y. pestis* and the reasons that control its virulence have been discovered. In the year 2001 the broad genome sequence of *Y. pestis* containing 4.65 million base pairs and three plasmids has been available by a working group from the United Kingdom[27]. Once relating the chromosomal DNAs from wild-type Y. *pestis* and Y. *pseudo tuberculosis* a very high degree of connection can be found. The chromosomal DNA of *Y. enterocolitica* is not that closely related. Phylogenetically three biovars of *Y. pestis* can be famous: Antiqua, Medievalis and Orientalis, each connected with one pandemic wave[28].

CONCLUSION

Y. pestis is amongst the oldest reported and lethal human pathogens. Plague is transmitted to humans when fleas bite them, due to direct contact with infected animals, or inhalation of infectious respiratory droplets. In mostly, plague can be prevented by using combination of personal protective measures; insecticidal treatment of pets; applications of insecticides to home. Based on the broad scrutiny presented, it is obvious that the plague remains one of the fatal transmittable diseases identified to humankind. The widespread past accounts of its desolating things and the inherited description together with the genomic analysis of its spread offer important insights into the epidemiology of modern epidemics. The Justinian Plague (541 to 750/767) remains controversial as to its demographic, political, and social significances, dissimilar the second pandemic (1346 to 18th century), which killed at least 30% of the European people, made a thoughtful impression on the joint imagination, and led to a deep restructuring of medieval humanity. The death rates resultant from the existing third pandemic (1772 to present) have been extremely low (except at its beginning) due to the chief treatments and the use of antibiotics, which made it possible to decrease the death rate from 50 to 100% in the absence of treatment to approximately 10 to 25%.

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RESEARCH ARTICLE

Phytochemical Analysis of Flowers from *Russelia equisetiformis* Schltdl. and Cham. by UV-VIS, FTIR, LC-ESI-MS and its Antioxidant Power

Kavitha K R[1], Sandhya Ramesh R[2] and Sushama Raj R V[3]*

[1]Associate Professor, Department of Botany, Sree Narayana College, Chempazhanthy, (Affiliated to University of Kerala), Thiruvananthapuram, Kerala, India.
[2]Post Graduate Student, Department of Botany, Sree Narayana College, Chempazhanthy, (Affiliated to University of Kerala), Thiruvananthapuram, Kerala, India.
[3]Associate Professor, Department of Botany, Velu Thampi Memorial Nair Service Society College, Dhanuvachapuram, (Affiliated to University of Kerala), Thiruvananthapuram, India.

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***Address for Correspondence**
Sushama Raj R V
Associate Professor,
Department of Botany,
Velu Thampi Memorial Nair Service Society College, Dhanuvachapuram,
(Affiliated to University of Kerala),
Thiruvananthapuram, India.
E.Mail: drsushamarajrv@gmail.com

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ABSTRACT

Russelia equisetiformis is conventionally utilized as a medicinal plant, recognized for its analgesic, anti-inflammatory, and membrane-stabilizing properties. The current investigation was conducted to evaluate the secondary 2.3 ± 0.07. The ash content was 8.78 ± 0.32%. The carbohydrate content was 3.1% dry weight, respectively. However, the plant exhibited minimal fat content, which may diminish palatability. The overall anthocyanin concentration in this study was 23.65 mg CYE per 100 g. The qualitative UV-VIS spectral profile of the methanol extract was chosen at a wavelength range of 200 to 2500 nm because of the distinct peaks and appropriate baseline. The profile exhibited peaks at 254, 680, and 1044 nm, with corresponding absorptions of 0.524, 0.110, and 0.154, respectively. The FTIR exhibited a large peak at 3418.85 cm-1, indicating the presence of N-H stretching. A pronounced peak was observed at 2856.12 and 2922.66 cm-1, indicating C-H stretching, with a peak at 1244.58 cm-1 corresponding to O-H bending vibrations, and another peak near 1319 cm-1, associated with metabolites analyzed using UV-Vis, FTIR, and LC-ESI-MS, along with an assessment of the antioxidant characteristics of *R. equisetiformis*. The dried flower underwent proximate examination, revealing a moisture content of 5.32 ± 0.02. The crude protein content was 4.2 percent. The lipid content was determined to be 0.6 ± 0.012%. The crude fiber result of 8 cm-1 is attributed to the alkyl group, 1621.67 cm-1 corresponds to alkenyl C=C stretching, 1731.61 cm-1 denotes the stretching vibration of the halo formyl group, the peak at 2346.42 cm-1 signifies the presence

of C≡N stretching, and the peaks at 1376.7 and 1423.96 cm-1 indicate the presence of nitrates and carbonates. The FTIR spectrum validated the existence of alcohols, phenols, alkanes, alkynes, alkyl halides, aldehydes, aromatics, nitro compounds, and amines in the powder particle. The total antioxidant activity measured by AOX characteristics was 92.95, although the DPPH approach demonstrated the highest antioxidant capacity, with 83.1% inhibition at a dosage of 100 µg/mL. The FRAP assay of *Russelia equisetiformis* yielded a result of 70.2% with a 100 µg/mL extract. Phytochemicals in *R. equisetiformis* were identified using liquid chromatography-electrospray ionization-tandem mass spectrometry. This investigation identified phenolic compounds, including Chlorogenic acid at 360 m/s, Methyl protocatechuate at 155.85, 4-Hydroxybenzoic acid at 139.85, Caftaric acid at 314.90, and Catechin at 290.5. The findings demonstrate that the methanolic extract of *Russelia equisetiformis* may serve as a nutraceutical in combating oxidative stress and associated disorders.

Keywords: Anthocyanins, Antioxidant properties, FTIR, LC-MS, Phytochemicals, proximal composition, *Russelia equisetiformis*, UV-Vis

INTRODUCTION

Numerous contemporary non-communicable diseases are associated with oxidative stress, mostly resulting from the imbalance between the production and neutralization of prooxidants. Antioxidants are agents, both natural and manmade, that impede or postpone oxidation processes. Polyphenols constitute a varied array of phytochemicals celebrated for their antioxidant characteristics. *Russelia equisetiformis* Schltdl. and Cham, a prominent ornamental flowering plant formerly categorized within the polyphyletic family Scrophulariaceae, has been reclassified into the monophyletic family Plantaginaceae. It is commonly referred to as *Russelia juncea*, firecracker plant, coral plant, fountain bush, or coral fountain. It is a perennial, pendulous shrub indigenous to Tropical South America, particularly Mexico. [1]. The genus Russelia was named after Scottish naturalist Alexander Russell (1715-1768) by Dutch botanist Baron Nikolaus von Jacquin (1727-1817) [2]. This plant, in addition to its visual allure, has historically been employed in numerous medicinal applications, suggesting its possible therapeutic benefits. Due to the growing interest in natural products with antioxidant properties, a thorough examination of *Russelia equisetiformis* flowers is justified.

MATERIALS AND METHODS

Plant material

The flowers of *Russelia equisetiformis* were taken from their native habitat in Thiruvananthapuram, Kerala. The gathered flowers were washed with double distilled water to eliminate surface debris and subsequently diced into minute fragments. The flowers were permitted to air dry for approximately 7 to 10 days.

Proximate analyses

The proximate analysis of flowers was carried out for moisture content, crude protein, crude fat, crude fiber, total ash, and carbohydrate levels.

Determination of total anthocyanins

Total anthocyanin content from the flowers of *Russelia equisetiformis* was quantified using pH differential method with a two-buffer system - potassium chloride buffer, pH 1.0 (0.025 M) and sodium acetate buffer, pH 4.5 (0.4 M) as described by [3].

Liquid chromatography combined with electrospray ionization mass spectrometry (LCESI-MS)
The methanolic flower extract of *R. equisetiformis* were analyzed by liquid chromatography combined with electrospray ionization mass spectrometry (LCESI-MS).

Free Radical Scavenging Assays
Total Antioxidant Capacity Assay: The total antioxidant capacity of the methanol extract of *Russelia equisetiformis* was measured using the phosphor molybdenum technique as outlined by [4]. The antioxidant activity is quantified as the number of equivalents of ascorbic acid.

DPPH Radical-Scavenging Assay: The DPPH scavenging activity was assessed using a modified spectrophotometric approach based on [5]. The absorbance of DPPH diluted in methanol was designated as the control. The reduction in absorbance was quantified at 517 nm. The antioxidant capacity to neutralize the DPPH radical was determined using the following equation: Scavenging impact (%): [(1 - (absorbance of sample / absorbance of control)) × 100]. Results were presented as Mean ± SD from three experiments conducted in triplicate.

Ferric reducing antioxidant power (FRAP)
The ferric reducing antioxidant power was assessed using the method outlined by [6]. Ascorbic acid served as the reference standard.

UV-VIS and FTIR Spectroscopic analysis
UV-visible spectrophotometric examination of the flower extract from *Russelia equisetiformis* was performed utilizing a UV-visible spectrophotometer (Perkin Elmer, USA Model: Lambda 950) with a slit width of 2 nm, employing a 10-mm cell at ambient temperature. The extract was analyzed under visible and ultraviolet light within the wavelength range of 200 to 2500 nm for proximate analysis. The extract was subjected to centrifugation at 3000 rpm for 10 minutes and subsequently filtered using Whatman No. 1 filter paper for UV-VIS spectrophotometer analysis. The sample is diluted to a ratio of 1:10 using the identical solvent.

Fourier transform infrared (FTIR) The Fourier transform infrared technique was employed to discover the distinctive functional groups present in the flowers of *Russelia equisetiformis*. A little amount of floral extracts was combined with anhydrous potassium bromide (KBr). The amalgamation was meticulously combined in a mortar and subjected to a pressure of 6 bars for 2 minutes to produce a KBr thin disc. The disc was subsequently positioned in a sample cup of a diffuse reflectance accessory. The infrared spectrum was acquired utilizing a Bruker Vertex 70 spectrometer from Germany. The sample was scanned from 4000 to 400 cm^{-1}. The maximum values of the UV-VIS and FTIR were documented.

Statistical analysis
The primary phytochemical and antioxidant studies were conducted in triplicate, and the findings were presented as mean with standard deviation. IC50 values were assessed utilizing Microsoft Excel 2013. The inhibitory percentages for each dosage in each assay were statistically evaluated using one-way analysis of variance.

RESULT AND DISCUSSION

Proximal composition
The proximal composition of the powdered flowers from *Russelia equistiformis* revealed the following constituents: Total carbohydrates: 3.1 ± 0.03; Crude protein: 4.2 ± 0.01; Crude fat: 0.6 ± 0.012 Crude fiber 2.3 ± 0.07 Ash content: 8.78 ± 0.32; Moisture content: 5.32 ± 0.02. The nutrients found in plants include carbohydrates, proteins, and lipids. The flower exhibited a favorable composition of primary metabolites with minimal fat content.

FTIR analysis

The FTIR spectroscopy technique is employed to evaluate the functional groups in the methanolic extract of *Russelia equisetiformis* flowers. The spectral band seen at 3277.39 cm^{-1} (Fig. 1) is attributed to O-H stretching, characterized by prominent and broad peaks, indicating its association with the alcohol group in the plant extract. A peak at around 1604.41 cm^{-1} in the figure indicates the presence of a N¼O stretching group, characteristic of the nitro compound class. The spectral signature of the functional group verifies that the phytocompound in the floral extract acted as a reducing and capping agent. The peak at 1734.45 in the spectrum corresponds to C¼N. Stretching is associated with the imine/oxime group, with the imine group being significant in the floral extracts of the plant *Russelia equisetiformis* due to its chemical synthesis properties. Robust bonds were identified at 3277.39 cm−1, 2917.75 cm−1, and 1734.45 cm−1, but the remaining bonds exhibited weak to moderate strength. The results indicated the existence of hydroxyl groups, lipids, alkanes, amino acids, and phosphorus compounds. The FTIR analysis revealed pronounced C-H out-of-plane bending (loop bend) vibrations associated with a substituted benzene ring, signifying the presence of phenols and flavonoids in the crude extract. Flavonoids are polyphenolic compounds distinguished by two benzene rings connected by a linear carbon chain. The identification of benzenoid chemicals by FTIR spectrophotometry corroborated the results from the phytochemical screening, which revealed the presence of phenols and flavonoids. The amines, imines, alkanes, and phenols identified were regarded as the principal functional groups of bioactive substances. The peak at around 1734–1745 cm−1 was attributed to the C=O ester functional group. This investigation indicates that the peak at 1734.45 cm−1 corresponds to carotenoids and is attributed to the C=O conjugate. The conjugated double bond in carotenoids is identified as the structure responsible for light absorption [7]. A prior investigation indicated that a peak at 1604.41 cm−1 is attributable to chlorophyll and protein content. A variety of approaches exist to identify phytochemical components in plant extracts. Fourier transform infrared spectroscopy (FTIR) is employed to identify functional groups in gaseous, liquid, and solid substances by infrared radiation beams [8]. Hemmalakshmi *et al*. [9] conducted an analysis of the phytochemicals in *Erythrina variegata* utilizing Fourier transform infrared spectroscopy. Rajiv *et al*. [10] employed FTIR to analyze phytochemicals in the fruit extracts of *Myristica dactyloids*. Maobe and Nyarango [11] documented the therapeutic properties of *Urtica dioica* with a Fourier transform infrared spectrophotometer. Pharmawati and Putu Wrasiati [12] conducted phytochemical screening and FTIR spectroscopy on the crude extract of Inhauls acoroides leaves. Pratheeba *et al*. [13] demonstrated the larvicidal, pupicidal, and adulticidal efficacy of *Ocimum gratissimum* leaf extracts and observed comparable peak ranges by FTIR analysis. Li *et al*. [14] examined the characteristics of green tea using FTIR. Mezzomo and Ferreira [7] additionally examined the utilization of FTIR in the analysis of carotenoids. The FTIR spectrum of the flower displays the majority of the distinctive peaks associated with anthocyanin (Table 1). The peak at 2917.75 cm^{-1} corresponds to the asymmetric and symmetric C-H stretching modes, respectively. The spectra exhibits a peak at 1724 cm-1, indicative of the typical C=O stretching vibration of anthocyanins [15][16]. The FTIR spectra of anthocyanins displays distinctive absorption peaks associated with functional groups including O-H (hydroxyl), C-H (alkyl), C=O (carbonyl), C-C (carbon-carbon), C=C (carbon-carbon double bond), and C-O (ether). The absorption peaks facilitate the identification and characterization of the functional groups inside the anthocyanin molecules [17].

Total anthocyanin

The anthocyanin concentration of *Russelia equisetiformis* was quantified as mg CYE per 100 g of dried powder. The overall anthocyanin concentration in this study was 23.65 mg CYE per 100 g. Flowers of numerous species are abundant in polyphenolic chemicals, including flavonoids and anthocyanins, which are recognized for their antioxidant properties. The total anthocyanin concentration can further validate the antioxidant capacity of a sample, as the employed approach incorporates electron transfer reactions. Rafi *et al*. [18] examined the total phenolic, flavonoid, and anthocyanin contents of six Rhododendron species from Indonesia and compared these with their antioxidant properties. The concentrations of total phenolics, flavonoids, and anthocyanins ranged from 11.98 to 48.11 mg equivalent gallic acid per gram of dried powder, 0.78 to 8.92 mg equivalent quercetin per gram of dried powder, and 0.12 to 0.29 mg equivalent cyanidin-3-glucoside per gram of dried powder, respectively. The findings acquired from *Russelia equistiformis* was more noteworthy than that from Rhododendron variants.

UV-VIS spectra of the floral extract

The UV-VIS spectra of the extracts were analyzed to verify the existence of phytocompounds that absorb light in the UV region due to the n–π* electronic transition of aromatic moieties and chromophores [19]. Consequently, absorption peaks in the UV–VIS spectrum signify the existence of alkaloids, flavonoids, phenolic acids, and tannins in plant extracts [20]. The absorption spectrum of the ethanol and methanol extracts of the flowers is presented in Table 2. Numerous different absorption peaks were identified in the spectra. Of those, three correspond to anthocyanins or polyphenols. The initial peak occurred at 206–230 nm, varying according to the solvent employed. The peaks were achieved at shorter wavelengths using the ethanol extracts. The second and third peaks were contiguous, with one identified at 270–293 nm and the other at 314–320 nm. A modest elevation in absorbance has also been noted at 384–422 nm. Gallic acid exhibited two peaks at 216–219 nm and 260–271 nm, but quercetin demonstrated four peaks at approximately 204–219 nm, 256–272 nm, 305–322 nm, and 374–382 nm. The peaks in the ultraviolet region (210–320 nm) validated the existence of polyphenolic chemicals in the flower extracts. Comparable UV-VIS spectra were documented for the extracts of bearberry leaves, *Dillenia pentagyna*, and Physalis [19][20][21]. Research indicates that plant extracts with elevated antioxidant capabilities typically have greater absorbance peaks at 280 and 358 nm [19]. Flavonoids function as UV filters, safeguarding plants from biotic and abiotic stressors. All flavonoids exhibit an absorption peak at 240–290 nm due to conjugated ring A, while those with conjugated rings B and C display an absorption peak at 300–500 nm [22].

Characterization of constituents by LC-MS

In LC-MS, the molecular ion (M+) is generated when a molecule undergoes ionization by the loss of an electron. The m/z of this ion correlates with the molecular weight (MW) of the sample, which is potentially valuable for elucidating the structure. M denotes mass, while Z represents the charge number of ions. ESI MS in negative and positive ion modes. The distinction lies in the fact that the negative ion mode charges the analyte by deprotonation, whereas the positive ion mode charges it through protonation. Small compounds possessing a singular functional group typically yield singly charged ions. In positive ion mode, singly charged ions result from the addition of a proton, whereas in negative ion mode, they arise from the loss of a proton. Conversely, bigger molecules like peptides and proteins possess many charged functional groups. Numerous functional groups create an ionic envelope surrounding the molecule. The various charged states assist in identifying analytes with elevated molecular weights. The LC-MS in both positive and negative ionization modes is illustrated in the figures by aggregating the intensities of mass spectrum peaks from the whole scan (Figures 2, 3, and Table 3).

The Positive ionization shows the following

In the figure, peak 1 had an ion peak at M+ in the mass spectrum, which yielded MS product ions at m/z 366.95 (Relative Intensity 28.62) and peak 2 at 382.350 (Relative Intensity 42.31). The compounds are unidentified in the literature. In the figure, peak 1 had an ion peak at M+ in the mass spectrum, which yielded MS product ions at m/z 202.850 (Relative Intensity 43.30) and peak 2 at 218.800 (Relative Intensity 37.10). The compounds are unidentified in the literature. In the figure, peak 1 had an ion peak at M+ in the mass spectrum, which yielded MS product ions at m/z peak 1 at 276.900 (Relative Intensity 19.90) and peak 2 at 292.900 (Relative Intensity 19.17). The compounds are unidentified in the literature. In the figure, peak 1 had an ion peak at M+ in the mass spectrum, which yielded MS product ions at m/z 526.950 (Relative Intensity 19.69) = acylated Malvidin-3-O-(6″-O-acetyl) glucoside, peak 2 at 622 (Relative Intensity 19.9) = acylated Peonidin-3-(caffeoyl) glucoside. Other m/z compounds are unidentified in the literature.

The negative ionization shows the following

In the figure, peak 1 had an ion peak at M- in the mass spectrum, which yielded MS product ions at m/z 112.8 (Relative Intensity 44.68), peak 2 at 214.75 (Relative Intensity 60.55), peak 3 at 378.85 (Relative Intensity61.55), peak 4 403.85 (Relative Intensity 26.61) = Cyanidin-3-O-arabinoside, and peak 5 at 454.85 (Relative Intensity 16.94) = Petunidin-3-O-arabinoside. Other m/z compounds are unidentified in the literature. In the figure, peak 1 had an ion peak at M- in the mass spectrum, which yielded MS product ions at m/z 216.750 (Relative Intensity 37.68), peak 2 at 224.80 (Relative Intensity 20.1), peak 3 at 226.8 (Relative Intensity 14.63). The compounds are unidentified in the

literature. In the figure, peak 1 had an ion peak at M- in the mass spectrum, which yielded MS product ions at m/z 366.85 (Relative Intensity 24.78), peak 2 at 378.85 (Relative Intensity 61.55), peak 3 at 386.85 (Relative Intensity 18.76). The compounds are unidentified in the literature. In the figure, peak 1 had an ion peak at M- in the mass spectrum, which yielded MS product ions at m/z 403.85 (Relative Intensity 26.61), peak 2 at 454.85 (Relative Intensity 16.94) = Petunidin-3-O-arabinoside, peak 3 at 538.90 (Relative Intensity 5.66) = Malvidin-3-O-(6″-O-acetyl) glucoside. Rest of the m/z compounds is unidentified in the literature. In the present study, some anthocyanin compounds were identified by comparison with reference standards, and others were tentatively characterized based on their retention times and MS spectra referring to the literature Gavrilova *et.al.* [23], Wu and Prior [24], Grace *et.al.* [25] and Saha *et.al.* [26]. 12 major anthocyanin compounds were identified in the flower samples. The identification fractions with their retention time, and molecular formulae were shown below.

Cyanidin glycosides
Non-acylated (6 numbers)
1.418.90 Cyanidin-3-O-arabinoside C20H19O10
2.438.90 Cyanidin-3-O-glucoside C21H21O11
3.454.90 Cyanidin-3-O-galactoside C21H21O11
4.584.00 Cyanidin-3-O-sambubioside C22H29O15
5.622.95 Cyanidin-3,5-O-diglucoside C27H31O16
6.701.15 Cyanidin-3-O-(2″-O-xylosyl)rutinoside C32H39O19
Acylated (3 numbers)
1.454.90 Cyanidin-3-(6-acetoyl)galactoside C23H23O12 and Cyanidin-3-(6-acetoyl)glucoside C23H23O12
2.542.90 Cyanidin 3-(3″-malonoyl)glucoside C24H23O14
3.584.00 Cyanidin-3-(p-coumaroyl) glucoside C30H27O13

Delphinidin glycosides
Non-acylated (1 only)
1. 418.90 Delphinidin-3-O-arabinoside C20H19O11
Acylated (1 only)
1. 523.05 Delphinidin-3-(6-acetoyl) glucoside

Peonidin glycosides
Acylated (1 only)
1. 523.05 Peonidin-3-O-(6″-O-acetyl)glucoside C24H25O12
The LC-ESI-MS analysis of the *Russelia equisetiformis* sample revealed the presence of phytochemicals, including Chlorogenic acid at 360 m/z, Methyl protocatechuate at 155.85 m/z, 4-Hydroxybenzoic acid at 139.85 m/z, Caftaric acid at 314.90 m/z, and Catechin at 290.5 m/z (Figures 2 and 3). The combination of mass spectrometry and liquid chromatography is optimal for the analysis of phenolics present in plants. LC-MS/MS offers the benefit of continuous separation and structural elucidation of molecules. Electrospray ionization is a widely employed technique for LC-ESI-MS/MS analyses. In the ESI negative ion mode, the analysis of small compounds with free carboxyl groups predominantly produces the ion [M-H]-, corresponding to their carboxylate anion [27]. The identification of phenolic components in the extract of *Russelia equisetiformis* was conducted using chromatograms derived from HPLC and ESIMS, with a comparison with existing literature data.

The total antioxidant capacity (TAC)
TAC involved the reduction of Mo(VI) to Mo(V) by the extract, followed by the formation of a green phosphate/Mo(V) complex at acidic pH. It assesses both hydrophilic and lipophilic antioxidants (total antioxidant capability). The findings demonstrate an elevated total antioxidant capacity (TAC), quantified as ascorbic acid equivalents, of the methanol extracts, specifically 92.95 mg/g equivalents of ascorbic acid at a dosage of 100 g/mL (Table 4). This indicates the possible analogous antioxidant components of the methanol extract, since the antioxidant capacity of ascorbic acid serves as a reference standard for comparing plant extracts with potential antioxidant

properties [28]. Anthocyanins are water-soluble pigments that impart blue, purple, and red hues to various fruits and vegetables. Anthocyanins are chemically classified as flavonoids and are glycosides including a sugar moiety and an aglycone unit [29]. Multiple studies suggest that this family of flavonoids may reduce the risk of cardiovascular disease, cancer, hyperlipidemia, and other chronic diseases by the consumption of anthocyanin-rich foods. The TAC data of anthocyanin extract from *Russelia equistiformis* corroborates its medicinal effects. The free radical chain reaction results in lipid peroxidation. Radical scavengers can directly interact with and neutralize peroxide radicals to impede peroxidation chain events [30], which are essential in the development of different illnesses.

DPPH and FRAP assay

The antioxidant capacity differed between the two methodologies utilized in this investigation (Figures 4 and 5). The DPPH technique demonstrated the highest antioxidant capacity, with 83.1% inhibition at a dose of 100 µg/mL. The FRAP method indicated an antioxidant capacity of 70.2% at a concentration of 100 µg/mL. The variations in antioxidant capacity results resulted from the distinct techniques employed in measuring the antioxidant activities of each method. The DPPH method measures antioxidant capacity through the transfer of electrons and hydrogen from antioxidant molecules to the DPPH radical, influenced by the solvent type and the redox potential of the involved chemicals. The DPPH radical possesses a dark purple hue, with a peak wavelength near 517 nm; this color transitions to yellow when receiving an electron or hydrogen from antioxidant molecules, resulting in a stable DPPH molecule [31]. The FRAP method assesses the reducing capacity of antioxidant chemicals in converting Fe3+ to Fe2+, paralleling their efficacy in neutralizing free radicals through electron transfer. The FRAP approach involves the reduction of iron (III) to iron (II) and the subsequent production of the iron (II)-tripyridyltriazine (Fe (II)-TPTZ) complex at low pH [32]. The decolorization of DPPH signifies the free radical scavenging ability of the samples. Notably, in the current study, its activity was markedly greater than that of ascorbate (50 µg/mL resulted in 53.2% inhibition, utilized as the reference). The findings of this study unequivocally indicate that anthocyanin extract from *Russelia equisetiformis* has superior antioxidant activity as measured by DPPH radical scavenging. The sample's ferric reducing power was evaluated, and the results are presented in the figure. The FRAP assay assesses antioxidant potential by measuring the ability of sample extracts to convert ferric tripyridyltriazine (Fe (III)-TPTZ) complexes into ferrous tripyridyltriazine (Fe (II)-TPTZ). The results indicate that the ferric reducing capacity of anthocyanin extract from *Russelia equisetiformis* was measured at 70.2% with a concentration of 100 µg/mL extract. The relationship between antioxidant activities and their ferric ion reduction abilities has been previously documented for specific plant extracts by [33]. The prior study by Oloumi *et al*. [34] shown the efficacy of Hibiscus sabdariffa calyx extract in comparison to the reference antioxidant ascorbic acid, utilizing the DPPH technique. The elevated antioxidant activity noted in Roselle petal extracts is attributed to the presence of polyphenolic components, including phenolic acids, flavonoids, and anthocyanins [35]. The DPPH scavenging method is employed to assess the antioxidant activity of compounds because of its simplicity, rapidity, sensitivity, and reproducibility [29]. The current findings indicate that the extracts are effective free radical scavengers, particularly against peroxy radicals, and may possess the capacity to inhibit lipid autoxidation, potentially offering therapeutic benefits for diseases where lipid peroxidation plays a critical role in pathogenesis. The electron donating capacity, indicative of the reducing power of bioactive substances, is correlated with antioxidant activity. Antioxidants may function as reductants, and the inactivation of oxidants by reductants can be characterized as redox reactions, wherein one reactant is reduced while the other is oxidized. The presence of reductants, including antioxidant compounds in the samples, facilitates the reduction of the Fe3+/ferricyanide complex to its ferrous form. The reducing power of the extracts augmented at higher concentrations, indicating that their electron-donating capacity is concentration-dependent. Salem *et al*. [36] conducted a review on the biological activities and phytochemical composition of Hibiscus species. Sánchez-Moreno *et al*. [37] evaluated the free radical scavenging capacity of several red, rosé, and white wines. Škrovánková *et al*. [38] examined the antioxidant activity and health consequences of prevalent medicinal species.

CONCLUSION

This study aimed to evaluate the phytochemical characterisation of *Russelia equisetiformis* flowers using UV-Vis, FTIR, and LC/MS analysis. Additionally, the extract was evaluated for its antioxidant capabilities. The UV-Vis spectrum exhibited many absorption bands between 200 and 2500 nm, signifying the presence of flavonoids, phenolic compounds, tannins, terpenoids, carotenoids, chlorophyll, and alkaloids. FTIR spectra validated the existence of alkaloids, flavonoids, terpenes, anthraquinones, and phenolic chemicals. FTIR and LC-ESI-MS analyses confirm the existence of several polyphenolic compounds. This work represents the inaugural publication on the floral phytochemical fractionation of *R. equisetiformis*. Additional inquiries into lead compounds and their implications are essential.

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Table 1: FTIR standard Peak position, major functional group and the class.

Peak Position	Group	Class	Peak Position	Group	Class
3584-3700	O-H stretching	alcohol	1626-1662	C=C stretching	alkene
3200-3550	O-H stretching	alcohol	1648-1658	C=C stretching	alkene
3500	N-H stretching	primary amine	1600-1650	C=C stretching	conjugated alkene
3300-3400	N-H stretching	aliphatic primary amine	1580-1650	N-H bending	amine
3310-3350	N-H stretching	secondary amine	1566-1650	C=C stretching	cyclic alkene
2500-3300	O-H stretching	carboxylic acid	1638-1648	C=C stretching	alkene
2700-3200	O-H stretching	alcohol	1610-1620	C=C stretching	α, β-unsaturated ketone
2800-3000	N-H stretching	amine salt	1500-1550	N-O stretching	nitro compound
3267-3333	C-H stretching	alkyne	1465	C-H bending	alkane
3000-3100	C-H stretching	alkene	1450	C-H bending	alkane
2840-3000	C-H stretching	alkane	1380-1390	C-H bending	aldehyde
2695-2830	C-H stretching	aldehyde	1380-1385	C-H bending	alkane
2550-2600	S-H stretching	thiol	1395-1440	O-H bending	carboxylic acid
2349	O=C=O stretching	carbon dioxide	1330-1420	O-H bending	alcohol
2250-2275	N=C=O stretching	isocyanate	1380-1415	S=O stretching	sulfate
2222-2260	C≡N stretching	nitrile	1380-1410	S=O stretching	sulfonyl chloride
2190-2260	C≡C stretching	alkyne	1000-1400	C-F stretching	fluoro compound
2140-2175	S-C≡N	thiocyanate	1310-1390	O-H bending	phenol

	stretching				
2120-2160	N=N=N stretching	azide	1335-1372	S=O stretching	sulfonate
2150	C=C=O stretching	ketene	1335-1370	S=O stretching	sulfonamide
2120-2145	N=C=N stretching	carbodiimide	1342-1350	S=O stretching	sulfonic acid
2100-2140	C≡C stretching	alkyne	1300-1350	S=O stretching	sulfone
1990-2140	N=C=S stretching	isothiocyanate	1266-1342	C-N stretching	aromatic amine
1900-2000	C=C=C stretching	allene	1250-1310	C-O stretching	aromatic ester
2000	C=C=N stretching	ketenimine	1200-1275	C-O stretching	alkyl aryl ether
1650-2000	C-H bending	aromatic compound	1020-1250	C-N stretching	amine
1818	C=O stretching	anhydride	1200-1225	C-O stretching	vinyl ether
1785-1815	C=O stretching	acid halide	1163-1210	C-O stretching	ester
1770-1800	C=O stretching	conjugated acid halide	1124-1205	C-O stretching	tertiary alcohol
1775	C=O stretching	conjugated anhydride	1085-1150	C-O stretching	aliphatic ether
1770-1780	C=O stretching	vinyl / phenyl ester	1087-1124	C-O stretching	secondary alcohol
1760	C=O stretching	carboxylic acid	1050-1085	C-O stretching	primary alcohol
1735-1750	C=O stretchin	esters	1030-1070	S=O stretching	sulfoxide
1735-1750	C=O stretching	δ-lactone	1040-1050	CO-O-CO stretching	anhydride
1745	C=O stretching	cyclopentanone	985-995	C=C bending	alene
1720-1740	C=O stretching	aldehyde	960-980	C=C bending	alkene
1715-1730	C=O stretching	α, β-unsaturated ester	885-895	C=C bending	alkene
1705-1725	C=O stretching	aliphatic ketone	550-850	C-Cl stretching	halo compound
1706-1720	C=O stretching	carboxylic acid	790-840	C=C bending	alkene
1680-1710	C=O stretching	conjugated acid	665-730	C=C bending	aklene
1685-1710	C=O stretching	conjugated aldehyde	515-690	C-Br stretching	halo compound
1690	C=O stretching	primary amide	500-600	C-I stretching	halo compound
1640-1690	C=N stretching	imine / oxime	860-900	C-H bending	1,2,4-trisubstituted
1666-1685	C=O stretching	conjugated ketone	860-900	C-H bending	1,3-disubstituted
1680	C=O stretching	secondary amide	790-830	C-H bending	1,4-disubstituted
1680	C=O stretching	tertiary amide	790-830	C-H bending	1,2,3,4-tetrasubstituted
1650	C=O stretching	δ-lactam	760-800	C-H bending	1,2,3-trisubstituted
1668-1678	C=C stretching	alkene	735-775	C-H bending	1,2-disubstituted
1665-1675	C=C stretching	alkene	730-770	C-H bending	monosubstituted
1665-1675	C=C stretching	alkene	680-**720**		Benzene derivatives

Table 2: UV-VIS spectra of the floral extract of *Russelia equistiformis flowers.*

Wavelength (nm)	Absorbance
2500	0.582434
2400	0.451067
2300	0.442077
2200	0.372655
2100	0.441016
2000	0.352334
1900	0.312737
1800	0.23512
1700	0.239004
1600	0.258887
1500	0.295471
1400	0.205659
1300	0.196228
1200	0.245262
1100	0.295289
1000	0.379153
900	0.483901
700	0.897499
600	1.167755
500	1.359528
400	1.502328
300	1.560395
200	1.307921

Table 3: The Phytoconstituents are Evaluated on the Basis of their Registered Mass Spectra M/Z Databases Except the Constituents Indicating with Superscript * Are Proposed on the Basis of their Molecular Mass.

Sl. No.	Proposed Compounds	RT (Min)	MW observed	Ionization Mode	Theoretical (m/z)	Relative intensity (%)
1.	Kaempferol 4′-methyl ether	0.79	298.90	$[M-H]^+$	301.0707	7.51
2.	3′,4′,5,7-tetramethylquercetin	0.79	360	$[M+H]^+$	359.1082	11.77
3.	Xanthosine 5′-monophosphate	0.79	364.90	$[M+H]^+$	365.0498	100
4.	6 ethoxy-3 (4′hydroxyphenyl)-4 methyl coumarin	2.73	292.90	$[M+H]^+$	296.0806	19.17
5.	Sodium salt of 6 ethoxy-3 (4′hydroxyphenyl)-4 methyl coumarin*	2.73	316.95	[M+H]	318.1048	5.56
6.	Phytol sodium*	2.73	316.95	$[M+H]^+$	318.3079	5.56
7.	α-D-Glucose-1,6-diphosphate potassium salt hydrate	2.73	338.2	$[M]^+$	340.2924	10.29
8.	Myricetin sodium*	2.73	340.85	[M-H]	340.0375	13.84
9.	Phytol disodium*	2.73	342.85	[M-H]	340.3079	12.21
10.	Myricetin disodium*	2.73	360	$[M+H]^+$	362.0375	11.7
11.	Catalpol*	2.73	360	$[M]^+$	362.1212	11.7
12.	Myricetin trisodium*	2.73	384.90	$[M+H]^+$	384.1375	9.98
13.	Campestanol*	2.73	404.85	[M+H]	384.3861	6.6

14.	Astringin	2.73	404.85	[M+H]	406.1263	6.6
15.	Catalpol trisodium*Or Cycloartanol	2.73	438.9	$[M+H]^+$	428.2212428.4018	11.82
16.	Myricetin-3-o-Galactoside	2.73	454.9	$[M+H]^+$	481.0982	12.23
17.	Dihydroquercetin-glucosyl-rhamnoside*	2.73	606	$[M+H]^+$	613.1162	13.56
18.	3-O-methylgallic acid	3.11	180.8	[M-H]	185.0445	9.57
19.	6methylflavonol sodium salt*	3.61	268.75	[M-H]	274.0786	6.78
20.	Kaempferol	3.61	288.8	$[M-H]^+$	287.2700	6.17
21.	3 cyclopentylpropionic acid*	3.89	214.75	[M-H]	213	60.55
22.	Dihydroquercetin	4.65	314.9	$[M+H]^+$	304.0583	5.99
23.	Dihydromyricetin3-O-xyl*	4.80	454.85	[M-H]	467.1000	16.94
24.	Delphinidin 3-O-xyl*	4.90	438.9	$[M+H]^+$	437.1000	11.82

Abbreviations used as; Rha- rhamnosyl, mal-malonyl, gly-glycoside and xyl-xyloside

Table 4: Total Antioxidant Activity from anthocyanin extract of *Russelia equistiformis*

Concentration	(Total antioxidant activity mg/g equivalents of ascorbic acid)
10	38.4 ± 0.0321
20	61.1 ± 0.165
40	74.2 ± 0.246
60	83 ± 1.309
80	90.2 ± 0.897
100	92.95 ± 0.427

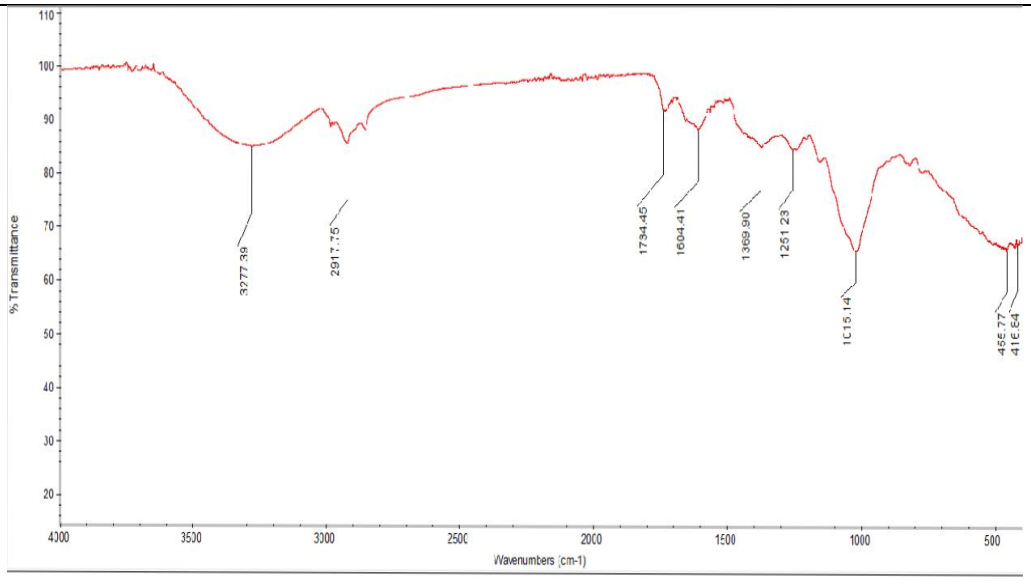


Figure 1- FTIR of *Russelia equisetiformis* flower methanolic extract

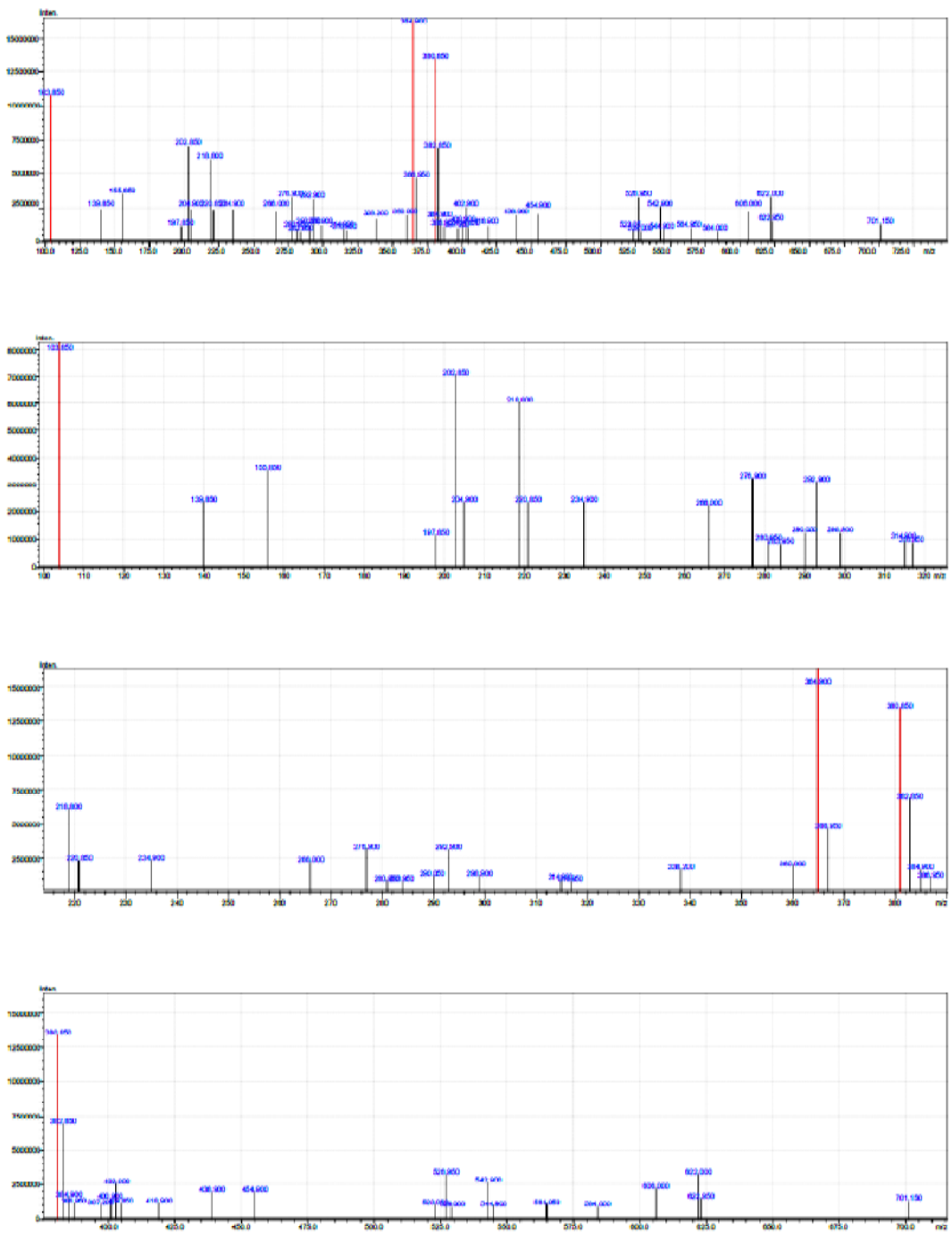


Figure 2: The chromatogram of *Russelia equisetiformis* flower analyzed by liquid chromatography (M+H)

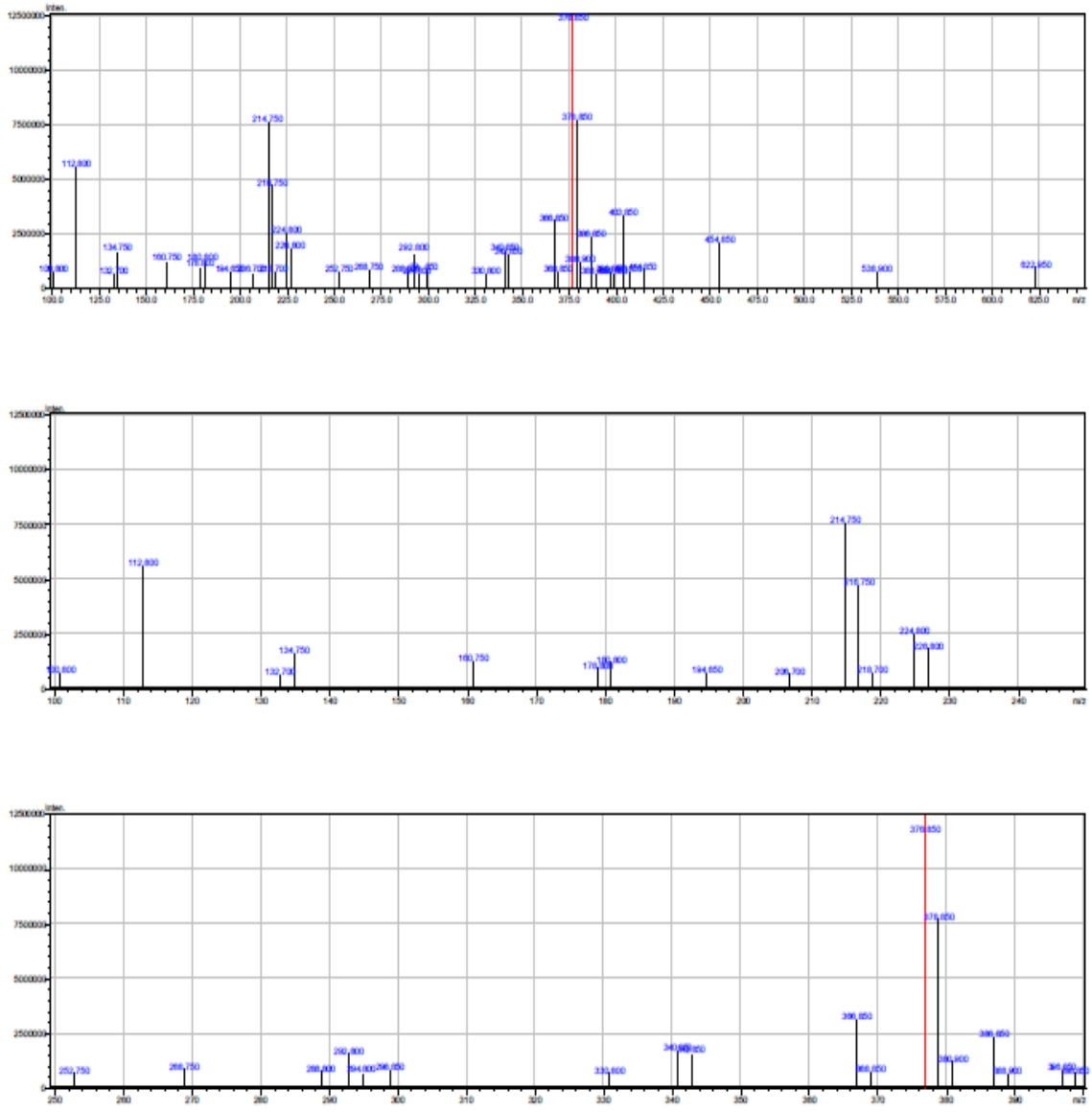


Figure 3: The chromatogram of *Russelia equisetiformis* flower analyzed by liquid chromatography (M-H)

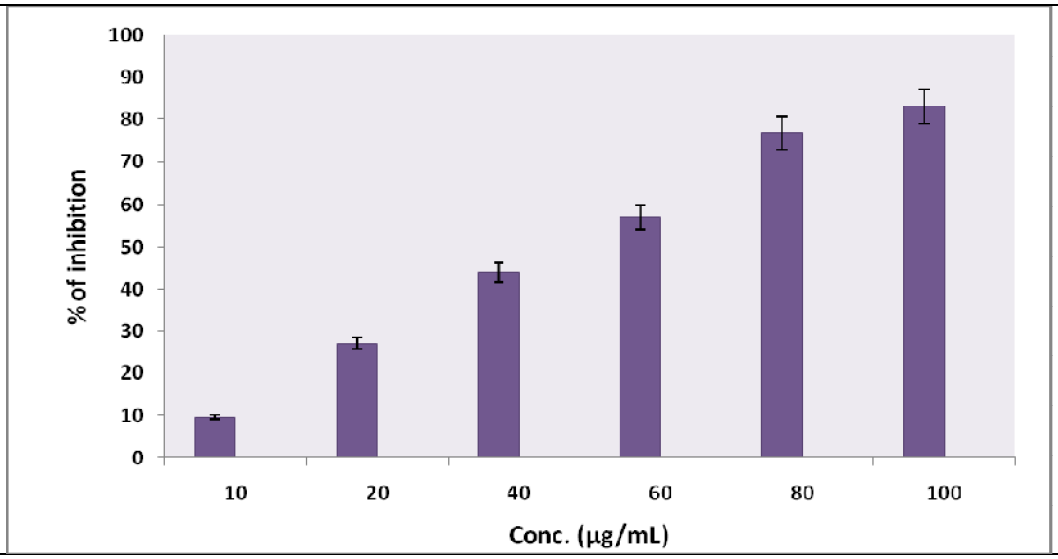


Figure 4: DPPH radical scavenging potentiality from various concentrations from anthocyanin extract of *Russelia equisetiformis*

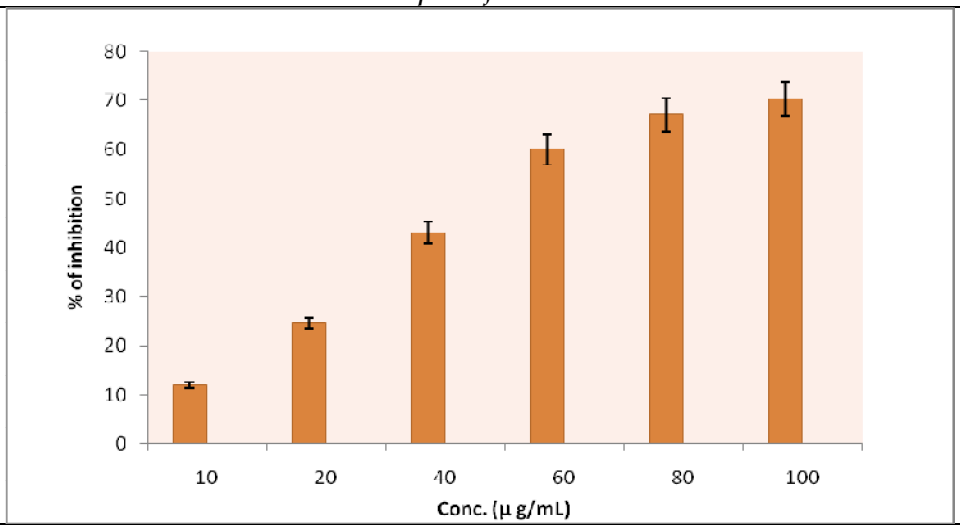


Figure 5: FRAP scavenging potentiality from various concentrations from anthocyanin extract of *Russelia equisetiformis*

RESEARCH ARTICLE

Comprehensive Phytochemical and GC-MS Profiling of *Withania somnifera* Extracts: A Comparative Analysis of Root, Stem, Leaf and Seed Constituents

Neha Singh[1]*, Nisha Singh[2] and Anita R J Singh[3]

[1]Ph.D Research Scholar, Department of Biotechnology, Women's Christian College, (Affiliated to University of Madras), Chennai, Tamil Nadu, India.
[2]Academic Consultant, Department of Academic Consultant, Central Institute of Educational Technology, NCERT, New Delhi, India.
[3]Associate Professor, Department of Biotechnology, Women's Christian College, (Affiliated to University of Madras), Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Neha Singh
Ph.D Research Scholar,
Department of Biotechnology,
Women's Christian College,
(Affiliated to University of Madras),
Chennai, Tamil Nadu, India.
E.Mail: neha0128singh@gmail.com

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ABSTRACT

Withania somnifera, the scientific name for Ashwagandha, is a long-used medicinal herb with a 3,500-year history. Well known for its healing qualities, it has been used for ages to treat a variety of illnesses, such as diabetes mellitus, ulcers, stress, inflammation, sleeplessness, and TB. It has also been used to strengthen the immune system. To find the existence of important secondary metabolites, a thorough qualitative analysis of a variety of plant extracts was done for the current study. These constituents play a pivotal role in augmenting the medicinal worth and therapeutic capacities of Ashwagandha, hence accentuating its significance in contemporary phytotherapy. Thirty different peaks were found during GC-MS analysis, and several noteworthy chemicals were identified. Among these, methylparaben, 1-Carbomethoxy-3,3-dimethyldiaziridine, 3-Amino-2-oxazolidinone, and 3-Deoxy-D-mannoic lactone show antibacterial qualities. 2,5-Dimethylfuran-3,4(2H,5H)-dione is an antifungal chemical. Additional compounds that have been identified include dihydro-5-propyl-, 5-heptyldihydro-, 2(3H)-furanone, and (S)-5-Hydroxymethyl-2[5H]-furanone. These compounds exhibit a variety of biological activities, including those that are antibacterial, antifungal, antioxidant, antiulcer, anti-TB, analgesic, anti-inflammatory, anticancer, and anticonvulsant. Furthermore, acetamide and other derivatives of 4H-Pyran-4-one exhibit anti-inflammatory qualities. The formation of phosphomycins is known to require

the precursor cis-4-hydroxycyclohexanecarboxylic acid, whereas 1.alpha, or 1,2,3,5-cyclohexanetetrol, functions as a glycosidase inhibitor.

Keywords: *Withania somnifera,* antibacterial, antifungal, antioxidant, antiulcer, and anti- TB.

INTRODUCTION

The three doshas- Pitta, Kapha, and Vata—are balanced by plant secondary metabolites to preserve health. These metabolites are essential for the treatment of many illnesses. For example, the compounds in *Withania somnifera* (ashwagandha) support vigor and stress. Supporting Systemic Functions: By encouraging optimal operation and correcting imbalances, they also support the digestive, respiratory, and circulatory systems—three essential bodily systems. In Ayurvedic medicines, combining various plant metabolites can improve their bioavailability and medicinal effects. *Withania somnifera* (L.) Dunal, popularly known as Ashwagandha or Indian ginseng, is a significant medicinal herb utilized in Ayurvedic, Unani, and various other traditional medical systems. The extensive therapeutic uses, including anti-inflammatory, antistress, antioxidant, immunomodulatory, and neuroprotective properties, have been thoroughly established and ascribed to a diverse array of secondary metabolites (Singh *et al .*, 2011[1]; Mir *et al .*, 2021[2]). Different components of the plant, such as roots, leaves, stems, and seeds, are utilized in both traditional medicine and modern herbal preparations. The plant is recognized for its diverse array of phytochemicals, including alkaloids, flavonoids, saponins, tannins, steroids, phenolics, terpenoids, and withanolides, which substantially enhance its therapeutic effectiveness (Saleem *et al .*, 2020[3]). Alkaloids and withanolides are acknowledged for their anti-cancer, adaptogenic, and anxiolytic properties (Mishra *et al .*, 2000[4]; Dar *et al .*, 2015[5]). Phytochemical screening, especially by solvent extraction techniques, is essential for the identification and isolation of these molecules. The selection of solvent markedly affects the type and quantity of bioactives extracted, dependent upon the polarity and solubility of the phytochemicals (Sasidharan *et al .*, 2011[6]). While several studies have identified significant phytochemicals in *Withania somnifera*, the majority have concentrated predominantly on the roots, with few comparable evaluations of other plant components, including stems, leaves, and seeds. Due to the rising global demand for plant-derived therapeutic medicines, a thorough phytochemical investigation of all significant plant components is crucial to fully exploit the medicinal potential of *Withania somnifera*. This study is to conduct a solvent-based comparative phytochemical analysis of the roots, leaves, stems, and seeds to ascertain the distribution patterns of bioactive compounds. The results will establish a significant basis for forthcoming pharmacological, nutraceutical, and product development initiatives related to *Withania somnifera*.

MATERIALS AND METHODS

For Biochemical analysis

Plant extracts**,** hexane, Whatman's filter paper, Test tubes, HCl solution, funnel, picric acid, acetic acid, Glass pipettes, FeCl3. Finally, H_2SO_4, methanol, liquid ammonia, chloroform, oil, and distilled water.

Qualitative estimation of Various Biochemical constituents in different plant parts root, leaves, bark, and seeds

Phytochemical Screening

standard methods were used for qualitative phytochemical analyses of *Withania somnifera* root extracts, as reported by Edeoga *et al .* (2005)[7], Harborne (1998)[8], Sofowora (1993)[9], and Trease and Evans (1989)[10]. Below are the steps used to find different phytoconstituents:

Fehling's Test for Reducing Sugars

About 0.2 g of powdered plant material was mixed with 1 mL of solvent. We added 3 mL of distilled water and 1 mL each of Fehling's solutions A and B to this. The mixture was heated, and the appearance of a brick-red precipitate showed that there were reducing sugars.

Benedict's test for non-reducing sugars, a few drops of hydrochloric acid were added to one milliliter of plant extract and cooked. The solution was neutralized with sodium bicarbonate when it had cooled down. Then, Benedict's reagent was added. When heated again, the colour changed to yellow or orange, which meant that there were non-reducing carbohydrates present.

Starch (Iodine Test)

A few drops of iodine solution were added to 0.5 mL of plant extract. The blue-black tint that formed showed that starch was present.

Phenolic Compounds (Ferric Chloride Test)

We boiled one gram of plant material with a solvent for ten minutes and then filtered it. We added a few drops of a 1% ferric chloride solution to the filter. The presence of phenolic chemicals was shown by a dark green or blue tint.

Cardiac Glycosides (Keller–Kiliani Test)

Two milliliters of plant extract were combined with 1.5 mL of glacial acetic acid and 1 mL of ferric chloride solution. Concentrated sulfuric acid was meticulously introduced along the perimeter of the test tube. The emergence of a brown ring at the contact signified the existence of cardiac glycosides.

Flavonoids (Ammonia Test)

Approximately 200 mg of powdered botanical material was extracted using a solvent. Three milliliters of weak ammonia solution were added to the extract. The yellow hue observed upon standing signified the presence of flavonoids.

Saponins (foam test)

0.5 grams of powdered material were boiled in 10 mL of solvent for 10 minutes and subsequently filtered. Five milliliters of the filtrate were combined with 2.5 mL of distilled water and agitated vigorously. Continuous foaming signified the existence of saponins. The incorporation of three drops of a saturated oil solution, accompanied by agitation, led to the creation of an emulsion, therefore corroborating the presence of saponins.

Alkaloids (Picric Acid Test)

Approximately 0.1 g of the powdered material was combined with 2 mL of hexane, thoroughly agitated, and subsequently filtered. The filtrate was subjected to treatment with 3 mL of 2% hydrochloric acid, gently heated, and subsequently filtered once more. A little quantity of picric acid was included into the final solution. The emergence of a yellow precipitate signified the existence of alkaloids.

Antioxidant Activity (Phosphomolybdenum Assay)

Ten milligrams of methanolic plant extract were solubilized in 10 mL of distilled water. One milliliter of 0.6 M sulfuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate was combined in a beaker, and the total volume was adjusted to 50 mL with distilled water. Thereafter, 0.3 mL of the extract and 3 mL of the reagent were incubated at 97 °C for 90 minutes. The change to a green hue signified the existence of antioxidant chemicals.

Salkowski Test for steroids

Two milliliters of plant extract were combined with two milliliters of acetic acid, followed by the meticulous addition of 2 to 3 drops of pure sulfuric acid. The emergence of a blue-green ring signified the existence of steroids.

Tannins test (Ferric Chloride Test)

About 0.1 g of the plant sample was boiled in 4 mL of solvent and subsequently filtered. A little quantity of 0.1% ferric chloride was introduced to the filtrate. The emergence of a brownish-green or blue-black hue signified the existence of tannins.

Phlobatannins
An aqueous extract of the material was subjected to boiling with 1% aqueous hydrochloric acid. The emergence of a crimson precipitate at the base of the test tube signified the presence of phlobatannins.

Terpenoids (Salkowski Test)
Three milliliters of plant extract were placed in a dry test tube and combined with 2 mL of ferric chloride solution, followed by the addition of several drops of pure sulfuric acid. The emergence of a reddish-brown hue validated the existence of terpenoids.

Anthraquinones (Bornträger's Test) Two milliliters of aqueous plant extract were combined with 4 mL of concentrated sulfuric acid and subjected to heating. Subsequent to boiling, 3 mL of chloroform was added. The chloroform layer was isolated and transferred to a separate test tube containing 1 mL of diluted ammonia (1:1). The ammonia layer had a pink to violet hue, indicating the presence of anthraquinones.

For GCMS analysis of aqeous extract of *Withania somnifera*
Plant Material Collection and Authentication
Root samples of *Withania somnifera* including both wild and cultivated varieties—were collected from various regions across India to ensure broad genetic representation. The taxonomic identity of the plant specimens was authenticated by Dr. P. Jayaraman, Ph.D., Director of the Plant Anatomy Research Centre (PARC) and former Professor of Botany, Presidency College, Chennai. A voucher specimen was deposited at the PARC Herbarium under the registration number PARC/2021/4565. All collected accessions were subsequently cultivated under controlled field conditions in Chennai, Tamil Nadu, to ensure uniform growth for biochemical and genetic analyses.

Preparation of Aqueous Root Extract
Freshly collected roots of *Withania somnifera* were thoroughly washed with distilled water to remove any adhering soil or debris. The cleaned roots were shade-dried at ambient temperature until constant weight was achieved and then pulverized into a fine powder using a mechanical grinder. For extraction, 20 g of root powder was loaded into a Soxhlet apparatus and extracted with double-distilled water for 6–8 hours until exhaustion. The resulting aqueous extract was concentrated under reduced pressure using a rotary evaporator at 40–45°C to remove excess water. The concentrated extract was then stored at 4°C in airtight containers for further phytochemical and GC–MS analysis. Prior to GC–MS analysis, the extract was diluted using sterile double-distilled water and filtered through Whatman filter paper No. 41 to obtain a clear, particle-free solution. This filtration step ensured that no particulates interfered with chromatographic detection and facilitated accurate identification of bioactive compounds.

GC–MS Analysis of *Withania somnifera* Root Extract
The concentrated aqueous root extract of *Withania somnifera* was further diluted with sterile double-distilled water and filtered through Whatman filter paper No. 41 to obtain a particle-free solution suitable for Gas Chromatography–Mass Spectrometry (GC–MS) analysis. This analysis was conducted to identify the bioactive phytochemical constituents present in the sample.

Instrumentation and Chromatographic Conditions
GC–MS analysis was performed using a Shimadzu NX Series system, comprising a Nexis GC-2030 gas chromatograph coupled with an AOC-20i automatic injector. The system was equipped with a SH-I-5Sil MS capillary column (30 m length × 0.25 mm internal diameter × 0.25 μm film thickness). A split injection mode was used with a split ratio of 10:1, and helium served as the carrier gas at a constant flow rate of 2.0 mL/min**.**
The oven temperature program was configured as follows

- Initial temperature**:** 35°C, held for 2 minutes
- Ramp: 20°C/min to 450°C
- Final hold**:** 5 minutes at 450°C

Mass Spectrometry Conditions Mass spectrometric detection was carried out using a metal quadrupole mass analyzer connected directly to the GC column. The system operated in Electron Ionization (EI) mode. Spectral data acquisition and instrument control were performed using GCMS Solution Software Version 2.6**.**

Two modes of resolution were applied
Low-resolution spectra
Resolving power**:** 1000 (at 20% peak height definition)
Scan range: m/z 25–1000
Scan rate**:** 0.3 seconds per scan
Inter-scan delay: 0.2 seconds

High-resolution spectra
Resolving power: 5000
Scan range: m/z 65–1000
Scan rate: 1 second per scan

Compounds Identification
Phytochemical constituents were identified by comparing the mass spectral data to those in the NIST Mass Spectral Library Version 11**,** embedded within the instrument software. Further confirmation of compound identities was performed using GC-MS-based metabolomics**,** incorporating retention index comparisons for enhanced accuracy.

RESULT AND DISCUSSION

Phytochemical analysis of *Withania somnifera* root sample
The roots contained several bioactive chemicals, with significant quantities of alkaloids detected in various solvents, including chloroform, diethyl ether, and ethanol. Antioxidants were very abundant in methanol, with modest concentrations detected in ethanol and aqueous solutions. Cardiac glycosides had substantial detection in ethanol, although their presence in other solvents was negligible (Table: 1). Flavonoids were detected in moderate concentrations in methanol, ethanol, and ethyl acetate. Saponins were significantly present in ethanol (++), whereas steroids showed the greatest quantity in chloroform (+++). We detected phenols in ethanol, but not in other solvents. Anthraquinones were prominently detected in water, methanol, and ethanol solutions. We consistently identified reducing sugars, tannins, and terpenoids in many solvents, with terpenoids being most prevalent in aqueous and methanol solutions.

The phytochemical analysis of *Withania somnifera* leaf samples
Revealed the presence of various bioactive compounds extractable in different solvents (Table: 2). Alkaloids demonstrated a significant presence in diethyl ether (++), whereas their presence was moderate in aqueous and ethanol solutions. Antioxidants were identified in methanol, ethanol, and petroleum ether. Ethanol exhibited a notable presence of cardiac glycosides (++), and flavonoids were strongly detected (++), with moderate levels found in aqueous and methanol extracts. Phenols were extensively detected in multiple solvents, especially in petroleum ether and ethyl acetate, where their presence was significantly pronounced (++). Saponins were identified in both aqueous and ethanol extracts, whereas chloroform exhibited a notable presence of steroids (++). Phenolatannins were significantly identified in both methanol and ethanol. All solvents tested consistently identified tannins and reducing sugars. Terpenoids were abundant in methanol and found in moderate concentrations in other solvents. Aqueous media exhibited a significant concentration of anthraquinones, while other solvents identified them at moderate levels.

The phytochemical analysis of *Withania somnifera* stems

Indicated the presence of alkaloids in all solvents, with the exception of petroleum ether. Significant concentrations were identified in water, methanol, and ethanol. Antioxidants were detected in methanol and ethanol (Table 3). Ethanol exhibited a significant presence of cardiac glycosides, whereas aqueous, methanol, and ethanol solvents reliably identified flavonoids and phenols. Saponins were significantly present in ethanol (++), while steroids showed the highest concentration in chloroform (+++), followed by ethanol and ethyl acetate (++). Multiple solvents reliably identified anthraquinones, tannins, and reducing sugars in moderate amounts. Terpenoids demonstrated significant abundance (+++) in chloroform, with moderate levels observed in alternative solvents.

Phytochemical analysis of *Withania somnifera* seed samples

Seeds represent the reproductive units of angiosperms, encompassing the embryo along with a nutrient reservoir. Their structure is surrounded by a protective seed coat. They are essential for plant propagation and the maintenance of genetic diversity. The seeds exhibited notable phytochemical activity across different solvents (Table 4). Alkaloids were identified in all solvents, with the exception of petroleum ether. Antioxidants were identified in both methanol and ethanol, whereas cardiac glycosides exhibited strong detection in ethanol. Flavonoids were consistently detected in aqueous, methanol, and ethanol extracts. Phenols were detected at moderate levels in methanol and ethanol, whereas Saponins exhibited a strong presence in ethanol (++). Steroids demonstrated the highest concentration in chloroform (+++), followed by ethanol and ethyl acetate (++). Anthraquinones were identified at moderate concentrations in both ethanol and chloroform. Reducing sugars, Tannins, and Terpenoids were consistently identifiable. Terpenoids were identified in significant quantities (+++) in chloroform.

GCMS Analysis of root aqueous extract of *Withania somnifera* (Fig.1)

The phytochemical analysis of *Withania somnifera* aqueous root extract in this study identified thirty unique chemical peaks, signifying a diverse array of bioactive chemicals (Table- 5). These findings indicate a complex phytochemical composition, corroborating the plant's extensive medicinal uses documented in traditional medicine. Among the discovered chemicals, 3-Amino-2-oxazolidinone, 3-Deoxy-D-mannoic lactone, methylparaben, and 1-Carbmethoxy-3,3-dimethyldiaziridine are recognized for their antibacterial properties, consistent with other observations about the antimicrobial efficacy of *Withania somnifera* root extracts. Specifically, 2,5-Dimethylfuran-3,4(2H,5H)-dione was identified, a chemical recognized for its antifungal properties, hence reinforcing the ethno pharmacological use of the plant against fungal diseases. Moreover, many furanone derivatives, including 2(3H)-Furanone, 5-heptyldihydro-, 2(3H)-Furanone, dihydro-5-propyl-, and (S)-5-Hydroxymethyl-2[5H]-furanone, were recognized. These chemicals are shown to exhibit extensive biological activity, including antibacterial, antifungal, antioxidant, antiulcer, anti-tubercular, analgesic, anti-inflammatory, anticancer, and anticonvulsant properties. Their presence may substantially enhance the many pharmacological effects associated with *Withania somnifera*. Furthermore, the identification of acetamide, 2-(1-pyrrolidinyl)-N-(3-chlorophenyl)-, in conjunction with 4H-Pyran-4-one, 2,3-dihydro-3,5-di-, and 4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-, corroborates prior research emphasizing their anti-inflammatory properties. These findings indicate a potential function in regulating inflammatory pathways, rendering them intriguing subjects for more pharmacological research. Another significant molecule found was cis-4-Hydroxycyclohexanecarboxylic acid, an essential precursor in the manufacture of phosphomycins, a category of polyketide antibiotics. This suggests a potential antibacterial mechanism intrinsic to the plant's phytochemical composition. Furthermore, 1α,2,3,5-Cyclohexanetetrol, noted for its glycosidase inhibitory properties, was also identified, indicating possible anti-diabetic uses via enzyme inhibition. The results together indicate that *Withania somnifera* aqueous root extract has a variety of bioactive substances exhibiting several pharmacological characteristics. The discovery of both established and new bioactive highlights the therapeutic potential of the plant and endorses its ongoing application in traditional and contemporary pharmaceutical formulations. Subsequent research should concentrate on the isolation, measurement, and confirmation of compounds' biological activity via in vitro and in vivo experiments to thoroughly clarify their modes of action and therapeutic effectiveness.

CONCLUSION

The extensive phytochemical analysis of *Withania somnifera* identified a diverse array of bioactive compounds present in its roots, leaves, stems, and seeds, extracted using various solvents. Alkaloids were extensively detected, with notably elevated amounts in root and stem extracts utilizing ethanol, methanol, and chloroform. Antioxidants exhibited a considerable affinity for polar solvents, including methanol and ethanol, in all plant sections, suggesting their potential function in mitigating oxidative stress. Ethanol repeatedly proved to be an effective solvent for the extraction of cardiac glycosides, flavonoids, saponins, and phenolic chemicals, particularly from roots and seeds. Methanol and aqueous extracts exhibited substantial terpenoid content, however chloroform was particularly successful in separating steroids and terpenoids, especially from stem and seed samples. Anthraquinones and tannins were reliably identified in the majority of solvents, with aqueous and methanolic extracts exhibiting greater affinity. Each plant component had a distinct phytochemical profile, indicating tissue-specific production and storage of secondary metabolites. The root and seed extracts exhibited the greatest overall phytochemical variety and richness, underscoring their medicinal potential. These findings not only corroborate the conventional use of *Withania somnifera* but also establish a scientific basis for the precise extraction and development of phytopharmaceutical products. Subsequent study should to concentrate on measuring these components and assessing their pharmacological activity by bioassay-guided investigations. This research also offers significant insights into the GCMS phytochemical makeup of *Withania somnifera* root aqueous extracts. GCMS Phytochemical profiling revealed thirty bioactive chemicals, some of which had notable pharmacological effects, including antibacterial, antifungal, antioxidant, anti-inflammatory, and anticancer actions. The existence of chemicals like furanones, pyranones, and glycosidase inhibitors reinforces the plant's historical medicinal uses and indicates its promise for novel drug discovery and development. The phytochemical data reinforces the basis for conservation, selective breeding, and bioprospecting of *Withania somnifera*.

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Table 1: Qualitative analysis of various Phytochemicals of *Withania somnifera* Roots sample

Test	Aqueous medium	Methanol	Ethanol	Chloroform	Diethyl ether	Petroleum ether	Ethyl acetate
Alkaloid	+	-	+	+	+	-	-
Antioxidants	+	++	+	+	-	+	-
Cardiac glycoside	+	-	++	-	-	-	-
Flavonoid	-	+	+	-	-	+	+
Phenol	-	-	+	-	-	-	-
Saponin	+	+	++	-	-	-	-
Steroid	+	++	+	+++	+	-	+
Phlobatannin test	-	+	+	-	-	-	-
Reducing Sugar	+	+	+	-	-	-	-
Anthraquinones test	++	++	++	+	-	-	+
Tannins	+	+	+	-	-	-	-
Terpenoids	+++	+++	+	++	+	+	+

Table 2: Qualitative analysis of various Phytochemicals of *Withania somnifera* leaves sample.

Test	Aqueous medium	Methanol	Ethanol	Chloroform	Diethyl ether	Petroleum ether	Ethyl acetate
Alkaloid	+	-	+	-	++	-	-
Antioxidants	-	+	+	-	-	+	-
Cardiac glycoside	+	+	++	-	+	+	+
Flavonoid	+	+	++	-	-	+	-
Phenol	+	+	+	+	-	++	++
Saponin	+	-	+	-	-	-	-
Steroid	-	-	+	++	+	-	+
Phlobatannin test	+	++	++	-	-	-	-
Reducing Sugar	+	+	++	+	-	-	-
Anthraquinones test	++	+	+	+	-	-	-
Tannins	+	+	+	+	-	-	+
Terpenoids	-	++	+	+	+	-	+

Table 3: Qualitative analysis of various Phytochemicals of *Withania somnifera* Stem.

Test	Aqueous medium	Methanol	Ethanol	Chloroform	Diethyl ether	Petroleum ether	Ethyl acetate
Alkaloid	+	+	+	+	+	-	+
Antioxidants	-	+	+	-	-	-	-
Cardiac glycoside	-	-	++	-	+	-	-
Flavonoid	+	+	+	-	-	-	-
Phenol	-	+	+	-	-	-	-
Saponin	+	+	++	-	-	-	-
Steroid	-	+	++	+++	+	+	++
Phlobatannin test	-	+	+	-	-	-	-

Reducing Sugar	+	+	+	+	-	-	-
Anthraquinones test	-	-	+	+	-	-	-
Tannins	+	+	+	-	-	-	-
Terpenoids	+	+	++	+++	++	+	+

Table 4: Qualitative analysis of various Phytochemicals of *Withania somnifera* Seeds sample.

Test	Aqueous medium	Methanol	Ethanol	Chloroform	Diethyl ether	Petroleum ether	Ethyl acetate
Alkaloid	+	+	+	+	+	-	+
Antioxidants	-	+	+	-	-	-	-
Cardiac glycoside	-	-	++	-	+	-	-
Flavonoid	+	+	+	-	-	-	-
Phenol	-	+	+	-	-	-	-
Saponin	+	+	++	-	-	-	-
Steroid	-	+	++	+++	+	+	++
Phlobatannin test	-	+	+	-	-	-	-
Reducing Sugar	+	+	+	+	-	-	-
Anthraquinones test	-	-	+	+	-	-	-
Tannins	+	+	+	-	-	-	-
Terpenoids	+	+	++	+++	++	+	+

Table 5: Chromatographic profile showing thirty peaks corresponding to identified bioactive compounds.

S. No.	Ret. Time	m/z	Area	Compound Name	A/H	Event
1.	3.487	TIC	2124679	1,3-Dioxol-2-one,4,5-dimethyl-	6.307	utilized as an additive in the electrolyte of lithium-ion batteries. **[11]**
2.	3.725	TIC	6539743	4-Heptanone, 2-methyl-	7.241	utilized to frighten other ants. [12]
3.	3.970	TIC	13150434	2,5-Dimethylfuran-3,4(2H,5H)-dione	5.292	It is a metabolite found in human urine, a fumigant, a fuel, a bacterial metabolite, an antifungal agent, and a Maillard reaction product.[13]
4.	4.192	TIC	790109		4.244	
5.	4.347	TIC	1086562	Cyclohexanone, 3-hydroxy-	4.651	utilized to induce and sustain general anesthesia[14].
6.	4.630	TIC	578641	Acetamide, 2-(1-pyrrolidinyl)-N-(3-chlorophenyl)-	7.874	utilized to alleviate inflammation and pain [15].
7.	4.818	TIC	3994302	3-Amino-2-oxazolidinone	5.323	This compound is used to treat depression and as an antimicrobial

						agent [16].
8.	5.068	TIC	36112950	4H-Pyran-4-one, 2,3-dihydro-3,5-di	6.020	Because it can eliminate free radicals, it has antioxidant qualities.[17]
9.	5.410	TIC	988232	(S)-5-Hydroxymethyl-2[5H]-furanone	5.714	It contains antibacterial and antifungal, antioxidant, antiulcer and anti-TB, analgesic and anti-inflammatory, anticancer, anticonvulsant, and more.[18]
10	5.499	TIC	1071141	(S)-5-Hydroxymethyl-2[5H]-furanone	3.348	It possesses antibacterial, antifungal, antioxidant, antiulcer, anti-TB, analgesic, anti-inflammatory, anticancer, anticonvulsant properties, and more.[18]
11	5.670	TIC	777301	4H-Pyran-4-one, 3,5-dihydroxy-2-methyl-	3.413	Used as a Protective Agent (anti oxidant) [19]
12	5.745	TIC	2147156	2(3H)-Furanone, dihydro-5-propyl-	5.200	It possesses antibacterial, antifungal, antioxidant, antiulcer, anti-TB, analgesic, anti-inflammatory, anticancer, anticonvulsant properties, and more.[20]
13	6.186	TIC	6308923	2(3H)-Furanone, 5-heptyldihydro-	5.714	It possesses antibacterial, antifungal, antioxidant, antiulcer, anti-TB, analgesic, anti-inflammatory, anticancer, anticonvulsant properties, and more.[20]
14	6.404	TIC	72574882	5-Hydroxymethylfurfural	5.559	utilized in the food business as a flavoring agent and biomarker for food goods.[21]
15	6.662	TIC	9940112	1,2,3-Propanetriol, 1-acetate	5.209	utilized in lotions and ointments as a humectants [22]
16	6.899	TIC	1971250	1,2,3-Propanetriol, 1-acetate	5.953	utilized in lotions and ointments as a humectants [22]
17	7.362	TIC	11261277	3-Octanol, acetate	12.980	This substance functions as a pheromone, a flavoring agent, a human metabolite, and a plant metabolite.[23]
18	7.710	TIC	990399	Maltol	6.390	Often used in flavours, but it also serves as a "sweetener" and enhancer of flavor [24].
19	7.820	TIC	1496100	cis-4-Hydroxycyclohexanecarboxylic	4.443	initial ingredient in the production of phosphomycins, which are antibiotics of the polyketide type[25].
20	7.984	TIC	1439312	1-Carbmethoxy-3,3-dimethyldiaziridi	3.256	utilized as a broad-spectrum antiviral and antibacterial drug [26]
21	8.407	TIC	1537998	1,3-Dioxocane, 2-pentadecyl-	7.397	utilized in polyurethane, textile/leather, personal care, pharmaceutical, coatings and adhesives, and synthetics. [27]

22	8.629	TIC	404065	Phenol, 2,6-dimethoxy-	4.514	It is metabolite of a plant [28]. Used in nut, coffee, liquorice, tea, spice, savory, meat, shellfish, and smoky tastes [29].
23	8.782	TIC	1213497	8-Azabicyclo[3.2.1]octan-3-ol,8-met	4.403	It is an alkaloid [30] , used in plant extracts and natural flavoring for the food and beverage, cosmetic, and personal care industries [31].
24	9.234	TIC	979482	Butane, 1,1'-[methylenebis(oxy)]bis	6.539	utilized as emulsifying and cleaning agents [32] ,
25	10.219	TIC	1270666	(Z),(Z)-2,5-Dimethyl-2,4-hexadiened	3.034	It results in 9,10-dichloroanthracene photodechlorination.[33]
26	10.569	TIC	8489732	Methylparaben	2.787	Used as a preservative in toothpaste to stop microorganisms from growing [34].
27	11.060	TIC	15184227	2,2,3,3-Tetramethylcyclopropanecarb	15.191	The sponification reaction of a 2, 2, 3, 3-tetramethyl cyclopropane carboxylic acid ester with sodium hydroxide is carried out using this catalyst as a phase transfer. [35]
28	14.617	TIC	45112814	1,2,4,5-Cyclohexanetetrol, (1.alpha.,2	28.651	It is an important intermediate in the synthesis of voglibose.. [36]
29	11.487	TIC	29002276	1,2,3,5-Cyclohexanetetrol, (1.alpha.,	29.456	As an inhibitor of glycosidase. [36]
30	13.985	TIC	55969756	3-Deoxy-d-mannoic lactone	43.182	It exhibits antimicrobial properties. [37]

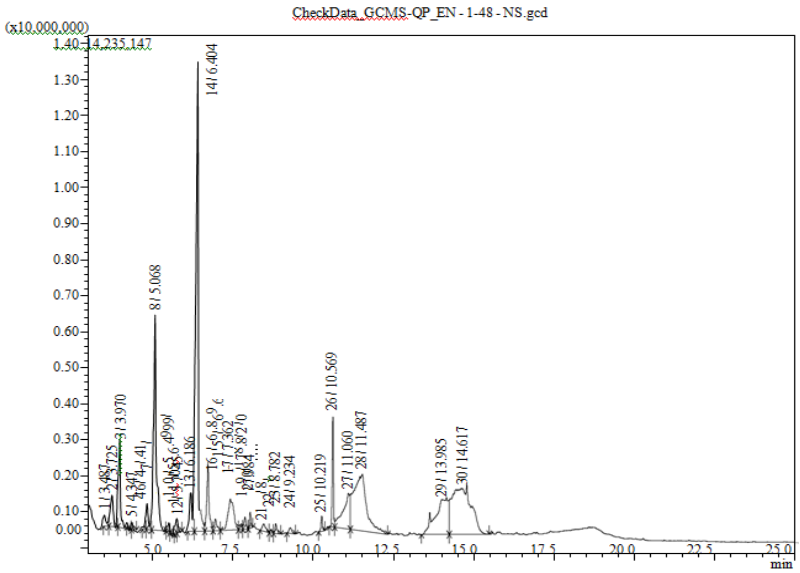


Figure 1: GC-MS Chromatogram of *Withania somnifera* Aqueous Root Extract

REVIEW ARTICLE

Mastic Gum: A Review on Nature's Proven Anticancer Agent

Pallabi Sarkar[1] and Biswa Prasun Chatterji[2*]

[1]Ph.D.Scholar, Department of Food, Nutrition and Dietetics, Assam Down Town University, Panikhaiti, Guwahati, Assam, India.
[2]Professor, Faculty of Science, Assam Down Town University, Panikhaiti, Guwahati, Assam, India.

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***Address for Correspondence**
Biswa Prasun Chatterji
Professor,
Faculty of Science,
Assam Down Town University,
Panikhaiti, Guwahati, Assam, India.
E.Mail: biswaprasun@gmail.com

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ABSTRACT

Throughout history, plants and herbs have been utilised for their therapeutic benefits. Natural herbal remedies are appealing substitutes for cancer therapy because of their therapeutic potential and low side effects. Numerous substances originating from plants, such as polyphenols, demonstrated lethal effects on rapidly proliferating cancer cells, indicating their potential as anticancer medicines. One such substance is mastic gum (MG), which is the dried resin produced by *Pistacia lentiscus* var. chia plant. MG has demonstrated encouraging anticancer properties against a number of cancer types, including colon, breast, prostate, and oral cancer. Research has shown that the essential oil of MG and its effects on cancer cell lines are both pro-apoptotic and antiproliferative. The anticancer mechanism includes downregulation of inflammatory pathways, induction of apoptosis, suppression of cell migration and proliferation, and modification of cell cycle regulation. Furthermore, encasing MG essential oil in nanoparticles has increased the oil's cytotoxic efficacy against cancer cells while reducing damage to healthy cells. MG is a natural substance with potential uses in the prevention and treatment of cancer, according to the majority of the current research. This study attempts to summarise research on MG's anticancer properties and comprehend its cytotoxic effects on different cancer cells.

Keywords: *Pistacia lentiscus*, mastic gum, anticancer agent, chemopreventive

INTRODUCTION

Plants and herbs have been used for their medicinal properties since ancient times. Many important drugs have been developed deriving the natural products from them. Since natural herbal products have therapeutic potential with

least to no side effects, hence they have emerged as attractive alternative treatments despite the remarkable evolution of medicine in recent times. In fact, several chemical compounds isolated from medicinal plants have shown to cause cytotoxic effect on rapidly dividing cells, hence revealing their considerable potential to be developed as anticancer agents (Rahman *et al.* 2018). Polyphenolic compounds like flavonoids, curcumin, tannins, resveratrol and gallacatechins are all considered to have anticancer potential and are compounds widely found in plants. Flavonoids itself is a class of diverse compounds largely distributed in grains, fruits, vegetables, tea, soybean and play a vital role for the treatment of large number of cancer. Curcumin is another leading phytochemical extracted from *Curcuma longa* (turmeric) having cytotoxic property over the growth of human glioblastoma cells. Tannins, the water soluble polyphenols are present in grapes, green tea and coffee. Resveratrol can be found in foods including peanuts, grapes and red wine. Gallacatechins are present in green tea. It is also believed that these polyphenols if included in the diet, can improve health as well as reduce risk of cancers due to their antioxidant properties (Grenwell and Rehman, 2015, Iqbal *et al.* 2017, Subramanian *et al.* 2019, Youness *et al.* 2021, Gahtori *et al.* 2023). Although various plants and their polyphenolic compounds have been investigated for their effect on cancer cells, yet there are more that have caught the attention of researchers for further studying their anticancer drug potential. One of them on the spotlight is mastic gum which needs thorough investigations and clinical trials for proving itself from having anticancer compounds to have effective anticancer activities. Chios mastic gum (CMG) or Mastiha is the dried exudate that forms as a resin and is produced by the plant *Pistacia lentiscus* var. chia, cultivated exclusively in Mediterranean and Middle Eastern countries. CMG originally is secreted as a colloidal clear liquid which then solidifies after 15- 20 days into various shapes. The solidified CMG appears as a crystalline shape of teardrops and falls to the ground under the tree. The material on the ground is collected by hand and further processed for packaging and use. The hardness of the CMG depends on the atmospheric temperature, exposure time in the open, and the size of the product (Dimas *et al.* 2012, Gortzi *et al.* 2022). Since CMG and its essential oils possess natural antimicrobial agents, they have been used extensively in Mediterranean and Middle Eastern countries not only as a dietary supplement but herbal remedy as well (Loizou *et al.* 2009, Li *et al.* 2011, Paraskev *et al.* 2016). CMG is one of the finest natural products having several phytochemical properties playing a remarkable role in treating diseases like gastrointestinal problems, inflammation, decreasing anxiety and stress, memory enhancement, antioxidant behavior and anti-inflammatory activity (Dadras *et al.* 2019, Im *et al.* 2017). Certain studies have reported that it is also known to have anticancer properties and can contribute in treating or preventing certain types of cancer (Shafiei *et al.* 2015, Li *et al.* 2011, Rahman 2018, Simitcioglu *et al.* 2018). These reports on CMG strongly support it to be potential solution for cancer treatment. This study aims at understanding the use and cytotoxic effect of mastic gum on cancer cells and summarizes the work done on their anticancer activities till date.

Mastic Gum: The resin with potential anticancer compounds

Cancer, among a range of diseases at tissue level, have emerged as the second greatest global health threat, with an estimated 10 million cancer deaths reported in 2020 (Sung, H. *et al.* 2021). Cancer treatment has always been a highly complex process. Some of the treatment modalities commonly used for cancer is chemotherapy, hormone therapy, hyperthermia, immunotherapy, photodynamic therapy, radiation therapy, stem cell transplant, surgery, targeted therapy and adjuvant therapy (De Souza and Chatterji, 2015, Debela *et al.* 2021). Although recent development in cancer studies has contributed in treatment options, yet the treatments have certain unavoidable side effects. Specific side effects of cancer treatment depend on different variables like tumour type, disease stage, severity and length of treatment. Patient variables such as age, co-morbidities, baseline health and functional status also may play a role (Üstündagˇ and Zencirci, 2015, Niedzwiedz *et al.* 2019, Wang *et al.* 2021, Alessy *et al.* 2022). In recent years, researchers have begun to focus on locally available plants in an effort to find sources of natural substances that might be used medically, particularly because of their potential to treat cancer. One of these natural components is CMG, the resin of *Pistacia atlantica* with the local name of Baneh (Rahbar *et al.* 2016, Fitsiou *et al.* 2019) CMG and its essential oil are a complex mixture of different bioactive compounds. The most researched group of Pistacia species components are the essential oils and both qualitative and quantitative differences in terms of chemical composition were found to be substantial. The chemical components of essential oils can be divided into two groups as terpene hydrocarbons (monoterpenes, sesquiterpenes and triterpenoids) and the oxygenated compounds, primarily phenols, aldehydes, ketones, esters, lactones, coumarins, ethers, and oxides. Monoterpene content is high in mastic gum,

whereas α-pinene (~80%) is the chief essential oil of CMG. Other compounds detected in their essential oils are β-Pinene, camphene, myrcene, and limonene. The significant sesquiterpene present in mastic gum is β-caryophyllene, while the major monoterpene alcohol is linalool (Hadjimbei *et al.* 2015, Pachi *et al.* 2020, Tabanca *et al.* 2020). Major bioactive compounds of mastic gum and their essential oil (Fig. 1). Due to presence of different phytochemical groups as well as phenolic compounds, CMG is associated with biological activities, like antimicrobial, antioxidant, anti-inflammatory and anticancer characteristics (Dimas *et al.* 2012, Paraschos *et al.* 2012, Moeini *et al.* 2019). Due to its ability to stop low-density lipoprotein (LDL) oxidation, mastic gum was suggested for cardiovascular protection (Pachi *et al.* 2020). By avoiding dental caries, chewing mastic gum showed antibacterial action against Streptococcus mutans that was substantial (Aksoy *et al.* 2006). Due to its pleasant aroma and beneficial effects, mastic gum has a notable reputation in food and pharmaceutical items. For instance, it is used in milky desserts and sweets, such as Turkish delight, halva, ice cream, yoghurts, candies and chewing gum, as well as in alcoholic beverages like soumatha and some specialty coffees that have a mastic flavour. Additionally, cosmetic formulations like soap, body lotions, shampoos, toothpastes, mouthwashes, and surgical adhesive strips use mastic gum components (Freedman 2011, Paraskevopoulou *et al.* 2016, Tabanca *et al.* 2020). Although compared to the fruit and leaves, Pistacia resins are poor in phenolic compounds, yet phenolic acids, specifically, p-hydroxyphenylacetic, vanillic, gallic, and trans-cinnamic acids are the main phenolic compounds in the gum. The triterpenoids present in mastic gum represent different skeleton types- the tetracyclic euphane- and dammarane skeleton type and the pentacyclic oleanane and lupane skeleton type. The main non-volatile natural products reported in the literature that have been isolated from the gum are: a) masticadienonic acid, b) tirucallol, c) oleanolic acid, d) isomasticadienonic acid, e) 3-o-28-norolean-12-en, f) 20(S)-3β-acetoxy-20-hydroxydammar-24-en, g) 3-oxo-dammara-20(21),24-diene, h) 3β-hydroxymalabarica-14(26),17E,21-triene, i) 3-oxo-malabarica-14(26),17E,21-triene, j) 3-β-hydroxy-28-norolean-12-en, k) 3-oxo-28-norlup-20(29)-en, l) (8R)-3-Oxo-8-hydroxy-polypoda-13E,17E,21-triene, m) 1,4-poly-β-myrcene (He *et al.* 2007, Hadjimbei *et al.* 2015, Assessment report on *Pistacia lentiscus* L., resin (mastix), 2015).

Supporting this, studies have reported that high content of polyphenol compounds and presence of triterpenoids are mainly responsible for the anticancer potential of mastic gum has (Hadjimbei *et al.* 2015, Rahman *et al.* 2018, Fitsiou *et al.* 2019). Ramot *et al.* 2018, conducted a study aimed to assess the systemic toxic potential of RPh201, a mastic gum extract formulated for neurological indications, administered to minipigs for 39 weeks followed by a 6-week recovery period. Treatment-related findings at injection sites included abscesses, chronic inflammation, and subcutaneous fibrosis, with marked severity abscesses in the high-dose group considered adverse. Minimal-to-slight subcutaneous and lymph node abscesses in control and lower dose groups were related to the vehicle (cottonseed oil) and not considered adverse. This study determined the no-observed-adverse-effect level of RPh201 to be 12.5 mg/kg/occasion. Studies have also shown effects of orally administered mastic oil in mice by inhibition of specific cytochrome P450 enzymes in various tissues, with no significant weight loss or toxicity observed in tests. The leaves, gums and oil of *Pistacia lentiscus* can be used in the treatment of various skin, respiratory, and gastrointestinal disorders and they also exhibit effective antiviral and cytotoxic activity (Attoub *et al.* 2014, Boke *et al.* 2023). The assessment of diet supplements' potential as anticancer agents in vivo is subject to several limitations due to the broad range of cancer types, their distinct clinical courses, and their underlying signalling. Additionally, the co-administration of cytotoxic drugs creates additional challenges. But, there is a lot of scientific interest in using food-related ingredients as an adjuvant to treat certain cancers, with the goal of improving the effectiveness and lowering the toxicity of traditional therapies. Mechanism of mastic gum extract inducing cell growth inhibition and apoptosis (Fig. 2). Several studies conducted till date have proven the fact that mastic gum components can exert anticancer activities via various mechanisms, including the induction of apoptosis and autophagic pathways activation, angiogenesis inhibition, and downregulating inflammatory mediators against breast cancer, colorectal cancer, prostate cancer, lung carcinoma and oral cancer. The hexane extract of mastic causes HCT116 human colon cancer cells to undergo apoptosis in vitro via a caspase-related mechanism. A key factor in the onset and spread of prostate cancer, mastitis attenuates the expression and activity of the androgen receptor. Furthermore, mastic suppresses the advancement of the cell cycle in prostate cancer cells via suppressing NF-κB activity and the NF-kB signalling pathway (He *et al.* 2006, He *et al.* 2007, Balan *et al.* 2007). Mastic oil has also been shown in vitro to have both proapoptotic and antiproliferative effects on human K562 leukaemia cells. Additionally, it was discovered to inhibit

the vascular endothelial growth factor, which reduced the in vivo and in vitro production of microvessels from mouse melanoma cells (Loutrari *et al.* 2006). Mastic gum extracts also shows significant antimicrobial activity. In a study by Gortzi *et al.* 2022, liposome encapsulation enhanced stability and absorption efficiency and both the extracts-acidic mastic gum extract—AMGE—and the neutral one—NMGE, resulted in effective inhibition of liver cancer cell growth. NGME extract exhibited the highest potency against cancer cells and the results supported potential chemopreventive uses of mastic extracts. Studies have also examined leaves and gums from different aged trees and analyzed their chemical components which resulted in exhibited effective antiviral and cytotoxic activity.

Mastic Gum against breast cancer

Breast cancer is a significant public health concern, and the search for effective and safe therapeutic options continues to be a priority in oncological research. Natural products, such as plant extracts and their bioactive compounds, have garnered increasing attention as potential anticancer agents due to their structural diversity and potential to selectively target cancer cells. The anticancer effects of mastic gum and its essential oil have been investigated in various studies. Mastic gum has been reported to exhibit antiproliferative and pro-apoptotic properties in breast cancer cell lines, suggesting its potential as a therapeutic agent. The mechanisms underlying these effects may involve the modulation of cell cycle regulation and the induction of apoptosis in cancer cells. Furthermore, the essential oil extracted from mastic gum has also demonstrated potent anticancer activities against breast cancer cells, potentially through the inhibition of cell proliferation and the induction of cell cycle arrest. Additionally, the anticancer properties of mastic gum and its essential oil may be enhanced through the use of delivery systems, such as polymeric nanoparticles, which can improve the bioavailability and targeted delivery of the active compounds to the tumor site. This approach has been explored in various studies, with promising results in terms of improving the anticancer efficacy and reducing the side effects associated with conventional chemotherapeutics (Najjari *et al.,* 2022, Mateos *et al.,* 2022). Rezae *et al*. (2011) reported in one of their studies that the T47D human breast cancer cells were able to undergo apoptosis and stop proliferating when exposed to the pericarp methanolic extract of *Pistacia atlantica* (locally known as Baneh). The same research team's 2012 investigation sought to determine whether or not Baneh extract may affect the cell cycle progression of T47D cells. Flow cytometry and western blot analysis methods were used for the study, respectively, to assess the distribution of the cell cycle's regulatory proteins and to investigate any potential effects of Baneh extract on the cell cycle of T47D cells. The study reported that Baneh extract substantially correlated with exposure time-dependent G0/G1 cell cycle arrest, cyclin D1 and cdk4 expression reduction, and G0/G1 cell cycle arrest. Parallel to this, Dox-treated T47D cells accumulated in the S phase at early time points, but after 48 h cell cycle progression was blocked on the G2/M stage. Dox accelerated the remarkable accumulation of cyclin B1 and increased the amount of cyclin A. Overall, the findings showed that cell cycle arrest and downregulation of cyclin D1 and cdk4 expression play a role in the anticancer activity of the Baneh pericarp extract. In 2016, Pasban-Aliabadi *et al.* conducted a study to investigate the cytotoxic and anti-proliferative properties of mastic gum with and without combining with doxorubicin at different doses against breast cancer. Cytotoxicity of the gum was determined using MTT assay on MCF-7 human breast cancer cells. Western blotting was used for evaluating the cellular makers of apoptosis (caspase3 and P53) and cell proliferation (Cyclin-D1). Doxorubicin at doses of 10 nM and 200 nM was used as anticancer control drug in combination treatment. The result showed that mastic gum (100 μg/mL) significantly induced cell damage, activated caspase3, and increased P53 protein level. Additionally, Cyclin-D1 was significantly decreased in gum-incubated cells. Moreover, mastic gum (25 μg/mL) combined with doxorubicin (200 nM) for treatment of cells produced a significant cytotoxic effect compared to each drug alone. The study concluded that mastic gum extract has a potential anti-proliferative and anti-apoptotic property in MCF-7 cells and can be a potent modality to treat breast cancer. In a study conducted by Fahmy *et al.,* 2023, a green extraction technique was used to extract the essential oil from the resins of P. lentiscus, or chios mastic. GC-MS analysis was used to establish the chemical composition of P. lenticus essential oil (PO), which was subsequently encapsulated into a niosomal formulation using a thin-film hydration process. The produced PO/Ns had exceptional negative surface charge, low PDI, high EE%, nanoscale size (less than 120 nm), and amazing stability. Additionally, they demonstrated the imprisoned PO's sustained release behaviour over the course of 72 hours. Without harming normal breast epithelial cells, the produced PO/Ns' cytotoxic efficacy against breast and ovarian cancer cells significantly outperformed that of pure PO. PO/Ns showed maximum cytotoxic effect against cancerous

cells while posing little to no hazard to healthy cells. Following cell cycle study, it was discovered that PO/Ns had a great ability to stop the cell cycle, trap cells in the sub-G1 phase, and promote apoptosis. In another study by Alabrahim and Azzazy, 2024, using GC-MS, the chemical makeup of the isolated essential oils of *Pistacia lentiscus* (PLEO) was ascertained. To explore the anticancer and potential synergistic effects of PLEO and 5-Fluorouracil (5FU) on triple-negative breast cancer cells (MDA-MB-231), human adenocarcinoma breast cancer cells (MCF-7), and human skin melanoma cell line (A375), the compounds were electrospun into poly-ε-caprolactone nanofibers (PCL-NFs), with a diameter ranging from 290.71 nm to 680.95 nm. The synthesised nanofibers (NFs) demonstrated exceptional tensile strength and physical integrity along with improved thermal stability. According to biodegradability tests, the NFs' use as a localised therapy for melanoma or breast tissues (postmastectomy) is supported by their prolonged stability over a 42-day period. Studies on release demonstrated sustained release patterns over 168 hours, with more levels of 5FU and PLEO released at pH 5.4, suggesting enhanced targeting skills towards cancerous areas. NFs that were loaded with PLEO exhibited significant antioxidant capabilities. Lastly, when compared to free chemicals, NFs loaded with either PLEO or 5FU showed stronger anticancer activity. When PLEO and 5FU were co-loaded into NFs, the strongest anticancer effects were seen. According to the study's findings, the created 5FU-PLEO-PCL-NFs have the potential to be used locally to treat melanoma and breast cancer tissues (after mastectomy) in order to reduce the likelihood of recurrence.

Mastic Gum against prostate cancer

Prostate cancer is one of the most prevalent forms of cancer affecting men worldwide. Several studies have investigated the anticancer activity of mastic gum and its essential oil against prostate cancer. Mastic gum has been shown to exhibit antiproliferative and proapoptotic effects in prostate cancer cell lines, suggesting its potential as an effective anticancer agent. Additionally, the essential oil of mastic gum has been found to suppress the migration and invasion of prostate cancer cells, which are key processes in metastasis (Giaginis and Theocharis, 2011). Furthermore, the polyphenolic compounds in mastic gum, including gallic acid, have been shown to inhibit the migration and invasion of prostate cancer cells, suggesting their potential utility in preventing metastasis. The therapeutic potential of mastic gum and its essential oil for prostate cancer is further supported by their ability to selectively target cancer cells without significantly affecting the viability of normal cells, a crucial characteristic for an effective and safe anticancer agent (He *et al.* 2006, He *et al.* 2007, Rahman 2018). Mastic gum reduces the expression and operation of the androgen receptor (AR) in prostate cancer cells, according to He *et al.* (2006). The study discovered that androgens, and the androgen receptor, are key players in the emergence of prostate cancer. This study showed that the components of mastic gum suppressed androgen receptor transcriptional expression, which decreased levels of androgen receptor mRNA and protein. The decreased expression of all three androgen-regulated genes—NKX3.1, hK2, and prostate-specific antigen—confirmed the suppression of androgen receptor function. The same research team also conducted another study in 2007 to check the impact of mastic gum on androgen-independent prostate cancer. They employed PC-3 prostate cancer cells, which do not express AR. Mastic gum reduced nuclear factor kappa B (NF-B) activity and the NF-kB signalling pathway, which in turn prevented PC-3 cells from growing and stopping the cell cycle in the G1 phase. The results showed that mastic gum thereby prevented the growth of prostate cancer in both androgen-dependent and -independent prostate cancer cells. Additionally, He *et al.* 2007 found that prostate cancer cells express more maspin, prostate cancer tumor-suppressing gene, when mastic gum is present. 8 μg/mL of mastic gum caused a 1.5-fold increase in maspin expression. Further it was shown that mastic gum significantly boosted the expression of the maspin mRNA and protein. The study finally concluded that transcriptional factors are mostly responsible for the enhanced effects of mastic gum on maspin expression.

Mastic Gum against oral cancer

Oral cancer is a significant health concern, with a growing incidence and often poor prognosis. Surgical techniques, radiation therapy, and chemotherapy have improved, but the overall 5-year survival rate remains around 50-60% (Le *et al.*, 2020). Additionally, a significant portion of oral cancer cases have unknown origins, and may be attributed to various pathogens, including bacteria (Huang and Sullivan, 2013, Chen *et al.*, 2022). Mastic gum has been shown to inhibit the growth of various oral bacteria, including Fusoacterium nucleatum, which has been linked to the development of oral cancer. Additionally, mastic gum has demonstrated the ability to induce apoptosis in oral cancer

cells, potentially offering a novel therapeutic approach (Naidu *et al.,* 2004). While more research is needed to fully elucidate the mechanisms by which mastic gum may benefit oral cancer patients, the available evidence suggests it may be a promising complementary therapy to conventional treatment modalities. Incorporating mastic gum into oral hygiene routines and exploring its use in targeted cancer therapies could offer new avenues for improving outcomes for individuals diagnosed with oral cancer. Li *et al.* 2011 investigated in vitro anti-tumor effect of CMG extract on oral squamous cell carcinoma. The study was designed to examine oral squamous cell carcinoma cell line, YD-10B and the standard chemotherapy drug Paclitaxel (Taxol, Bristol-Myers Squibb) was used as a control to see if the extracts could cause apoptosis by activating caspase-3. The findings demonstrated that both compounds displayed growth inhibitory effects in the MTT experiment that were dose- and time-dependent, with CMG extracts potentially having a higher impact than taxol at low concentrations (5–10 μg/mL). Furthermore, fragmentation of the genomic DNA was caused at 24 hour following the addition of 10 μg/mL of CMG and 50 μg/mL of Taxol. Lastly, western blot analysis revealed that 10 μg/mL of CMG and 50 μg/mL of Taxol caused procaspase-3 to cleave. Hence, in vitro growth inhibition and induction of apoptosis of YD-10B oral cancer cells were seen using CMG extracts and Taxol, and it was determined that CMG extracts exhibited stronger antitumor activity than Taxol. Another study by Kim *et al.* 2016, examined the effects of mastic gum on human oral cancer cells. YD-10B cells were cultured for 24 hours in mastic concentrations of 0, 1, 2, 5, and 10 mg/mL. Analysis was done on cell number, viability, morphology, colony-forming assay, and DAPI staining. The results showed that when YD-10B cells were treated with mastic, cell proliferation was inhibited in a dose-dependent manner, and nearly all of the cells in the 10 mg/mL culture were dead. The YD-10B cells had morphological changes and nuclear fragmentation after being treated with mastic, and this inhibition of cell colonization was dose-dependent. DAPI staining results showed that destruction of the shape of the nucleus was caused in a concentration dependent manner with increased mastic treatment. Considering the results, the study concluded that mastic gum can be used as an anticancer agent against oral cancer.

Mastic Gum against colon cancer

Colorectal or colon cancer is a significant global health concern, with increasing incidence rates worldwide. Recent studies have highlighted the potential of mastic gum in preventing and managing colon cancer. The xanthones isolated from mastic gum, including α-mangostin, β-mangostin, and γ-mangostin, have shown potent antiproliferative and pro-apoptotic activities in colon cancer cell lines (Pan-In *et al.,* 2014). Moreover, mastic gum has been found to exhibit anti-inflammatory properties, which are closely linked to the development and progression of colorectal cancer. The anti-inflammatory effects of mastic gum may be mediated through the inhibition of key inflammatory mediators, such as cyclooxygenase-2 (COX-2) and nuclear factor-κB (NF-κB) (Choi *et al.,* 2022). Using components of a supplement made from *Pistacia lentiscus* L. var. chia, Balan *et al.* (2007) studied the antiproliferative efficacy and induction of apoptosis in human colon cancer cells treated in vitro. According to the study, a 50% ethanol extract of the plant-derived substance chios mastic gum (CMG) contains substances that prevent the growth and cause the demise of HCT116 human colon cancer cells in a test tube. The CMG treatment promotes cell arrest at G1, cell separation from the substrate, activation of pro-caspases-8, -9, and -3, and various morphological alterations in cell organelles that are indicative of apoptosis. Additionally, despite being time- and dose-dependent, these events are independent of p53 and p21. The lack of inhibition of apoptosis by CMG in HCT116 cell clones that overexpress the anti-apoptotic protein Bcl-2 or the dominant-negative form of FADD suggests that CMG kills cells via a different pathway that is unrelated to the death receptor- and mitochondrion-dependent pathways.

CONCLUSION

Mastic gum, the dried resin produced by the *Pistacia lentiscus* var. chia plant, has been investigated for its potential as an anticancer agent. Studies have shown that mastic gum's high content of polyphenols and triterpenoids may contribute to its anticancer properties. Mastic gum has demonstrated antiproliferative and pro-apoptotic effects on various cancer cell lines, including breast, colorectal, prostate, lung, and oral cancers. The proposed mechanisms of action include the induction of apoptosis and autophagy, inhibition of angiogenesis, and downregulation of inflammatory mediators. Additionally, the encapsulation of mastic gum essential oil in nanoparticles has improved

its cytotoxic efficacy against breast and ovarian cancer cells while minimizing harm to normal cells. Overall, the available evidence suggests that mastic gum is a promising natural compound with potential applications in cancer treatment. However, there needs to be more in vitro research for the development of supplement or medicine to ensure the chemopreventive activity of mastic gum and its essential oil.

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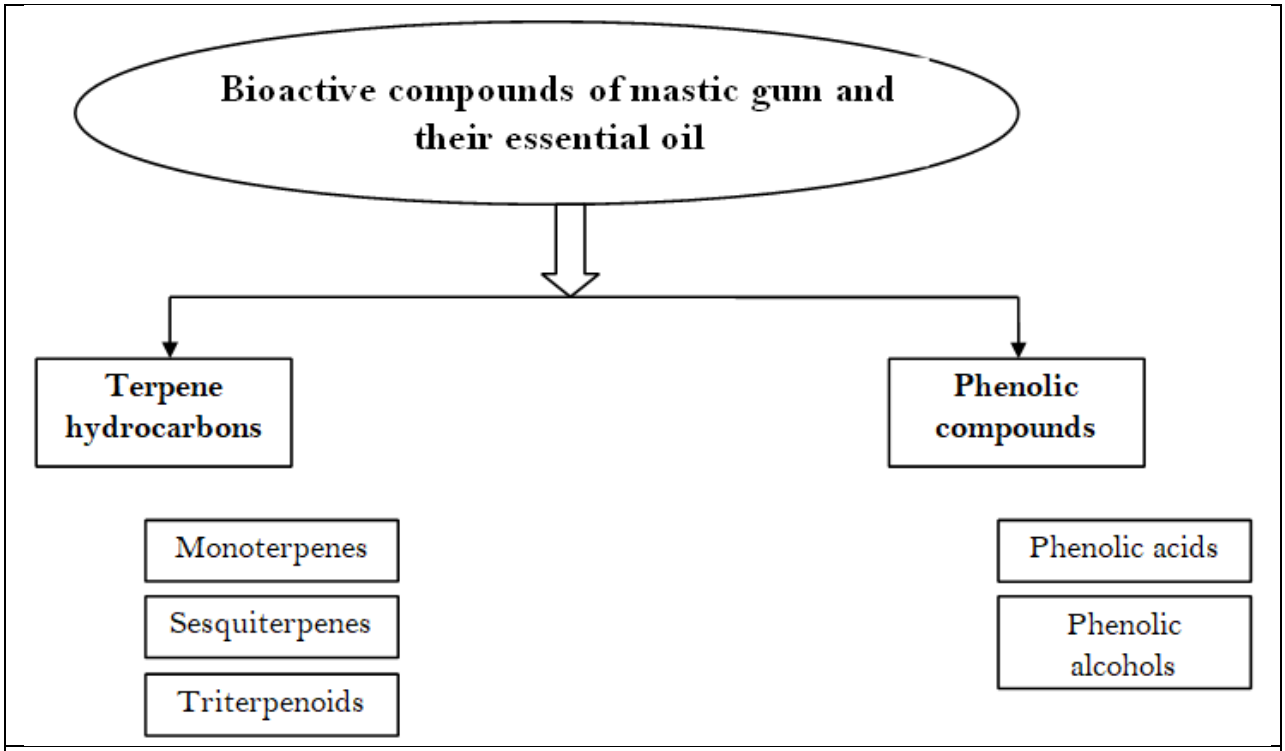


Fig. 1: Major bioactive compounds of mastic gum and their essential oil

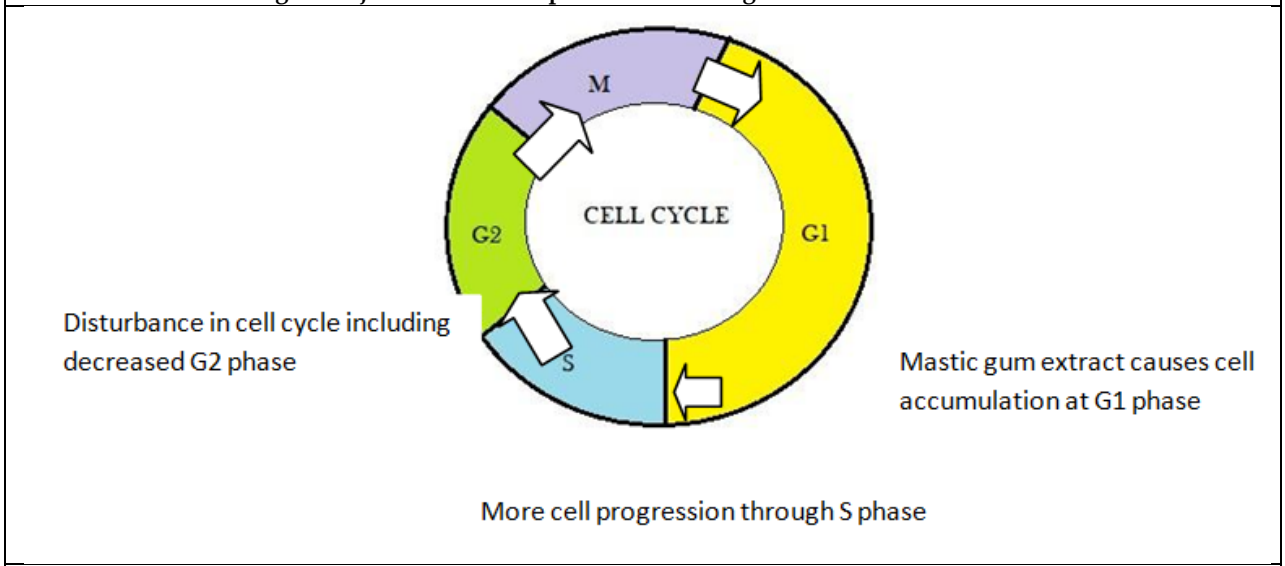


Fig. 2: Mechanism of mastic gum extract inducing cell growth inhibition and apoptosis

RESEARCH ARTICLE

An Eco Epidemiological Prey Predator SEI Model with Disease in Prey Only

R. Sivakumar[1,3*] and S. Vijaya[2]

[1]Research Scholar, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Professor, Department of Mathematics, Annamalai University, Annamalai Nagar, Chidambaram, Cuddalore, Tamil Nadu, India.
[3]Assistant Professor, Department of Mathematics, Sri Manakula Vinayagar Engineering College, School of Arts and Science, (Affiliated to Pondicherry University), Puducherry, India.

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***Address for Correspondence**
R. Sivakumar
Assistant Professor,
Department of Mathematics,
Sri Manakula Vinayagar Engineering College,
School of Arts and Science,
(Affiliated to Pondicherry University),
Puducherry, India.
E.Mail: sivakumarmaths.sas@smvec.ac.in

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ABSTRACT

In this paper, we create and analyze a predator-prey model that includes a disease in the prey population. This model takes into account the dynamics of Susceptible, exposed, and infected prey, as well as their interactions with predator species. The prey population is split into three categories: susceptible, exposed, and infected, and the disease spreads by interaction between susceptible and infected individuals. The predator species preferentially targets prey based on their health level, which affects each prey class differently. We investigate the impact of disease transmission, predation pressure, and natural mortality on system stability and the survival of prey and predator populations. We use mathematical analysis and numerical simulations to discover crucial requirements for ecosystem coexistence, extinction, and oscillatory behavior.

Keywords: Predator-prey model, eco-epidemiology, disease dynamics, stability analysis, SEI model, prey-predator interactions, mathematical modeling, population dynamics.

MSC (MATHEMATICAL SUBJECT CLASSIFICATION)
• **92D30**: Epidemiology

- **92D25**: Population dynamics (general)
- **92D40**: Ecology
- **34D20**: Stability of solutions to ordinary differential equations
- **37N25**: Dynamical systems in biology
- **37N35**: Dynamical systems in ecology

INTRODUCTION

Predator-prey interactions are critical components of ecological systems, determining population dynamics and ecosystem stability. Traditional predator-prey models, such as the Lotka-Volterra model, make the assumption that the prey population is healthy and has no internal structure. However, in genuine ecosystems, illnesses frequently influence prey populations, altering their interactions with predators and influencing overall system dynamics. Disease in prey can cause reduced reproduction rates, higher mortality, and behavioral changes, all of which effect predator feeding rates and survival. To improve our understanding of these complex systems, eco-epidemiological models incorporate disease dynamics into predator-prey interactions. By introducing disease compartments into the prey population, researchers can investigate the combined impacts of predation and disease on population stability and persistence. In this study, we extend the standard predator-prey framework by categorizing the prey population as susceptible, exposed, or infected, resulting in a model that incorporates the impact of disease transmission and predation on both prey and predator populations. The goal of this study is to investigate how sickness in the prey population influences ecosystem stability and behavior. We hope to find scenarios under which the disease can benefit or hurt predator and prey populations. We analyze the system's equilibrium points, conduct stability analyses, and look at how characteristics like disease transmission rate, predation rate, and recovery rate affect population dynamics. This study advances our understanding of the variables that determine biodiversity, population control, and ecosystem resilience by shedding light on the relationship between disease and predation.

Mathematical model and the Basic Assumption

The prey population is separated into three compartments based on disease dynamics: susceptible (S), Exposed(E), infected (I), and maybe recovered (R).Susceptible prey can get infected through contacts with diseased people, based on a transmission rate parameter. In addition to natural predation, infected prey may die at a faster rate as a result of disease. Given limited natural resources, the susceptible prey population may continue to expand logistically. Infected people may have lower or even no reproduction rates since the condition affects fertility. The disease is thought to have no latency period, which means that once infected, prey have an instantaneous impact on disease spread. Environmental aspects such as seasonality and habitat change are not considered (in a basic model).

$$\frac{dD}{dt} = rD\left(1 - \frac{D+E+F}{K}\right) - \frac{c_1 DG^2}{a + (D+E+F)G} - \lambda D$$

$$\frac{dE}{dt} = \lambda D - \sigma E - \frac{c_3 EG^2}{a + (D+E+F)G}$$

$$\frac{dF}{dt} = \sigma E - \frac{c_2 FG^2}{a + (D+E+F)G} - \gamma F$$

$$\frac{dG}{dt} = e\frac{(c_1 D + c_3 E + c_2 F)G^2}{a + (D+E+F)G} - dG$$

We have some basic assumption for

D→ susceptible prey

E→ exposed prey

F→ infected prey

G→predator

r→growth rate

K→ carrying capacity

λ→ force of infection

c_1→ search rate of susceptible prey
c_2→ search rate of infected prey
c_3→ search rate of exposed prey
a→ half saturation point or constant
γ→ total death rate of infected prey (natural death rate+death rate due to infection
e→ conversion factor
d→natural death rate for predator population

Roundedness of the System
Proposition 1
The sound prey population is limited.

Proof.
From equation (2), we get
$\frac{dD}{dt} = rD\left(1 - \frac{D+E+F}{K}\right) - \frac{c_1 DG^2}{a+(D+E+F)G} - \lambda D < rD\left(1 - \frac{D+E+F}{K}\right) < \frac{rD}{K}(k - D)$
Using a simple yet typical argument, we may conclude that limsupt!+1 D(t) 0 such that for any t > T1, D(t) < k + ~ = W.

Theorem 1 states that all system (2)-(4) solutions starting in $R_+^3$are uniformly bounded.

Proof.
We defined a function.
Ω= D + E + F+G
The time derivative of equation (5) is provided by $\frac{d\Omega}{dt} = \frac{dD}{dt} + \frac{dE}{dt} + \frac{dF}{dt} + \frac{dG}{dt}$ for each μ> 0. The following inequality holds
$\frac{d\Omega}{dt} + \mu\Omega = rD\left(1 - \frac{D+E+F}{K}\right) - \frac{c_1 DG^2}{a+(D+E+F)G} - \lambda D + \lambda D - \sigma E - \frac{c_3 EG^2}{a+(D+E+F)G}$
$+\sigma E - \frac{c_2 FG^2}{a+(D+E+F)G} - \gamma F + e\frac{(c_1 D + c_3 E + c_2 F)G^2}{a+(D+E+F)G} - dG + \mu\Omega$
$\leq K\frac{(\mu+r)^2}{4r} - (\gamma - \mu)F - (d - \mu)G \; since \; e < 1$
$\leq K\frac{(\mu+r)^2}{4r} = \phi \; if \; \mu < min(\gamma, d).$
Therefore, using the preceding equation, we have$\frac{d\Omega}{dt} + \mu\Omega \leq \emptyset$. Applying the theory of differentiated inequality. We obtain 0<$\Omega(D,E,F,G) < \left(\frac{\phi}{\mu}\right)(1 - e^{-\mu t}) + \Omega\left(D_0, E_0, F_0, G_0\right) \times e^{-\mu t}$ and for t→ ∞ *we have* $0 < \Omega < \frac{\phi}{\mu}$Hence, all the results of the system of equations (2)–(4) that originate in$R_+^3$are limited to the region B={(D,E,F,G) ϵ R_+^3 : $\Omega = \frac{\phi}{\mu} + \epsilon$ for any ϵ> 0}.

Equilibrium
The system of equations (2)–(4) exhibits the following equilibria.

1. The trivial equilibrium point
   {D=0,E=0, F=0,G=0}
2. Exposed, Infected prey free and predator free equilibrium point
   {D=K, E=0, F=0, G=0}
3. Susceptible, Infected prey free and predator free equilibrium point
   {D=0, E=$\frac{\lambda K}{\sigma}, F = 0, G = 0$}
4. Susceptible, Exposed prey free and predator free equilibrium point
   {D=0, E=0, F=$\frac{\lambda K}{\gamma}, G = 0$}
5. Susceptible, Exposed and Infected prey free equilibrium point

{D=0,E=0, F=0, G=$\frac{e(c_1K)}{(a+K)d}$}

6. Infected prey free and predator free equilibrium point
{D=$\frac{\sigma K}{r}$, E = $\frac{\lambda K}{\sigma}$, $F = 0, G = 0$}
7. Exposed prey free and predator free equilibrium point
{D=K, E=0, F=$\frac{\lambda K}{\gamma}$, $G = 0$}
8. Predator free equilibrium point
{D=$\frac{\sigma K}{r}$, E = $\frac{\lambda K}{r}$, F=$\frac{\lambda K}{\gamma}$, $G = 0$}
9. Susceptible prey and predator free equilibrium point
{D=K, E= $-\frac{\lambda K}{\gamma}$, F=$\frac{\lambda K-\lambda K}{\sigma}$,G=$\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}$
10. Infected prey free and predator free equilibrium point
{D=K, E= $-\frac{\lambda K}{\gamma}$, $F = 0, G = 0$}
11. Exposed prey free and predator free equilibrium point
{D=K, E=0, F=$\frac{-r\lambda K}{\sigma}$, $G = 0$}
12. Non- trivial equilibrium point
{D=$\frac{\sigma K}{r}$, E = $\frac{\lambda K}{\sigma}$, F=$\frac{-r\lambda K}{\sigma}$, $G = \frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}$}

Stability analysis

In this section, we will look at the critical point's stability characteristics.
The Jacobian matrix becomes

J=$\begin{bmatrix} J_{11} & J_{12} & J_{13} & J_{14} \\ J_{21} & J_{22} & J_{23} & J_{24} \\ J_{31} & J_{32} & J_{33} & J_{34} \\ J_{41} & J_{42} & J_{43} & J_{44} \end{bmatrix}$

$J_{11} = r\left(1-\frac{D+E+F}{K}\right) - rD - \frac{c_1G^2}{a+(D+E+F)G} + \frac{c_1DG^2}{(a+(D+E+F)G)^2} - \lambda$

$J_{12}= -\frac{rD}{K} + \frac{c_{1D}G^3}{(a+(D+E+F)G)^2}$

$J_{13} = -\frac{rD}{K} + \frac{c_{1D}G^3}{(a+(D+E+F)G)^2}$

$J_{14}= \frac{-2c_1DG}{a+(D+E+F)G} + \frac{c_{1D}G^2(D+E+F)}{(a+(D+E+F)G)^2}$

$J_{21} = \lambda$

$J_{22}= -\sigma - \frac{c_3G^2}{a+(D+E+F)G} + \frac{c_3EG^2}{a+(D+E+F)G}$

$J_{23} = \frac{c_3EG^2}{(a+(D+E+F)G)^2}$

$J_{24} = \frac{-2c_3EG}{a+(D+E+F)G} + \frac{c_3EG^2(D+E+F)}{(a+(D+E+F)G)^2}$

$J_{31} = 0$

$J_{32} = \sigma$

$J_{33} = -\gamma - \frac{c_2G^2}{a+(D+E+F)G} + \frac{c_2FG^2}{a+(D+E+F)G}$

$J_{34} = \frac{-2c_2FG}{a+(D+E+F)G} + \frac{c_2FG^2(D+E+F)}{(a+(D+E+F)G)^2}$

$J_{41} = \frac{e\,c_1G^2}{a+(D+E+F)G} - e\frac{(c_1D+C_3E+c_2F)G^2}{(a+(D+E+F)G)^2}$

$J_{42} = \frac{e\,c_3G^2}{a+(D+E+F)G} - e\frac{(c_1D+C_3E+c_2F)G^2}{(a+(D+E+F)G)^2}$

$J_{43} = \frac{e\, c_2 G^2}{a+(D+E+F)G} - e\,\frac{(c_1D+C_3E+c_2F)G^2}{(a+(D+E+F)G)^2}$

$J_{44} = \frac{2e\,(c_1D+C_3E+c_2F)G}{a+(D+E+F)G} - e\,\frac{(c_1D+C_3E+c_2F)G^2(D+E+F)}{(a+(D+E+F)G)^2}$

Theorem 5.1

Considering the linear version of the nonlinear differential equation (1), the equilibrium point {D=0, E=0, F=0, G=0} is unreliable and saddle point.

Proof

The changes applied to the Jacobian matrix are

$J_1 = \begin{bmatrix} r-\lambda & 0 & 0 & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$

The eigen values are $\lambda_1 = r-\lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma, \lambda_4 = 0$

The equilibrium point, Ξ1 (0,0,0,0), is considered unstable. If Atleast one of the eigenvalues of matrix J_1 have positive real components and atleast one of the eigenvalue with negative real components called saddle point. The eigen values $\lambda_1 = r-\lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma, \lambda_4 = 0$ are unreliable and represent a saddle point.

Theorem 5.2

Consider the linear version of equations. Therefore, the equilibrium point {D=K, E=0, F=0, G=0} is stable.

Proof

The changes made to the Jacobian matrix are

$J_2 = \begin{bmatrix} -rk-\lambda & -r & -r & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$

The eigen values are $\lambda_1 = -rk-\lambda < 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0.$

The equilibrium point is known as the saddle point If All of the eigenvalues of matrix J_2 have negative real components called stable point Thus, the eigen values $\lambda_1 = -rk-\lambda < 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma, \lambda_4 = 0$ form a stable point.

Theorem 5.3

Consider the linear version of the nonlinear differential equation(1), with the equilibrium point {D=0, E=$\frac{\lambda K}{\sigma}, F = 0, G = 0$} as the sink and saddle point.

Proof

The modifications made to the Jacobian matrix include

$J_3 = \begin{bmatrix} r\frac{\lambda}{\sigma}-\lambda & 0 & 0 & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$

The eigen values are $\lambda_1 = r\frac{\lambda}{\sigma}-\lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0.$

The equilibrium point is known as the saddle point. If Atleast one of the eigenvalues of matrix J_1 have positive real components and atleast one of the eigenvalue with negative real components called saddle point. Thus, the eigen values $\lambda_1 = r\frac{\lambda}{\sigma}-\lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0$ represent saddle point.

Theorem 5.4

Consider the linear version of the nonlinear differential equation(1), with the equilibrium point {D=0, E=0, F= $\frac{\lambda K}{\gamma}, G = 0$} as the sink and saddle point.

Proof

The modifications made to the Jacobian matrix include

$$J_4 = \begin{bmatrix} r\frac{\lambda}{\gamma} - \lambda & 0 & 0 & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

The eigen values are $\lambda_1 = r\frac{\lambda}{\gamma} - \lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0$

The equilibrium point is known as the saddle point. If all of the eigenvalues of matrix J_4 are negative real components. Thus, the eigen values $\lambda_1 = r\frac{\lambda}{\gamma} - \lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0.5$

Theorem 5.5

Consider the linear version of the nonlinear differential equation(1), with the equilibrium point {D=0, E=0, F=0, G= $\frac{e(c_1K)}{(a+K)d}$}as the sink and saddle point.

Proof

The modifications made to the Jacobian matrix include

$$J_5 = \begin{bmatrix} r - \frac{c_1(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} - \lambda & 0 & 0 & 0 \\ \lambda & -\sigma - \frac{c_3(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} & 0 & 0 \\ 0 & \sigma & -\gamma - \frac{c_2(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

The eigen values are$\lambda_1 = r - \frac{c_1(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} - \lambda > 0, \lambda_2 = -\sigma - \frac{c_3(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} < 0, \lambda_3 = -\gamma - \frac{c_{12}(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} < 0, \lambda_4 = 0.$

.

The equilibrium point is known as the saddle point. If Atleast one of the eigenvalues of matrix J_1 have positive real components and atleast one of the eigenvalue with negative real components called saddle point. Thus, the eigen values $\lambda_1 = r - \frac{c_1(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} - \lambda > 0, \lambda_2 = -\sigma - \frac{c_3(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} < 0, \lambda_3 = -\gamma - \frac{c_{12}(\frac{e(c_1K)}{(a+K)d})^2}{a+(\frac{e(c_1K)}{(a+K)d})} < 0, \lambda_4 = 0$ represent saddle point.

Theorem 5.6

Consider the linear version of the nonlinear differential equation(1), with the equilibrium point {D= $\frac{\sigma K}{r}$, E = $\frac{\lambda K}{\sigma}, F = 0, G = 0$} is asymptotically stable in the linear system of the nonlinear differential equation(1).

Proof

The changes to the Jacobian matrix are

$$J_6 = \begin{bmatrix} r\left(\frac{K - \frac{\sigma K}{r} + \frac{\lambda K}{\sigma}}{K}\right) - \sigma - \lambda & -\sigma & -\sigma & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

The eigen values are $\lambda_1 = r\left(\frac{K-\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}}{K}\right) - \sigma - \lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma, \lambda_4 = 0.$

If all of the eigen values of matrix J_6 have negative real components. Thus, the eigen values $\lambda_1 = r\left(\frac{K-\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}}{K}\right) - \sigma - \lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma, \lambda_4 = 0$ are asymptotically stable.

Theorem 5.7

Consider the linear version of the nonlinear differential equation(1), the equilibrium point {D=K, E=0, F= $\frac{\lambda K}{\gamma}, G = 0$}are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$J_7 = \begin{bmatrix} r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right) - r - \lambda & -\sigma & -\sigma & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$

The eigen values are $\lambda_1 = r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right) - r - \lambda > 0, \lambda_2 = -\sigma < 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0$

If all of the eigenvalues of matrix J_7 have negative true components. Thus, the eigen values $\lambda_1 = r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right) - r - \lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0$are asymptotically stable.

Theorem 5.8

Consider the linear version of the nonlinear differential equation(1), the equilibrium point {D= $\frac{\sigma K}{r}, E = \frac{\lambda K}{r}$, F= $\frac{\lambda K}{\gamma}, G = 0$}are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$J_8 = \begin{bmatrix} r\left(1-\frac{\frac{\sigma K}{r}+\frac{\lambda K}{r}+\frac{\lambda K}{\gamma}}{K}\right) - \sigma - \lambda & -\sigma & -\sigma & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$

The eigen values are $\lambda_1 = r\left(1-\frac{\frac{\sigma K}{r}+\frac{\lambda K}{r}+\frac{\lambda K}{\gamma}}{K}\right) - \sigma - \lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0.$

The equilibrium point is referred to as the asymptotically stable. If all of the eigenvalues of matrix J_8 have negative real components. Thus, the eigen values $\lambda_1 = r\left(1-\frac{\frac{\sigma K}{r}+\frac{\lambda K}{r}+\frac{\lambda K}{\gamma}}{K}\right) - \sigma - \lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma < 0, \lambda_4 = 0.$ are asymptotically stable.

The characteristic equation is $\wedge_1 (\lambda) = B_1\lambda^3 + B_2\lambda^2 + B_3\lambda + B_4$

Where,

$B_1 = 1 \quad , B_2 = -(m_{33} + m_{22} + m_{11}), \quad B_3 = -(-m_{11}m_{22} - m_{11}m_{33} + m_{12}m_{21} + m_{13}m_{31} - m_{22}m_{33} + m_{23}m_{32}), \quad B_4 = m_{11}m_{22}m_{33} + m_{11}m_{23}m_{32} + m_{12}m_{21}m_{33} - m_{13}m_{23}m_{31} - m_{13}m_{21}m_{32} + m_{13}m_{22}m_{31}.$

Routh Hurwitz criterions, all the eigenvalues of J_8have negative real elements if

i. $B_1 > 0$
ii. $B_3 > 0$

iii. $B_1B_2B_3 > B_3^2 + B_1^2B_4$.

Thus, given the structure of nonlinear differential equations is locally asymptotically stable around equilibrium point {D= $\frac{\sigma K}{r}$, E = $\frac{\lambda K}{r}$, F= $\frac{\lambda K}{\gamma}$, $G = 0$} if conditions stated.

Theorem 5.9

consider the linear version of the nonlinear differential equation(1), the equilibrium point {D=K, E= $-\frac{\lambda K}{\gamma}$, F= $\frac{\lambda K-\lambda K}{\sigma}$,G= $\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}$}are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$$J_9 = \begin{bmatrix} J_{11} & J_{12} & J_{13} & J_{14} \\ J_{21} & J_{22} & J_{23} & J_{24} \\ J_{31} & J_{32} & J_{33} & J_{34} \\ J_{41} & J_{42} & J_{43} & J_{44} \end{bmatrix}$$

$$J_{11} = r\left(1 - \frac{K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}}{K}\right) - r - \frac{c_1\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)} + \frac{c_1K\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)\right)^2} - \lambda$$

$$J_{12} = -r + \frac{c_1K\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^3}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)\right)^2}$$

$$J_{13} = -r + \frac{c_1K\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^3}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)\right)^2}$$

$$J_{14} = \frac{-2c_1K\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)} + \frac{c_1K\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2\left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)\right)^2}$$

$$J_{21} = \lambda$$

$$J_{22} = -\sigma - \frac{c_3\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)} + \frac{c_3 - \frac{\lambda K}{\gamma}\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{23} = \frac{c_3 - \frac{\lambda K}{\gamma}\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^3}{a + \left(K - \frac{\lambda K}{\gamma} + \frac{\lambda K - \lambda K}{\sigma}\right)\left(\frac{-(\lambda K - rK + a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{24}=\frac{-2c_3-\frac{\lambda K}{\gamma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^3}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+\frac{-2c_3-\frac{\lambda K}{\gamma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{31}=0$$

$$J_{32}=\sigma$$

$$J_{33}=-\gamma-\frac{c_2\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+\frac{c_2\frac{\lambda K-\lambda K}{\sigma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{34}=-\frac{2c_2\frac{\lambda K-\lambda K}{\sigma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+\frac{c_2\frac{\lambda K-\lambda K}{\sigma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{41}=-\frac{ec_1\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}-\frac{e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{42}=-\frac{ec_3\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}-\frac{e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{43}=-\frac{ec_2\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}-\frac{e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

$$J_{44}=-\frac{2e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}-\frac{e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$

The equilibrium point is considered asymptotically stable. If all eigenvalues of matrix J_9 have negative real components. Thus, the eigen values

$$\lambda_1=r\left(1-\frac{K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}}{K}\right)-r-\frac{c_1\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+\frac{c_1K\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}-\lambda>0,\lambda_2=-\sigma-$$

$$\frac{c_3\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+\frac{c_3-\frac{\lambda K}{\gamma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}>0,\lambda_3=-\gamma-\frac{c_2\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}+$$

$$\frac{c_2\frac{\lambda K-\lambda K}{\sigma}\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}<0,\lambda_4=$$

$$-\frac{2e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}-\frac{e\left(c_1K+c_3\left(-\frac{\lambda K}{\gamma}\right)+c_2\left(\frac{\lambda K-\lambda K}{\sigma}\right)\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)^2}{a+\left(K-\frac{\lambda K}{\gamma}+\frac{\lambda K-\lambda K}{\sigma}\right)\left(\frac{-(\lambda K-rK+a\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)}{c_1K}\right)}$$ are asymptotically stable.

Theorem 5.10

Consider the linear version of the nonlinear differential equation(1), the equilibrium point {D=K, E= $-\frac{\lambda K}{\gamma}$, $F = 0, G = 0$} are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$$J_{10} = \begin{bmatrix} r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right)-r-\lambda & -r & -r & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

The eigen values are $\lambda_1 = r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right)-r-\lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma -< 0, \lambda_4 = 0.$

The equilibrium point is referred to as the asymptotically stable. If all of the eigenvalues of matrix J_{10} have negative real components. Thus, the eigen values $\lambda_1 = r\left(1-\frac{K+\frac{\lambda K}{\gamma}}{K}\right)-r-\lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma -< 0, \lambda_4 = 0$ are asymptotically stable

Theorem 5.11

Consider the linear version of the nonlinear differential equation(1), the equilibrium point {D=K, E=0, F=$\frac{-r\lambda K}{\sigma}$, $G = 0$} are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$$J_{11} = \begin{bmatrix} r\left(1-\frac{K+\frac{-r\lambda K}{\sigma}}{K}\right)-r-\lambda & -r & -r & 0 \\ \lambda & -\sigma & 0 & 0 \\ 0 & \sigma & -\gamma & 0 \\ 0 & 0 & 0 & 0 \end{bmatrix}$$

The eigen values are $\lambda_1 = r\left(1-\frac{K+\frac{-r\lambda K}{\sigma}}{K}\right)-r-\lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma -< 0, \lambda_4 = 0.$

The equilibrium point is referred to as the asymptotically stable. If all of the eigenvalues of matrix J_{11} have negative real components. Thus, the eigen values $\lambda_1 = r\left(1-\frac{K+\frac{-r\lambda K}{\sigma}}{K}\right)-r-\lambda > 0, \lambda_2 = -\sigma > 0, \lambda_3 = -\gamma -< 0, \lambda_4 = 0.$ are asymptotically stable

Theorem 5.12

consider the linear version of the nonlinear differential equation(1), the equilibrium point {D=$\frac{\sigma K}{r}$, E = $\frac{\lambda K}{\sigma}$, F=$\frac{-r\lambda K}{\sigma}$, $G = \frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}$}are asymptotically stable.

Proof

The changes made to the Jacobian matrix are

$$J_{12} = \begin{bmatrix} J_{11} & J_{12} & J_{13} & J_{14} \\ J_{21} & J_{22} & J_{23} & J_{24} \\ J_{31} & J_{32} & J_{33} & J_{34} \\ J_{41} & J_{42} & J_{43} & J_{44} \end{bmatrix}$$

$$J_{11} = r\left(1 - \frac{\frac{\sigma K}{r} + \frac{\lambda K}{\sigma} + \frac{-r\lambda K}{\sigma}}{K}\right) - \sigma - \frac{c_1\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_1\frac{\sigma K}{r}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$-\lambda$$

$$J_{12} = \left(\frac{\frac{-r\,\sigma K}{r}}{K}\right) - \sigma - \frac{c_1\frac{\sigma K}{r}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^3}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{13} = \left(\frac{\frac{-r\,\sigma K}{r}}{K}\right) - \sigma - \frac{c_1\frac{\sigma K}{r}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^3}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{14} = \frac{2c_1\frac{\sigma K}{r}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} + \frac{2c_1\frac{\sigma K}{r}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{21} = \lambda$$

$$J_{22} = -\sigma - \frac{c_3\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_3\frac{\lambda K}{\sigma}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{23} = \frac{c_3\frac{\lambda K}{\sigma}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^3}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}$$

$$J_{24} = \frac{-2c_3\frac{\lambda K}{\sigma}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} + \frac{2c_3\frac{\lambda K}{\sigma}\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{31} = 0$$

$$J_{32} = \sigma$$

$$J_{33} = -\gamma - \frac{c_2\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_2\left(\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}$$

$$J_{34} = \frac{-2c_2\left(\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} + \frac{2c_2\left(\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e\left(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}$$

$$J_{41} = -\frac{ec_1\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}$$

$$J_{42} = -\frac{ec_3\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{\left(a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\right)^2}$$

$$J_{43} = -\frac{ec_2\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{\left(a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\right)^2}$$

$$J_{44} = -\frac{2e\left(\frac{e(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}{\left(a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\right)^2}\text{-d}$$

The eigen values are $\lambda_1 = r\left(1-\frac{\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}}{K}\right) - \sigma - \frac{c_1\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_1\frac{\sigma K}{r}\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} - \lambda >$

$$0, \lambda_2 = -\sigma - \frac{c_3\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} +$$

$$\frac{c_3\frac{\lambda K}{\sigma}\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} > 0, \lambda_3 = J_{33} = -\gamma - \frac{c_2\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_2\left(\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}$$

$$< 0, \lambda_4 == -\frac{2e\left(\frac{e(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)^2}{\left(a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)\right)^2}.$$

The equilibrium point is referred to as the asymptotically stable. If all of the eigenvalues of matrix J_{12} have negative real components. Thus, the eigen values

$$\lambda_1 = r\left(1-\frac{\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}}{K}\right) - \sigma - \frac{c_1\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_1\frac{\sigma K}{r}\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} - \lambda > 0, \lambda_2 = -\sigma -$$

$$\frac{c_3\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} + \frac{c_3\frac{\lambda K}{\sigma}\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2} > 0, \lambda_3 = J_{33} = -\gamma - \frac{c_2\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} +$$

$$\frac{c_2\left(\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)}$$

$< 0, \lambda_4 == -\frac{2e(\frac{e(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)})^2}{a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right)} - \frac{e\left(c_1\frac{\sigma K}{r}+c_3\left(\frac{\lambda K}{\sigma}\right)+c_2\left(\frac{-r\lambda K}{\sigma}\right)\right)(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d})\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)^2}{(a+\left(\frac{\sigma K}{r}+\frac{\lambda K}{\sigma}+\frac{-r\lambda K}{\sigma}\right)\left(\frac{e(c_2\left(\frac{\lambda K}{\sigma}\right)+c_3\left(\frac{\lambda K}{\gamma}\right)}{a+\left(\frac{\lambda K}{\sigma}+\frac{\lambda K}{\gamma}\right)d}\right))^2}$-d are asymptotically stable.

Numerical results

We drew the majority of our base parameter values from the analysis of an eco-epidemiological model of prey and predators. r = 0.2, k = 11000 , $a = 1500$,λ= 0.0066, γ = 0.061, c_1 = 0.00005,c_3 = 0.00004,c_2 = 0.00003and we assume $d = 0.00001$. In the first simulation, we assume variables as r = 0.8 and K= 50. With these values for parameters, the equilibria are (1,0.03,0.4,0.5,0.3), (2,0.01,0.09,0.4,0.2), (0.8,100,200,0.003,225.0) (Figure 1 ,2,3). In the second simulation, we assume variables as r = 0.2 and K= 1100. With these values for parameters, the equilibria are (15,0.03,0.4,0.5,0.3), (20,0.01,0.09,0.4,0.2), (13,100,200,0.003,225.0) (Figure 4). There are two equivalent interaction equilibriums. (44.916,0.07715,0.8999) and (44.988,0.01080,0.9000).To investigate the existence of limit cycles resulting frome (50,15,15,1400) (figure 5). We take r = 0.4, k = 2,000,000, λ = 0.006, γ= 0.24/day, p = 0, c = 0.05,h = 0.04 and m = 5.0.The numbers of susceptible and infected prey(Figure 6) and the number of predators (Figure 7) trend toward their a stable state equilibrium levels(41:654; 499:8646; 13; 537:98). Wetake as parameters r = 0.6, k = 45, λ = 0.007, γ = 0.0076, p =0,δ =0.09, h = 50.0 and m =5.0. The numbers of susceptible and Exposed prey(Figure 8) and the number of predators (Figure 9) trend toward their a stable state equilibrium levels(35:814; 999:9532; 18; 234:46).

CONCLUSION

In a predator-prey model with diseased prey, disease introduction reduces prey availability, affecting predator survival and potentially destabilizing the ecosystem. Stability and coexistence are determined by the rates of infection, recovery, and predation. Disease can create new equilibrium states or oscillations, demonstrating how infection influences population dynamics and environmental stability**.**

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Conflict of Interest

The author declares that there is no conflict of interest regarding the publication of this paper**.**

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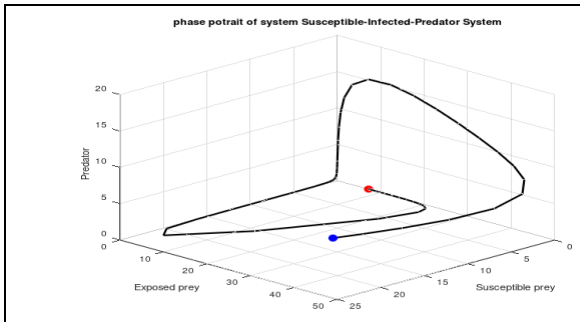


Figure 1: Phase portrait system of Susceptible Exposed Prey Predator

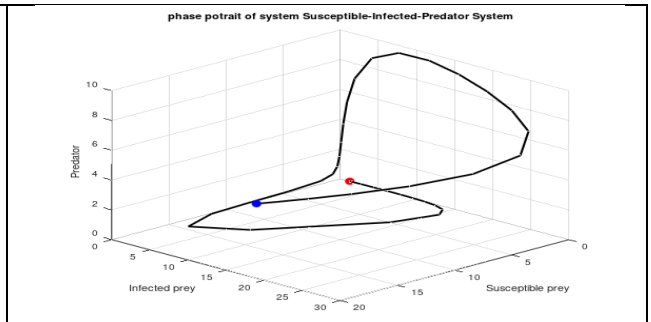


Figure 2: Phase portrait system of Susceptible Infected Prey Predator

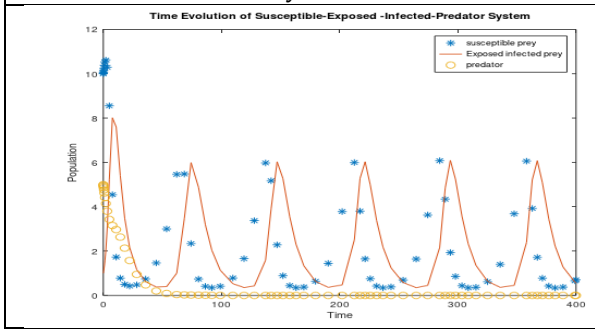


Figure 3: Time Evolution of Susceptible Exposed Infected Prey Predator system

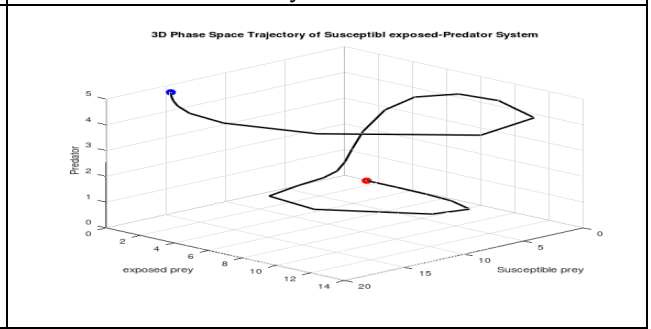


Figure 4: 3D Phase portrait of Susceptible Exposed Predator system

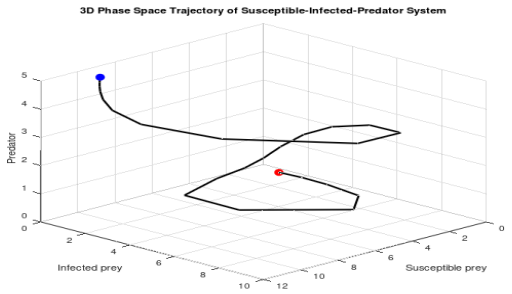


Figure 5: 3D Phase portrait of Susceptible Infected Predator system

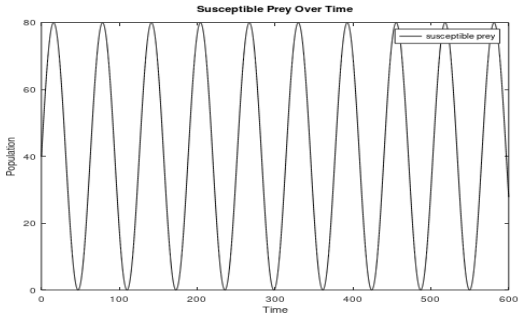


Figure 6: Susceptible Prey over time

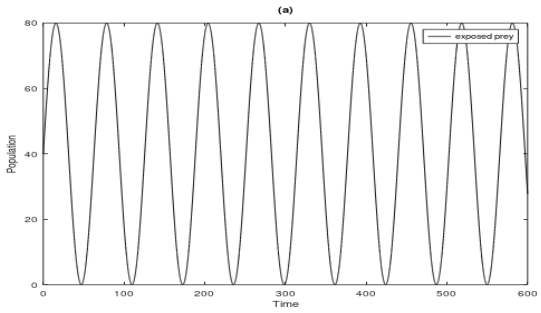


Figure 7: Exposed Prey over time

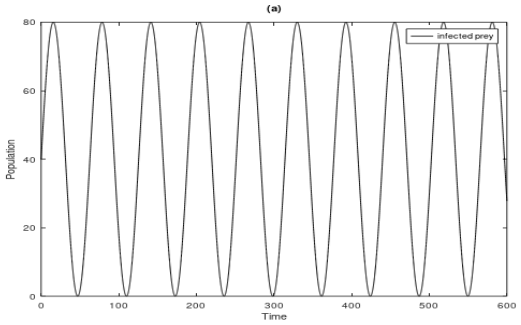


Figure 8: Infected Prey over time

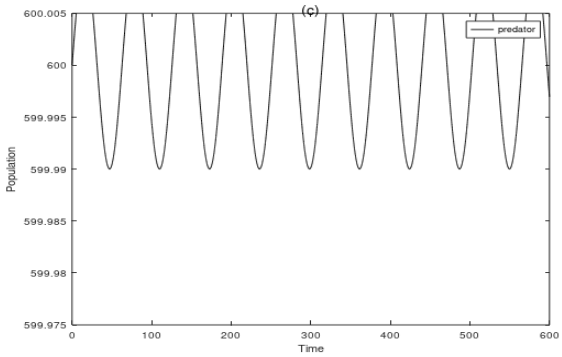


Figure 9: Predator over time

RESEARCH ARTICLE

A Study on Coupled Fixed Point Theorems in b-Multiplicative Metric Spaces

A. Mary Priya Dharsini[1] and J. Jarvisvivin[2]*

[1]Assistant Professor, Department of Mathematics, Holy Cross College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.
[2]Research Scholar (Reg. No. 22222102092010), Department of Mathematics, Holy Cross College, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
J. Jarvisvivin
Research Scholar (Reg. No. 22222102092010),
Department of Mathematics,
Holy Cross College,
(Affiliated to Bharathidasan University),
Tiruchirappalli, Tamil Nadu, India.
E.Mail: jarvisvivin3@gmail.com

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ABSTRACT

This manuscript aims to establish the existence and uniqueness of common coupled fixed points within the framework of $\mathfrak{b}$-multiplicative metric spaces.To achieve this, we construct suitable iterative sequences and utilize the inherent properties of $\mathfrak{b}$ - multiplicative metric spaces. The analysis is conducted under mixed-type contractive conditions, including a Geraghty-type contraction, to ensure the convergence of these sequences.We have proved the existence of coupled fixed points for two distinct types of contractions. We have introduced Geraghty-type contractions in the *c*ontext of *c*oupled fixed point theorems within $\mathfrak{b}$ -multiplicative metric spaces. We established *c*ommon *c*oupled fixed point theorems under mixed-type contractive mapping on $\mathfrak{b}$ -multiplicative metric spaces. An illustrative example is provided to support and clarify the theoretical results.

2020 Mathematics Subject Classification: 47H10, 54H25.

Keywords: Fixed Point, $\mathfrak{b}$-Multiplicative Metric Space($\mathfrak{b}-mms$), Coupled Fixed Point, Mixed-type contractive condition, Geraghty type contraction, Weakly Compatible(wc)

INTRODUCTION

Bhaskar and Lakshmikantham introduced the concept of coupled fixed points, establishing several *c*oupled fixed *p*oint theorems within the framework of standard metric spaces. Following their foundational work, numerous researchers have extended and refined coupled fixed point theorems in various settings. Coupled fixed point theorems are particularly useful in analysing systems where two variables are interrelated, such as in nonlinear systems of equations or differential equations with boundary conditions. The *w*eak *c*ompatibility was introduced by Jungck and Rhoades. They defined two mappings to be $\mathfrak{wc}$ if they commute at their points of coincidence, and showed that all compatible mappings are $\mathfrak{wc}$, although the converse is not necessarily true. (See[1,2]) Geraghty proposed a generalization of the Banach contraction principle by replacing the contraction constant with a function satisfying specific properties. This generalization has since been widely studied and extended to various types of distance spaces, and is commonly referred to as the Geraghty-type fixed point theorem. Bashirov introduced the concept of multiplicative metric spaces to transform the operations of addition and subtraction into multiplication and division, thereby facilitating computational processes. In 2017[3], Muhammad Usman Ali further extended this framework by proposing the notion of $\mathfrak{b}-\mathfrak{mms}$, which offer greater flexibility in the representation of distances and allow for the detection of certain nonlinear behaviors that may not be captured by traditional metric spaces. Subsequently, several mathematicians contributed to the development of this field, extensively documenting their work by considering various conditions on both mappings and spaces[4-9]. This paper aims to initiate the study of common coupled fixed point theorems for two contraction mappings within the framework of *b*-multiplicative metric spaces, focusing on a generalized mixed-type contractive condition as well as a Geraghty-type contraction. Here we extend the mixed-type contractive conditions in b-metric spaces[10] to $\mathfrak{b}-\mathfrak{mms}$, involve multiple distance terms and allow nonlinear, multivariable, or asymmetric control functions.The main purpose of proving a coupled fixed point theorem under a Geraghty-type contraction in the framework of $\mathfrak{b}-\mathfrak{mms}$ is to extend and generalize existing fixed point results.Here we extend Geraghty type contraction in bipolar metric spaces[11] to b-multiplicative metric spaces. Relaxes the contraction condition by introducing a function (a mapping ψ) that controls the contraction, making the theorem applicable in a broader class of problems.An example has been presented to demonstrate the validity of the results.

PRELIMINARIES

Definition 2.1[3]Let χ be a non-empty set and $\mu \geq 1$ be a given real number. A function $d: \chi \times \chi \to [1, \infty)$is called a $\mathfrak{b}$ −multiplicative metric with coefficientμ if the following conditions hold:
i) $d(x, y) > 1$ for all $x, y \in \chi$ with $x \neq y$ and $\mathrm{d}(x, y) = 1$ iff $x = y$
ii) $d(x, y) = d(y, x)$ for all $x, y \in \chi$
iii) $d(x, z) \leq [d(x, y).d(y, z)]^{\mu}$ for all $x, y, z \in \chi$
The triplet (χ, d, μ) is called$\mathfrak{b}-\mathfrak{mms}$.

Definition 2.2[3]Let (χ, d, μ) be a $\mathfrak{b}-\mathfrak{mms}$.
(i) A sequence $\{x_n\}$ is $\mathfrak{b}$-multiplicative convergent iff there exist $x \in \chi$ such that
$d(x_n, x) \to 1$ as $n \to \infty$.
(ii) A sequence $\{x_n\}$ is called $\mathfrak{b}$-multiplicative Cauchy iff
$d(x_m, x_n) \to 1$ as $n, m \to \infty$.
iii) A $\mathfrak{b}-\mathfrak{mms}(\chi, d, \mu)$ is said to be complete if every $\mathfrak{b}$-multiplicative cauchy sequencein χ is $\mathfrak{b}$- convergent to a point in χ.

Definition 2.3[10]An ordered pair $(x, y) \in \chi \times \chi$is referred to as a coupled fixed point of a function $\mathrm{P}: \chi \times \chi \to \chi$if it satisfies the conditions $\mathrm{P}(x, y) = x$ and$\mathrm{P}(y, x) = y$.

Definition 2.4[10] An ordered pair$(x, y) \in \chi \times \chi$is referred to as a coupled coincidence point of the functionsP : $\chi \times \chi \to \chi$ and q : $\chi \to \chi$if it satisfies the condition $P(x, y) = qx$ and $P(y, x) = qy$.

Definition 2.5 [10] An ordered pair$(x, y) \in \chi \times \chi$ is called a coupled fixed point of the functions P : $\chi \times \chi \to \chi$ and q : $\chi \to \chi$ if $P(x, y) = qx = x$and$P(y, x) = qy = y$.

Definition 2.6 [10]Let χ be a nonempty set. The mappings P : $\chi \times \chi \to \chi$ and q: $\chi \to \chi$ are said to commute if for all $x, y \in \chi$, the following holds:$qP(x, y) = P(qx, qy)$.

Definition 2.7 [1]Let(χ, d)be a multiplicative metric space, and let P and q be two self-maps on χ. The mappings P and q are said to be *c*ompatible if they satisfy the conditions for compatibilitywithin this framework.
(1)if$\lim_{n\to\infty} d(Pqx_n, qPx_n) = 1$, whenever $\{x_n\}$ is a sequence in χ such that$\lim_{n\to\infty} Px_n = \lim_{n\to\infty} qx_n = t$ for some t $\in\chi$.
(2) if $\lim_{n\to\infty} d(Pqx_n, qqx_n) = 1$and $\lim_{n\to\infty} d(qPx_n, PPx_n) = 1$, whenever $\{x_n\}$ is a sequence in χ such that $\lim_{n\to\infty} Px_n = \lim_{n\to\infty} qx_n = t$for some t $\in\chi$.

Definition 2.8 [2]Two functions P and q are considered *weakly compatible*if they commute at their points of coincidence. In other words, whenever$Px = qx$ for some $x \in \chi$, it must also hold that $Pqx = qPx$.

RESULTS AND DISCUSSION

Theorem 3.1
Let (χ, d, μ) be a complete $\mathfrak{b} - mms$ with *c*onstant $\mu \geq 1$. Consider two self-mappings P:$\chi \times \chi \to \chi$and q: $\chi \to \chi$. Assume there exist non-negative constants τ_i *for* $i = 1, 2, \ldots, 10$ such that for any x, y, u, v $\in \chi$, the following inequality holds
$d(P(x,y), P(u,v)) \leq d(qx, qu)^{\tau_1} . d(qy, qv)^{\tau_2} . d(P(x,y), qx)^{\tau_3} . d(P(y,x), qy)^{\tau_4} .$
$d(P(u,v), qu)^{\tau_5} . d(P(v,u), qv)^{\tau_6} . d(P(x,y), qu)^{\tau_7} .$
$d(P(y,x), qv)^{\tau_8} . d(P(u,v), qx)^{\tau_9} . d(P(v,u), qy)^{\tau_{10}}$
Suppose the following conditions are satisfied:

i. $\mu\tau_1 + \mu\tau_2 + \mu\tau_3 + \mu\tau_4 + \tau_5 + \tau_6 + \tau_7 + \tau_8 + (\mu^2 + \mu)\tau_9 + (\mu^2 + \mu)\tau_{10} < 1$
ii. q(x) is a complete subspace of x
iii. $P(\chi \times \chi) \subseteq q(\chi)$

Under these assumptions, the mappings P and q have acoupled coincidence point. Furthermore, if P and q are ωc, then P and q have a unique *c*oupled fixed *p*oint. Additionally, for any initial point$x_1 \in \chi$, the sequence $\{x_n\}$generated by $q(x_{n+1}) = P(x_n, x_n)$ converges to the common fixed point of P and q.

Proof
Let $x_0 \in \chi$. SinceP$(\chi \times \chi) \subseteq q(x)$, then $\exists x_1 \in \chi$ such that $P(x_0, x_0) = qx_1$. Again, Since
$P(\chi \times \chi) \subseteq q(\chi)$, then $\exists x_2 \in \chi$ such that $P(x_1, x_1) = qx_2$. By repeating this procedure, we generate a sequence$\{y_n\}$ in q(x), ensuring that $y_n = qx_{n+1} = P(x_n, x_n)$.
It follows that
$d(qx_{n+1}, qx_{n+2}) = d(P(x_n, x_n). P(x_{n+1}, x_{n+1}))$
$\leq d(qx_n, qx_{n+1})^{\tau_1} . d(qx_n, qx_{n+1})^{\tau_2} . d(P(x_n, x_n), qx_n)^{\tau_3} .$
$d(P(x_n, x_n), qx_n)^{\tau_4} . d(P(x_{n+1}, x_{n+1}), qx_{n+1})^{\tau_5} . d(P(x_{n+1}, x_{n+1}), qx_{n+1})^{\tau_6} .$
$d(P(x_n, x_n), qx_{n+1})^{\tau_7} . d(P(x_n, x_n), qx_{n+1})^{\tau_8} . d(P(x_{n+1}, x_{n+1}), qx_n)^{\tau_9} .$
$d(P(x_{n+1}, x_{n+1}), qx_n)^{\tau_{10}}$
Therefore,
$d(qx_{n+1}, qx_{n+2}) \leq d(qx_n, qx_{n+1})^{\tau_1} . d(qx_n, qx_{n+1})^{\tau_2} . d(qx_{n+1}, qx_n)^{\tau_3} .$
$d(qx_{n+1}, qx_n)^{\tau_4} . d(qx_{n+2}, qx_{n+1})^{\tau_5} . d(qx_{n+2}, qx_{n+1})^{\tau_6} .$
$d(qx_{n+1}, qx_{n+1})^{\tau_7} . d(qx_{n+1}, qx_{n+1})^{\tau_8} . d(qx_{n+2}, , qx_n)^{\tau_9} .$

$d(qx_{n+2},,qx_n)^{\tau_{10}}$
$= d(qx_n,\ qx_{n+1})^{\tau_1+\tau_2+\tau_3+\tau_4} \cdot d(qx_{n+1},qx_{n+2})^{\tau_5+\tau_6} \cdot d(qx_n,qx_{n+2})^{\tau_9+\tau_{10}}$

which implies that
$d(qx_{n+1},qx_{n+2})^{1-\tau_5-\tau_6} \leq d(qx_n,qx_{n+2})^{\tau_9+\tau_{10}} \cdot d(qx_n,qx_{n+1})^{\tau_1+\tau_2+\tau_3+\tau_4}$
Since
$d(qx_n,qx_{n+2}) \leq d(qx_n,qx_{n+1})^{\mu} \cdot d(qx_{n+1},qx_{n+2})^{\mu}$
We have
$d(qx_{n+1},qx_{n+2})^{1-\tau_5+\tau_6} \leq [d(qx_n,qx_{n+1}) \cdot d(qx_{n+1},qx_{n+2})]^{\mu(\tau_9+\tau_{10})} \cdot$
$d(qx_n,qx_{n+1})^{\tau_1+\tau_2+\tau_3+\tau_4}$

$d(qx_{n+1},qx_{n+2})^{1-\tau_5-\tau_6-\mu\tau_9-\mu\tau_{10}} \leq d(qx_n,qx_{n+1})^{\tau_1+\tau_2+\tau_3+\tau_4+\mu\tau_9+\mu\tau_{10}}$

Therefore, we obtain
$d(qx_{n+1},qx_{n+2}) \leq d(qx_n,qx_{n+1})^{\frac{\tau_1+\tau_2+\tau_3+\tau_4+\mu\tau_9+\mu\tau_{10}}{1-\tau_5-\tau_6-\mu\tau_9-\mu\tau_{10}}}$
Let us set
$$h = \frac{\tau_1+\tau_2+\tau_3+\tau_4+\mu\tau_9+\mu\tau_{10}}{1-\tau_5-\tau_6-\mu\tau_9-\mu\tau_{10}}$$
Since
$\mu h = \frac{\mu\tau_1+\mu\tau_2+\mu\tau_3+\mu\tau_4+\mu^2\tau_9+\mu^2\tau_{10}}{1-\tau_5-\tau_6-\mu\tau_9-\mu\tau_{10}} < 1$ and
$h \leq \mu h$, we get $h < 1$, then
$d(qx_{n+1},qx_{n+2}) \leq d(qx_n,qx_{n+1})^h$ (3.1)
$d(qx_n,qx_{n+1}) = d(\mathrm{P}(x_{n-1},x_{n-1}),\mathrm{P}(x_n,x_n))$
$\leq d(qx_{n-1},\ qx_n)^{\tau_1} \cdot d(qx_{n-1},\ qx_n)^{\tau_2} \cdot d(\mathrm{P}(x_{n-1},\ x_{n-1}),qx_{n-1})^{\tau_3}.$
$d(\mathrm{P}(x_{n-1},x_{n-1}),qx_{n-1})^{\tau_4} \cdot d(\mathrm{P}(x_n,x_n),qx_n)^{\tau_5}. d(\mathrm{P}(x_n,x_n),qx_n)^{\tau_6}.$
$d(\mathrm{P}(x_{n-1},x_{n-1}),qx_n)^{\tau_7} \cdot d(\mathrm{P}(x_{n-1},\ x_{n-1}),qx_n)^{\tau_8}. d(\mathrm{P}(x_n,\ x_n),qx_{n-1})^{\tau_9}.$
$d(\mathrm{P}(x_n,x_n),qx_{n-1})^{\tau_{10}}$

We get
$d(qx_n,qx_{n+1}) \leq d(qx_{n-1},\ qx_n)^{\tau_1} \cdot d(qx_{n-1},\ qx_n)^{\tau_2} \cdot d(qx_n,qx_{n-1})^{\tau_3}.$
$d(qx_n,qx_{n-1})^{\tau_4} \cdot d(qx_{n+1},\ qx_n)^{\tau_5}. d(qx_{n+1},qx_n)^{\tau_6}.$
$d(qx_n,qx_n)^{\tau_7} \cdot d(qx_n,qx_n)^{\tau_8}. d(qx_{n+1},qx_{n-1})^{\tau_9} \cdot d(qx_{n+1},qx_{n-1})^{\tau_{10}}$
Which leads us to
$d(qx_n,qx_{n+1}) \leq d(qx_{n-1},qx_n)^h$ (3.2)
Applying (3.1) and (3.2) repeatedly for n steps yields
$d(qx_{n+1},qx_{n+2}) \leq d(qx_{n-1},qx_n)^{h^2}$
$\vdots$
$\leq d(qx_0,qx_1)^{h^{n+1}}$ (3.3)

Given $n, m \in \mathrm{N}$, with $m > n$, then
$d(qx_n,qx_m) \leq d(qx_n,qx_{n+1})^{\mu} \cdot d(qx_{n+1},qx_m)^{\mu}$
$\leq d(qx_n,qx_{n+1})^{\mu} \cdot d(qx_{n+1},qx_{n+2})^{\mu^2} \cdot d(qx_{n+2},qx_m)^{\mu^2}$
$\leq d(qx_n,qx_{n+1})^{\mu} \cdot d(qx_{n+1},qx_{n+2})^{\mu^2} \ldots d(qx_{m-1},qx_m)^{\mu^{m-n}}$

From (3.3), we get
$d(qx_n,qx_m) \leq d(qx_0,qx_1)^{\mu h^n} \cdot d(qx_0,qx_1)^{\mu h^{n+1}} \cdot \ldots \cdot d(qx_0,qx_1)^{\mu^{m-n} r^{m-1}}$
$\leq d(qx_0,qx_1)^{\mu h^n[1+\mu h+\mu^2 h^2+\mu^3 h^3+\cdots]}$
$\leq d(qx_0,qx_1)^{\mu h^n \sum_{i=0}^{\infty}(\mu h)^i}$
$= d(qx_0,qx_1)^{\frac{\mu h^n}{1-\mu h}}$

Since $h^n \to 0$ as $n \to +\infty$, we find that
$\lim_{n,m\to+\infty} d(qx_n, qx_m) = 1$

So $\{qx_n\}$ is a ɓ-cauchy ꞩequence in $(q(\chi), d)$, due to the completeness of$q(\chi)\exists g \in q(\chi)$ such that
$\lim_{n\to+\infty} qx_n = qu = g$

We aim to prove that (u, u) serves as acoupled *c*oincidence *p*oint for the mappings P and q.
$d(qx_{n+1}, P(u,u)) = d(P(x_n, x_n), P(u,u))$
$\leq d(qx_n,\ qu)^{\tau_1} . d(qx_n,\ qu)^{\tau_2} . d(\mathrm{P}(x_n,\ x_n), qx_n)^{\tau_3}.$
$d(P(x_n,\ x_n), qx_n)^{\tau_4} . d(\mathrm{P}(u,u), qu)^{\tau_5}. d(P(u,u), qu)^{\tau_6}.$
$d(\mathrm{P}(x_n,\ x_n), qu)^{\tau_7} . d(P(x_n,\ x_n), qu)^{\tau_8}. d(\mathrm{P}(u,\ u), qx_n)^{\tau_9}.$
$d(P(u,\ \ \ u), qx_n)^{\tau_{10}}$

Which implies that
$d\big(qx_{n+1}, P(u,u)\big) \leq d(qx_n,\ qu)^{\tau_1} . d(qx_n,\ qu)^{\tau_2} . d(qx_{n+1}, qx_n)^{\tau_3}.$
$d(qx_{n+1}, qx_n)^{\tau_4} . d(P(u,u), qu)^{\tau_5}. d(P(u,u), qu)^{\tau_6}.$
$d(qx_{n+1}, qu)^{\tau_7} . d(qx_{n+1}, qu)^{\tau_8}. d(P(u,\ u), qx_n)^{\tau_9}.$
$d(P(u,u), qx_n)^{\tau_{10}}$ (3.4)

Note that
$d\big(qu, P(u,u)\big) \leq d(qx_{n+1}, qx_n)$

Taking the limit as $n \to +\infty$ in the above inequality, we derive
$d(qu, P(u,u))^{\frac{1}{\mu}} \leq \lim_{n\to\infty} d(qx_{n+1}, P(u,u))$ (3.5)
$d(qx_{n+1}, qx_n) \leq [d(qx_{n+1}, qu) . d(qu, qx_n)]^{\mu}$
As $n \to +\infty$, the inequality becomes
$\lim_{n\to\infty}(qx_{n+1}, qx_n) = 1$ (3.6)

Again,
$d(P(u,u), qx_n) \leq [d(P(u,u), qu) . d(qu, qx_n)]^{\mu}$

By allowing $n \to +\infty$, the inequality yields
$\lim_{n\to\infty} d(P(u,u), qx_n) \leq d(P(u,u), qu)^{\mu}$ (3.7)

Letting $n \to +\infty$ in (3.4) and using (3.5), (3.6), (3.7), we get
$d(qu, \mathrm{P}(u,u))^{\frac{1}{\mu}} \leq d(\mathrm{P}(u,u), qu)^{\tau_5}. d(\mathrm{P}(u,u), qu)^{\tau_6} . d(\mathrm{P}(u,u), qu)^{\mu\tau_9}$
$d(\mathrm{P}(u,u), qu)^{\mu\tau_{10}}$

Which implies that
$d\big(qu, P(u,u)\big) \leq d(P(u,u), qu)^{\mu\tau_5+\mu\tau_6+\mu^2\tau_9+\mu^2\tau_{10}}$
Since $\mu\tau_5 + \mu\tau_6 + \mu^2\tau_9 + \mu^2\tau_{10} < 1$, we get $d(qu, P(u,u) = 1$.

Hence $qu = P(u,u)$.
Thus,(u, u) servesas a coupled coincidence point of P and q. To establish the uniqueness of this point, suppose that (v, v) is another *c*oupled *c*oincidence *p*oint of P and q.
$d(qu, qv) = d\big(P(u,u), P(v,v)\big)$
$\leq d(qu,\ qv)^{\tau_1} . d(qu,\ qv)^{\tau_2} . d(\mathrm{P}(u,\ u), qu)^{\tau_3}.$
$d(\mathrm{P}(u,\ u), qu)^{\tau_4} . d(\mathrm{P}(v,v), qv)^{\tau_5}. d(\mathrm{P}(v,v), qv)^{\tau_6}.$
$d(\mathrm{P}(u,\ u), qv)^{\tau_7} . d(\mathrm{P}(u,\ u), qv)^{\tau_8}. d(\mathrm{P}(v,v), qu)^{\tau_9}.$

$d(\mathrm{P}(v,\ v), qu)^{\tau_{10}}$

Which implies that
$d(qu, qv) \leq d(qu, qv)^{\tau_1+\tau_2+\tau_7+\tau_8+\tau_9+\tau_{10}}$
Since $\tau_1 + \tau_2 + \tau_7 + \tau_8 + \tau_9 + \tau_{10} < 1$, then
$d(qu, qv) = 1$. So $qu = qv$.

Therefore, $(u,\ u)$ is the unique coupled coincidence point of P and q. Next, assume that P and q are ŵc. Then
$q\big(\mathrm{P}(u,u)\big) = \mathrm{P}(qu, qu)$
Put $w = qu$, then
$qw = q(qu) = q\big(\mathrm{P}(u,u)\big)$
$= \mathrm{P}(qu, qu)$
$= \mathrm{P}(w,w)$

So, (w,w) is a coupled coincidence point of P and q. Therefore $qw = qu$.
Hence $qw = w$ and $P(w,w) = qw = w$
So (w,w) is a coupled fixed point of P and q.

Theorem 3.2
Let (χ, d, μ) be acomplete ƀ-ϻϻs with constant $\mu \geq 1$. Let P:$\chi \times \chi \to \chi$and q: $\chi \to \chi$ be two mappings satisfying the following conditions

i. For all x, y, u, v $\in \chi$,
$\mathrm{d}\big(\mathrm{P}(x,y), \mathrm{P}(u,v)\big) \leq \psi\big(d(qx,qu), d(qy,qv)\big) \max\{d(qx,qu), d(qy,qv)\}$ whereψ: $[1,\infty) \times [1,\infty) \to [1,\infty)$
ii. $q(\chi)$ is a complete subspace of χ
iii. $P(\chi \times \chi) \subseteq q(\chi)$

Then, the mappings P and q have a coupled coincidence point. Moreover, if P and q are weakly compatible, then they have a unique coupled fixed point. Furthermore, for any $x_1 \in \chi$, the sequence $\{x_n\}$defined by $q(x_{n+1}) = P(x_n, x_n)$converges to theunique common fixed point of P and q.

Proof
Let $x_0 \in \chi$. Since $\mathrm{P}(\chi \times \chi) \subseteq q(x)$, then $\exists x_1 \in \chi$ such that $P(x_0, x_0) = qx_1$. Again, Since
$P(\chi \times \chi) \subseteq q(\chi)$, then $\exists x_2 \in \chi$ such that $P(x_1, x_1) = qx_2$. By continuing this process, we generate a sequence $\{y_n\}$ in q(x), where each term is defined as$y_n = qx_{n+1} = P(x_n, x_n)$.
It follows that
$d(qx_{n+1}, qx_{n+2}) = d(P(x_n, x_n)\ .\ P(x_{n+1}, x_{n+1}))$
$\leq \psi\big(d(qx_n, qx_{n+1}), d(qx_n, qx_{n+1})\big) \max\{d(qx_n, qx_{n+1}), d(qx_n, qx_{n+1})\}$
$d(qx_{n+1}, qx_{n+2}) \leq d(qx_n, qx_{n+1})^h$ (3.8)
$d(qx_n, qx_{n+1}) \leq d(qx_{n-1}, qx_n)^h$ (3.9)
Repeating (3.8) and (3.9) for n times, we get
$d(qx_{n+1}, qx_{n+2}) \leq d(qx_{n-1}, qx_n)^{h^2}$
⋮
$\leq d(qx_0, qx_1)^{h^{n+1}}$ (3.10)
$d(qx_n, qx_m) \leq d(qx_n, qx_{n+1})^{\mu}\ .\ d(qx_{n+1}, qx_m)^{\mu}$
$\leq d(qx_n, qx_{n+1})^{\mu}\ .\ d(qx_{n+1}, qx_{n+2})^{\mu^2}.\ d(qx_{n+2}, qx_m)^{\mu^2}$
$\leq d(qx_n, qx_{n+1})^{\mu}\ .\ d(qx_{n+1}, qx_{n+2})^{\mu^2} \ldots\ d(qx_{m-1}, qx_m)^{\mu^{m-n}}$
From (3.10), we get
$d(qx_n, qx_m) \leq d(qx_0, qx_1)^{\mu h^n}\ .\ d(qx_0, qx_1)^{\mu h^{n+1}} \ldots . d(qx_0, qx_1)^{\mu^{m-n_r m-1}}$
$\leq d(qx_0, qx_1)^{\mu h^n [1+\mu h+\mu^2 h^2+\mu^3 h^3+\cdots]}$
$\leq d(qx_0, qx_1)^{\mu h^n \sum_{i=0}^{\infty}(\mu h)^i}$

$= d(qx_0, qx_1)^{\frac{\mu h^n}{1-\mu h}}$

Since $h^n \to 0$ as $n \to +\infty$, we get that
$\lim_{n,m\to+\infty} d(qx_n, qx_m) = 1$

So $\{qx_n\}$ is a b-cauchy sequence in $(q(\chi), d)$, by completeness of $q(\chi) \exists g \in q(\chi)$ such that
$\lim_{n\to+\infty} qx_n = qu = g$

Show that (u, u) is coupled coincidence point of P and q.
$d(qx_{n+1}, P(u,u)) = d(P(x_n, x_n), P(u,u))$
$\leq \psi\big(d(qx_n, qu), d(qx_n, qu)\big)\max\{d(qx_n, qu), d(qx_n, qu)\}$

Let us take $n \to +\infty$ in the above inequality
$\leq d(qx_n, qu)$
$d(qx_n, qu) \leq [d(qx_n, qx_{n+1}) \,.\, d(qx_{n+1}, qu)]^{\mu}$

Letting $n \to \infty$ in above inequality, we get
$d(qx_n, qu) \leq [d(qu, qu) \,.\, d(qu, qu)]^{\mu}$
$d(qx_n, qu)^{\frac{1}{\mu}} \leq 1$
$d(qx_{n+1}, P(u,u)) \leq 1$
$d(qu, P(u,u)) = 1$
$qu = P(u,u)$
Hence $(u,\ u)$ is the coupled coincidence point of P and q.

To Prove uniqueness
$d(qu, qv) = d\big(P(u,u), P(v,v)\big)$
$\leq \psi\big(d(qu, qv), d(qu, qv)\big)\max\{d(qu, qv), d(qu, qv)\}$
Which implies that
$< \max\{d(qu, qv), d(qu, qv)\}$(a)
$d(qv, qu) = d\big(P(v,v), \mathrm{P}(u,u)\big)$
$\leq \psi\big(d(qv, qu), d(qv, qu)\big)\max\{d(qv, qu), d(qv, qu)\}$
$< \max\{d(qv, qu), d(qv, qu)\}$ (b)
Combine (a) and (b)
$< \max\{d(qu, qv), d(qv, qu)\}$
$\therefore d(qu, qv) = 1$ implies $u = v$.

Hence (u,u) is unique coupled coincident point of P and q.Now, suppose that P and q are ωc. Then
$q\big(\mathrm{P}(u,u)\big) = \mathrm{P}(qu, qv)$
Put $w = qu$, then
$qw = q(qu) = q\big(P(u,u)\big)$
$= P(qu, qu)$
$= P(w,w)$

So, (w,w) is a coupled coincidence point of P and q. Therefore $qw = qu$.
Hence $qw = w$ and $\mathrm{P}(w,w) = qw = w$
So (w,w) is a coupled fixed point of P and q.

Example 3.3
Let $\chi = [1,\infty)$ and $d: \chi \times \chi \to [1,\infty)$ by:
$d(x,y) = 1 + |\ln x - \ln y|$

Consider that $(\chi, d, \mu)$is a complete b-Multiplicative Metric spaces with constant $\mu = 2$.
Let $P(x, y) = \sqrt{xy}$and $q(x) = x$.
Check:$\mathrm{d}\big(\mathrm{P}(\mathrm{x},\mathrm{y}), \mathrm{P}(\mathrm{u},\mathrm{v})\big) \leq \psi\big(d(qx, qu), d(qy, qv)\big)\max\{d(qx, qu), d(qy, qv)\}$ (3.11)
$d(x, y) = 1 + |\ln x - \ln y|$
$\mathrm{d}\big(\mathrm{P}(\mathrm{x},\mathrm{y}), \mathrm{P}(\mathrm{u},\mathrm{v})\big) \leq 1 + \left|\ln\sqrt{xy} - \ln\sqrt{uv}\right|$
$\mathrm{P}(\mathrm{x},\mathrm{y}) = \sqrt{xy} \Longrightarrow \ln P(x, y) = \frac{1}{2}(\ln x + \ln y)$

Then $\left|\ln\sqrt{xy} - \ln\sqrt{uv}\right| = \left|\frac{1}{2}(\ln x + \ln y - \ln u - \ln v)\right|$
Define: $\psi(a, b) = \sqrt{\min\{a, b\}}$
$\psi\big(d(x, u), d(y, v)\big) = \sqrt{\min\{(x, u), d(y, v)\}}(\because q(x) = x)$

Here (3.11) becomes
$1 + \left|\frac{1}{2}(\ln x + \ln y - \ln u - \ln v)\right| \leq \sqrt{\min\{(x, u), d(y, v)\}}.\max\{d(x, u), d(y, v)\}$ (3.12)
Let $x = 4,\ y = 9, u = 16, v = 25$
LHS of (3.12) $\Longrightarrow 1 + \left|\frac{1}{2}(\ln 4 + \ln 9 - \ln 16 - \ln 25)\right|$
$= 1 + \frac{1}{2}|1.39 + 2.19 - 2.77 - 3.22| \Longrightarrow 1 + \frac{1}{2}|3.58 - 5.99|$
$= 1 + \frac{1}{2}\ (2.41) \Longrightarrow 2.2$
$\mathrm{P}(x, y) = \sqrt{36} = 6$ and $\mathrm{P}(\mathrm{u},\mathrm{v}) = \sqrt{400} = 20$
$d(6,20) = 1 + |\ln 6 - \ln 20| \approx 1 + |1.79 - 2.99| = 2.2$
Now,$d(4,16) = 1 + |\ln 4 - \ln 16| \approx 1 + |1.39 - 2.77| = 2.38$
$d(9,25) = 1 + |\ln 9 - \ln 25| \approx 1 + |2.19 - 3.22| = 2.03$
$\psi\big(d(x, u), d(y, v)\big) = \sqrt{\min\{2.38, 2.03\}} = \sqrt{2.03} \approx 1.43$
$\max\{d(x, u), d(y, v)\} = \max\{2.38, 2.03\} = 2.38$
RHS of (3.12) $\Longrightarrow 1.43 \times 2.38 \approx 3.4 > 2.2 = \mathrm{d}\big(\mathrm{P}(\mathrm{x},\mathrm{y}), \mathrm{P}(\mathrm{u},\mathrm{v})\big)$

which confirms that the contractive conditions of theorem 3.2 are met.
Here $\mathrm{P}(\mathrm{u},\mathrm{u}) = q(\mathrm{u}) \Longrightarrow \sqrt{\mathrm{u}^2} = \mathrm{u} \Longrightarrow \mathrm{u} = \mathrm{u}$
So any $\mathrm{u} \in \chi$ satisfies this. Hence, every point $(\mathrm{u}, \mathrm{u}) \in \chi \times \chi$ is a coupled coincidencepoint of P and q.

CONCLUSION

In this study, we have investigated the existence and uniqueness of common coupled fixed points in$\mathfrak{b} - \mathfrak{mms}$. By constructing appropriate iterative sequences and employing both mixed-type and Geraghty-type contractive conditions, we successfully established new fixed point results that generalize and extend existing theorems in the literature. The constructed example further illustrates the applicability of our findings.

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REVIEW ARTICLE

Novel Breakthroughs in Biodegradable Ocular Implants that Curb a Spectrum of Ocular Conditions: A Review

Pooja C[1], Sashtipriyaa R[1], Mariyam Susan Joji[1] and M. Sumithra[2]*

[1]Student, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Kattankulathur, Chengalpattu, Tamil Nadu, India.
[2]Associate Professor, Department of Pharmacology, SRM College of Pharmacy, SRM Institute of Science and Technology (Deemed to be University), Kattankulathur, Chengalpattu, Tamil Nadu, India.

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***Address for Correspondence**
M. Sumithra
Associate Professor,
Department of Pharmacology,
SRM College of Pharmacy,
SRM Institute of Science and Technology
(Deemed to be University),
Kattankulathur, Chengalpattu, Tamil Nadu, India.
E.Mail: sumithrm@srmist.edu.in

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ABSTRACT

Background: Given that our eyes serve as windows to the world, they play a key part in our health. Research on the administration of medications to the eyes has advanced significantly over the past 20 years, resulting in novel, secure, and user-friendly drug delivery techniques and technologies that may be able to get beyond these challenges and maintain drug levels in tissues. This review article gives a narrative account of biodegradable ocular implants which is a crucial element in creating secure and efficient drug delivery systems (DDS) it has entailed the generation of biocompatible polymers, which provide the flexibility to personalize drug release kinetics for particular medications and ocular conditions. A study search was performed using the key terms "Biodegradable ocular implants", "Drug delivery system", and "ocular inserts" using the database's clinical trials. API, Embase, PubMed and Google Scholar. Reviews, meta-analyses, randomized controlled trials, clinical trials, and observational studies were all part of the search approach. Only English literature was included in the search parameters. The current article was put together using the data that was found during the aforementioned search. The patient-friendly techniques, such as systemic and topical routes, occasionally have poor bioavailability, due to a variety of barriers, including the blood-aqueous barrier, cornea, sclera, tear film, and blood-retinal barrier. The emergence of innovative drug delivery methods for the human eye which employ biodegradable substances for sustained release as well as increased permeability has so attracted a lot of research attention. There are more advantages to biodegradable ocular implants than

to non- biodegradable ones. By injecting medication straight through the anterior chamber inside the eye, intraocular implants allow for the continual upkeep of therapeutic drug concentrations in the vitreous. Poly-hydroxy acid synthetic aliphatic polyesters, such as polylactic acids, polyglycolic acids, and polylactic-co-glycolic acids, are the polymers most frequently utilized in biodegradable delivery systems. The target of this review is to focus the light on the advantages of biodegradable ocular DDS and discuss potential future developments.

Keywords: Drug delivery system, Biodegradable ocular implants, polymers, sustained release, human eye, biodegradation

INTRODUCTION

The eye is a perplexing organ with an exclusive physiology and anatomy [1]. The eye is rendered immune-privileged, meaning it is highly protected from systemic circulation. Primarily, the anterior as well as the posterior segments comprise the structure of the eye. Anterior as well as the posterior segments of the eye can be affected by a variety of disorders that may cause vision impairment [2]. It is interesting to note that conditions impacting the anterior part of the eyes, likely kerato conjunctivitis, dry eye disease, and glaucoma, mostly impact the anterior part of the eyes. However, conditions akin to diabetic retinopathy, retinal vascular occlusions, and age-related macular degeneration frequently affect the posterior part of the retina [3]. In many controlled-release systems, biodegradable polymers are utilized as drug carriers. Enzymatic activity or hydrolytic reactions can break down the monomers that make up biodegradable polymers, often joined together by esters, amides, or ether linkages [1]. After they eventually break down and are absorbed by the body, biodegradable polymers release the medication. Numerous ocular medication delivery methods that employ various biodegradable polymer types have been investigated [4]. The field of ocular medication delivery technologies has quickly progressed, leading to improved therapeutic approaches for long-term eye conditions [5]. Any ocular drug-delivering system's major goals are to overcome different dynamic and static ocular barriers, lower dose frequency, and sustain the target location's therapeutic medication concentrations [6 , 7]. Above all, the drug-delivery method should try to maximize drug absorption while avoiding any negative ocular effects [1]. Various conventional and peculiar drug delivery techniques, such as aqueous gels, emulsions, ointments, suspensions, liposomes, dendrimers, nanoparticles, nano micelles, implants, contact lenses, nanoneedles, nanosuspensions, and in situ thermosensitive gels for ocular diseases, have been devised to conquer the obstacles associated with ocular drug delivery and enhance ocular bioavailability [8].

Background of the Study

The Human Eye: The Complicated Section of The Human System. The eye, which gives us awareness of our surroundings, is among the most complex and vital organs in the human body (Figure 1) [9]. One can distinguish between the anterior as well as posterior segments of the eye. The former segment is made up of the tear, cornea, iris, ciliary body, lens, anterior and posterior chambers, and aqueous humor, whereas the latter segment is made up of the sclera, retina, choroid, and vitreous humor [10, 11].

Barriers of the Human Eye

THE TEAR FILM
THE CORNEA
THE CONJUNCTIVA

One of the most complex and vital organs in the human body, the eye enables us to see our surroundings. Drug distribution into the eye is challenging since the entire eye acts as a natural barrier for a number of medications and proteins (Figure 2) [12]. Tears, a pre-corneal film made up of three layers and having a thickness of around 3 micrometers, are the most important ocular barrier now in use, according to various routes of administration. Contrarily, because of the makeup of the layers that make up the cornea, a transparent as well as avascular area of

the eye, both hydrophobic as well as hydrophilic medications naturally pass through it [13]. The conjunctiva, a fibrous layer covering one-third of the total eyeball, is another significant barrier that prevents medications with a molecular weight between 20 and forty kDa from passing through [10].

Biodegradation

The term "biodegradation" describes the hydrolysis of the polymer base chain that can be enzymatic or non-enzymatic and is frequently applied to goods that are metabolized or water- soluble [14]. Two main processes that contribute to biodegradation are degradation and erosion. In the case of hydrophobic polymers, the latter defines the process of breaking their primary chain into lower polymeric molecular fractions, whereas the former points out to the breaking of the polymer's primary chain compounds into water-soluble substances. The pace at which the polymer is eroding and its water permeability are both important factors in this process [15]. Employing biodegradable devices composed of polymers that decompose into harmless metabolites or macromolecules that may be distributed in vivo and harmlessly extracted from the body is one way to assure eye health [16]. Designing implants with adequate degradation rates and mechanical properties is a difficult task because of regulatory constraints, intricacy, and the expensive nature of appropriate degradation-rate polymers. A variety of factors, including the patient's age, alterations to the structure of the body, and physiological characteristics that may affect the device's performance, should be taken into account during the approval procedure for a biodegradable implant. Biodegradable objects such as sheets, discs, plugs, pellets, and rods can be shaped by using compression molding, extrusion, and/or solution casting [17].

Aspects that influence biodegradation

Polymer structure: When hydrolytic enzymes and microorganisms are present, the hydrolyzable linkages in the chain of biodegradable polymers are prone to breaking down [18].

Polymer morphology: Because the molecules in amorphous parts are far away from one another and therefore more vulnerable to degradation, enzymes can more readily target these sections of polymers than they can crystalline ones [19].

Chemical and radiation treatments: When polymers are photolyzed by UV and γ-ray radiation, radicals and/or ions are produced, which lead to the cleavage and cross linking of polymers [18].

Molecular weight: As a polymer's molecular weight rises, so does its biodegradability [19, 20].

The methods for accelerating biodegradable polymer degradation

The primary techniques for quickening the breakdown of biodegradable polymers center on altering the biotic and abiotic stages of the process. Abiotic phase methods typically include changing the characteristics of the polymer either during the production phase or prior to introducing it to the degradation process during the post-consumption stage [21]. On the other hand, methods pertaining to the biotic side mostly involve altering the environment; nonetheless, recent findings indicate that biotic techniques might also alter the settings during the processing stage [22].

Types of Biodegradable Polymers Used

The homogeneous dispersion of a drug, which may or may not be incorporated in the hydrophobic coat formed of bio-erodible polymers and is impermeable to the drug, makes up the bio-erodible inserts [23]. Ortho esters and orthocarbonates are two examples of bio-erodible compounds that have been employed successfully [24]. The foundation of biodegradable systems is made up of Polyglycolic acid (PGA), Poly (lactic-co-glycolic-acid) (PLGA), Polycaprolactone (PCL), and Polylactic acid (PLA) (Figure 3) [25].

Ocular Implants

Biodegradable (bioresorbable) and non-biodegradable (biodurable) are the two categories into which ocular implants fall; the former is the more advantageous. The biodegradable polymer- based devices decompose into innocuous byproducts or polymers that are soluble in living things and may be extracted from the body without risk [26]. The fact that biodegradable products don't need to be thrown away when their useful lives are over is their main advantage (Figure 4). In particular for the treatment of retinal illnesses that can impair vision, such systems present a potential remedy to many of the drawbacks of traditional methods for administering Ophthalmic Medications [27].

Techniques for Making Biodegradable Implants Designed for Intraocular Use

For ocular illnesses, controlled methods of drug delivery are necessary to provide the best possible medicinal intervention [2]. Polymeric materials have been largely researched for application in ocular drug delivery systems during the past 20 years [28]. These materials are essential for the controlled release of pharmaceuticals. Polymeric drug delivery systems possess the capability to mitigate a medication's side effects while simultaneously boosting its bioavailability. Biodegradable polymer-based ocular medication delivery systems represent one of the most significant areas of current research [29]. They can be produced in the shape of membranes, discs, or rods.

For example: - prolonged dexamethasone acetate (DA) release- Two of the methods used were – hot molding and compression [30]

Comparison and contradistinction of compression and hot molding methods

The hot molding process is used to first plasticize the polymeric cake before shaping it into rods. This approach reduces surface fractures because, as a variant with compression, the heat produces a chain coordination that increases the degree of crystallinity in the implants [31]. The matrices' pores and channels might encourage the compressed implants to absorb more water, which would quicken the degrading process. Nevertheless, the ultimate mechanical properties of the implant may deteriorate since the chain orientation does not happen whenever a polymeric cake compressed in a solid state [32]. According to a study by Sîlvia Ligório Fialho *et al*., a study of implants created with the same polymer type but prepared using various methods revealed that the compression systems enabled a faster dispersion of DA than the moulded ones. This outcome has been explained by the compressed devices' increased capacity to absorb water due to their larger pore count and channels observed in scanning electron microscopy (SEM) images. As DA exits the polymeric matrix and travels through the passages to the incubated setting, it increases the release of the drug [33]. Drugs that are crystalline, like DA, have the ability to change into an amorphous form while in the lyophilization process [29]. An exothermic transition, which is the result of drug recrystallization, can be facilitated by heating during differential scanning calorimetry analysis.

Biodegradable Implants

A method to maintain eye health is to utilize biodegradable devices made of polymers that can break down into harmless byproducts or that can be safely removed from the human body in vivo. Due to legal limitations, complexities, and the expensive nature of polymers with appropriate mechanical and degradation kinetics, designing implants that degrade is a challenging task. The alteration of abiotic and biotic phase could accelerate the biodegradation of polymers [10, 22].

Benefits of biodegradable over non-biodegradable

As biodegradable implants break down naturally, they don't require surgery, which is the main advantage over non-biodegradable implants.

- They are easily tolerated [34]
- PLA and/or PLGA polymers has acceptable biocompatibility [35]
- Compared to other tissues, polymers inserted in the vitreous cavity would lead to less inflammation [36]
- Pharmaceutical eyedrops administered topically have significant medication waste and are not effective for longer than a couple of hours. Therefore, biodegradable implants can be used to achieve patient compliance for chronic illnesses [37]
- Prolonged period of action/sustained release of action

- Increased bioavailability [38]

When it comes to addressing low medication bioavailability, drug variation, and patient compliance, sustained drug delivery provides the greatest solution [39].

Limitations

The main adverse circumstance of release systems that use biodegradable polymers encompass the supposedly last stage of the implant, which occurs when the polymeric material's hydrolysis attain a threshold and could result in the residual drug concentration releasing uncontrollably [40]. Furthermore, the movement of biodegradable implants in front of the retina or inside the anterior region of the eye may cause vision distortion. Polymers with varying molecular weights are mixed to create a more consistent delivering factor, hence reducing the negative consequences of this final phase [10, 41]. A further difficulty is obtaining serial drug concentration measurements in a live eye, as it is unethical to perform intrusive serial probe samples in human eyes [42].

Biodegradable Polymers PCL

Hexanoate repeat units are the basis of PCL, a semicrystalline polyester that is hydrophobic by nature and extremely soluble in organic solvents such as benzene, toluene, and chloroform. characteristic allows for regulated and prolonged medication release times [43]. For the purpose of delivering proteins into the back of the eye over an extended period of time, hybrid PCL thin-film devices were created by combining microporous as well as nonporous PCL and sealing the films together with heat [10, 44].

USES PCL copolymers are employed as drug carriers and to treat ophthalmic disorders. For instance, in an experiment, the glycolide-co-lactide-co-caprolactone copolymer (PGLC) device containing cyclosporine A was employed to cure chronic uveitis in the eyes of white rabbits [45].

PLA and PGA

Cornstarch and sugarcane are examples of renewable materials that are used to make PLA [46]. PLA polymers are thought to be compostable and biodegradable. An annual renewable resource can be used to manufacture polylactic acid, a polymer made from thermoplastic substances with outstanding strength and modulus [47, 48]. The potential for therapeutic usage of PGA is limited by its intrinsic characteristics, despite it being a biodegradable semicrystalline polymer. It also poorly solubilizes in typical polymer solvents and creates dangerous residues that may interact with the medication or the tissue. Furthermore, no solvent or melting method can be used to form them into films, rods, or capsules. On the other hand, its monomer can copolymerize with lactide to form the most extensively studied drug-delivery biomaterial to date [10].

USES: PLA has been utilized extensively in the management of eye conditions [10]. They have been used in the treatment of cytomegalovirus retinitis [49, 50].

PLGA

Lactic acid, also known as a-hydroxy propanoic acid, and glycolic acid, also known as hydroxy acetic acid, are synthesized to form Poly(lactide-co-glycolide). Poly(lactide-co-glycolide) breaks down to harmless compounds in vivo [51-53].

SYNTHESIS: The mixture of glycolic acid and lactic acid can be directly polycondensed to create poly(lactide-co-glycolide) [54]. However, the ring opening polymerization of lactide as well as glycolide is the most effective method for producing high molecular weight (MW) copolymers [55].

DEGRADATION: In vitro, poly(lactide-co-glycolide) breaks down by hydrolysing ester linkages. The end products of breakdown in vitro include glycolic and lactic acids [36]. It has been researched that the breakdown of poly(lactide-co-glycolide) occurs in two stages (Figure 5). Molecular weight decrease is the result of ester bond random hydrolytic cleavage that takes place during the first step. First-order kinetics appears to govern the chain

scission process. The start of mass loss through the polymer matrix and a rise in the pace of chain scission define the second stage.

The lactide: The three primary components that influence the pace at which PLGA copolymers break down are the lactide stereoisomeric constitution (i.e., the ratio of L-versus-DL-lactide), glycolide ratio, and molecular weight.

USES: Making polylactic acid (PLGA) into a range of shapes, such as vascular grafts and surgical meshes, stents and framework for tissue regeneration, and pins, plates, screws, and rods for orthopaedic and dental surgery, is a somewhat easy process [53].
Some of the properties of biodegradable polymers Table 1

Mechanisms of Drug Release
Drug release typically happens in three stages
Burst Release (Figure 8) The implant surface releases the medication, resulting in a brief period of intense drug release [56, 57].

Chain Scission and Diffusion (Figure 7, 9) Water from the tear fluid starts to seep into the matrix when the insert is inserted into the eye. This causes swelling, which in turn causes polymer chain relaxation and drug diffusion [24]. By hydrolytic cleavage, randomised chain scission among polymers takes place, increasing the porosity as well as surface area needed for drug diffusion [58].

Mass Loss and Biological Degradation In addition to drug release, there is biodegradation occurring inside the polymer matrix, a last burst in certain delivery systems, and a mass loss starting in the implant's central core [59, 60].

Osmosis: The transverse impermeable elastic membrane that makes up the insert for the Osmosis mechanism divides its interior into two compartments, designated first and second. The first compartment is surrounded by an elastic membrane that is impermeable and semi-permeable, while the second compartment is surrounded by an elastic membrane that is impermeable [61].

Bioerosion: The drug is dispersed in a framework of bioerodible substance that makes up the insert in the bioerosion mechanism. Through matrix bioerosion, the insert and tear fluid come into contact and cause a controlled, prolonged release of the medication [24, 62].
Some examples of currently marketed products (Table 2)

Applications
Long-term suppression of intraocular pressure
Glaucoma is the second most prevalent root of blindness worldwide, according to data from the World Health Organisation (WHO). [SOURCE: WHO] Retinal ganglion cells are lost in glaucoma, a multifactorial visual degenerative neuropathy [63]. The following groups are more likely to develop glaucoma: individuals over 60, diabetics, steroid users, relatives of an individual with a prior diagnosis of the illness, hypertension, excessive myopia, central corneal thickness of fewer than five millimeters, and eye injuries. It is estimated that 111.8 million people worldwide will be affected by glaucoma in the year 2040 [64]. Timolol maleate (TM)-incorporated biodegradable subconjunctival film from a combination of multilayering and blending with PEG copolymers, provides a therapeutic dose of 2.5 mcg for 90 days invitro [39]. Biodegradable elastomer poly(lactide-co-caprolactone) (PLC) is used for TM preparation. It provides both localized and sustained action. The copolymers' flexibility and mechanical properties make it ideal for an implant that patients will accept [65]. It is also biocompatible

Treatment of Cytomegalovirus (CMV) retinitis- in vitro retinal drug delivery

Within months of implantation, PLA dissolves, allowing connective tissue to repair the wound through healing [66] naturally. There won't be any indications of retinal detachment, inflammation, or anomalies in the retinal tissue (Figure 12) [67]. However, because of the matrix porosity created during the breakdown process, the plug contains an invasion of multinucleated giant cells, neutrophils, and fibroblasts with little retinal toxicity [49, 68].

Anti-inflammatory action

In a study performed by Okabe *et. al*, the characteristics of in vitro release of betamethasone phosphate exhibited a biphasic releasing pattern, consisting of an initial burst and another stage that was obtained from diffusional release [69, 70]. The drug's quick release, which was deposited in the matrix's water channels and on the implant's surface, might have caused the first burst [71, 72]. Slow drug release was seen during the second release phase, which may have been influenced by the polymer's rate of breakdown [73, 74]. Hydrolysis converts betamethasone phosphate into betamethasone [75]. These variables could be the cause of the discrepancy between drug concentrations in tissue and what is released from the implant (Figure 13) [76].

Post-operative cataract

In a study performed by Chennamaneni et. al., after up to six weeks, there was a bidirectional flow of dexamethasone from the endocapsular region, with notable amounts observed in the anterior as well as posterior chambers. In contrast, topical drops had a higher systemic exposure but limited exposure in all ocular regions [77]. All research groups had normal intraocular pressure, and slit-lamp biomicroscopy tests showed that both anterior as well as posterior chambers with implants did not produce any cells or fibrin, but the topical drop group did. Upon histological evaluation, all groups showed normal tissues and an absence of inflammation. The device did not hinder the visual axis or move to the middle of the eye (Figure 14) [78].

Treatment of diabetic macular edema

A typical microvascular consequence of diabetes called diabetic macular edema (DME) is defined by a breach within the blood-retinal barrier (BRB) (Figure 15). This causes microaneurysms and perifoveal aberrant capillary capillaries to leak intracellularly and extracellularly, thickening the retina in the process [79]. Center-involving DME is a primary cause of vision loss in people with diabetes and one of the main causes of substantial visual loss in the working-age population [80. 81]. A biodegradable as well as bioerodible implant with 0.7 milligrams of dexamethasone is called Ozurdex. Since triamcinolone acetonide is a more powerful glucocorticoid receptor agonist than dexamethasone, slow-release delivery techniques are necessary. To ensure a wound that seals itself, it is injected intravitally, deploying a 22-gauge applicator and a shelved injection technique. The solid polymer delivery system utilized in the implant is made up of poly-D, and L-lactide-co-glycolide as its polymer matrix. The prolonged distribution of dexamethasone over six months is made possible by the slow breakdown of this matrix into lactic and glycolic acids [82].

For orbital wall fracture reconstruction

Jang *et al*. and Kim *et al*. evaluated the precision of postoperative implantation between unsintered hydroxyapatite particles and poly-L-lactic acid (uHA/PLLA and PCL) meshes in ocular fracture repair [83]. After six months, they noticed no ocular movement restriction in the patients. Nevertheless, there were no notable variations in the surgical results, such as enophthalmos and diplopia, nor were there any problems with the two well-liked implants [84].

Future Impendings

The Food and Drug Administration (FDA) states that it is acknowledged that proving bio equivalency particularly for non-solution dosage forms can be difficult, particularly for locally acting ophthalmic medicines [85]. When creating a pharmaceutical bioequivalent implant, it's critical to understand how the physicochemical characteristics of ocular implants relate to their bioavailability [86]. To get beyond various ocular barriers, the best possible delivery system should be created with better medication bioavailability and dose-controlled release of the drug at the precise location of action. Future applications of long-acting ocular drug delivery technologies advances include

preventative treatment at an earlier stage of disease and economical treatment of ocular disorders in global health settings, as well as restricted therapeutic options for some conditions. The ongoing technological developments in the field of ocular implants, such as improved biocompatibility, safety, and product design, are also driving the market's expansion [90]. Though there are a number of positive aspects, the growth of the Ocular Implants market is expected to be hampered by a few critical factors, including low diagnosis rates, a shortage of skilled workers, and side effects from glaucoma and cataract surgeries that can cause long-term vision impairments.

Recent Approval of Biodegradable Implant

In order to treat ocular hypertension and open-angle glaucoma, the US Food and Drug Administration recently approved Durysta, an implant that contains bimatoprost. It offered a prolonged duration of release. It offers timolol (BD–twice daily) an alternate course of treatment. The intraocular pressure has progressively dropped. [SOURCE: MEDSCAPE]

CONCLUSION

A lot of promise exists for treating ocular illnesses with medication delivery technologies based on biodegradable polymers. In particular, these devices present a viable remedy for numerous drawbacks of traditional ocular medication delivery techniques, particularly in the management of visually impaired retinal disorders. Numerous ocular drug delivery methods have been studied, such as controlled drug release, drug targeting, and drug penetration augmentation. For controlled-release systems, biodegradable polymers have been frequently employed as the drug carrier. When biodegradable polymers break down and eventually become absorbed by the body, the medication is released from them. Numerous ocular medication delivery systems that employ various biodegradable polymer types have been investigated. When it comes to biodegradable drug delivery methods, some polymers are well-researched and have a good ocular safety profile, while others are still in the experimental stage of development. When new vehicles are developed that are designed for regulated and sustained drug administration to treat disorders that threaten vision, biodegradable polymers have the possibility to completely alter the way that drugs are delivered to the eyes. To improve the safety and effectiveness of biodegradable ocular formulations, assure quality control and safety, and comprehend the underlying mechanisms of action of these kinds of systems and research on the effectiveness and safety of these delivery methods in various eye conditions is now underway in clinical trials.

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Conflict of interest

The authors do not have any conflict of interest.

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Table 1 Some of the properties of biodegradable polymers

Properties	Unit	PCL	PLA	PGA
Molarmass	$g.mol^{-1}$	114	88	58.04
Physical state	_	Semicrystalline	Amorphous glassy polymer to semi-crystalline	Rigid material
Degree of crystallinity	%	69	<10	45-55
Melting temperature	˚C	60	150-160	224-230

Table 2 Some examples of currently marketed products:

Ocular Implant	COMPOSITION	INDICATIONS
Surodex®	Dexamethasone and PLGA with Hydroxypropyl Methylcellulose (HPMC)	Phacotrabeculectomy surgery, treatment of intraocular inflammation
Verisome™	Triamcinolone Acetonide (TA)	Treatment of chronic cystoid macular oedema
Ozurdex®	Dexamethasone0.7 mg	Treatment of Irvine-Gass syndrome, diabetic macular oedema

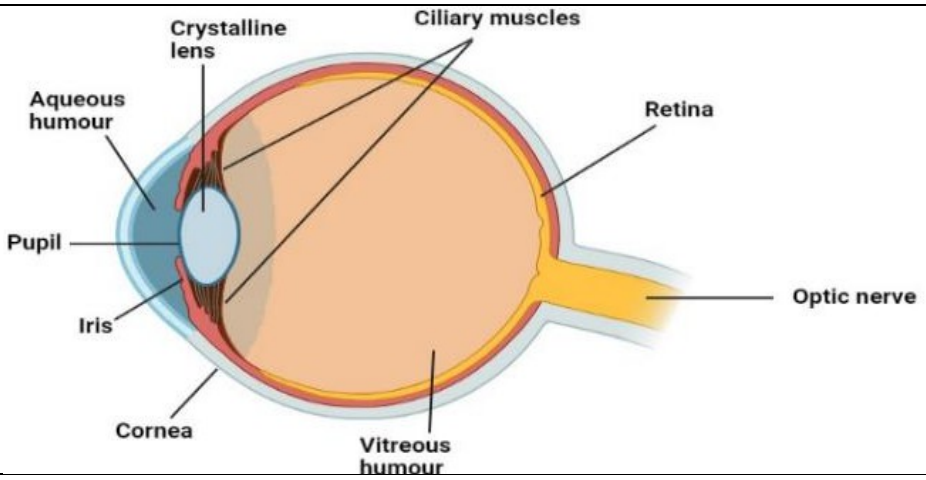


Figure 1: The Human eye-our windows to the outside world

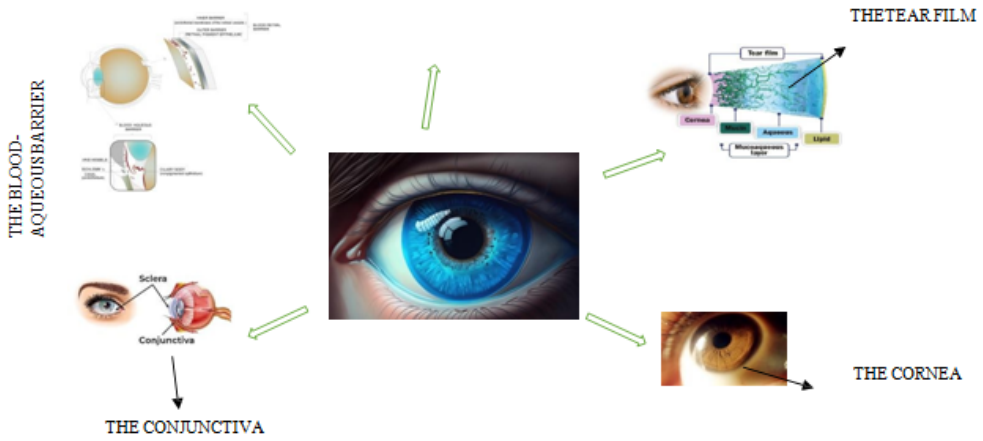


Figure 2: Barriers of the human eye to various medications used in the management of eye

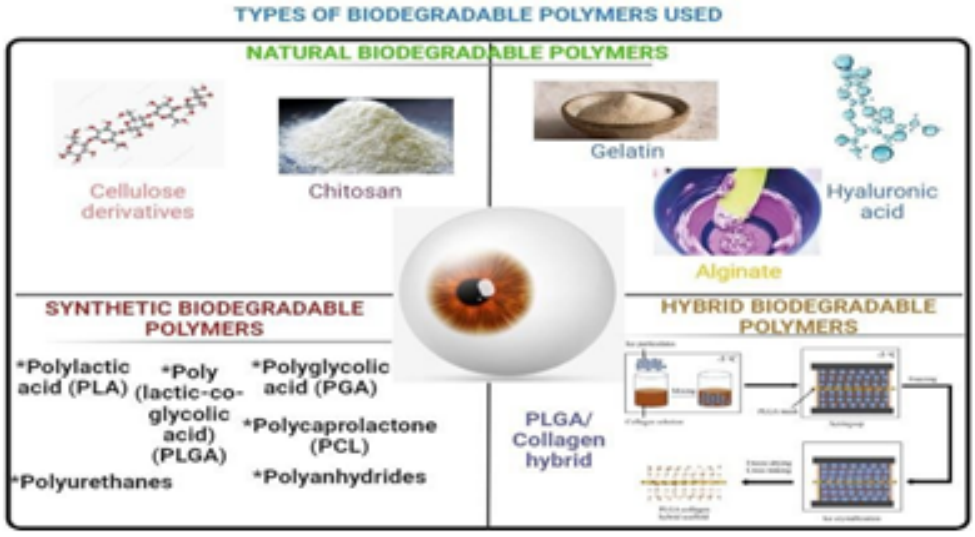


Figure 3: Types of biodegradable polymers used for the ocular implants

RESEARCH ARTICLE

Beyond the Itch: Exploring Miasm's Role in Psoriasis through a Homoeopathic Lens

Joshi Nidhi P.[1]* and Shah Kamlesh B.[2]

[1]Ph.D Scholar, Department of Organon of Medicine, Parul University, Vadodara, Gujarat, India.
[2]Professor and Guide (Ph.D Supervisor), Department of Organon of Medicine, Parul University, Vadodara, Gujarat, India.

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***Address for Correspondence**
Joshi Nidhi P.
Ph.D Scholar,
Department of Organon of Medicine,
Parul University, Vadodara, Gujarat, India.
E.Mail: dr.nidhijoshi21@gmail.com, 200400301001@paruluniversity.ac.in

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ABSTRACT

Psoriasis is a common, chronic and non-infectious skin disease characterized by well-defined slightly raised, dry erythematous macules with silvery scales and typical extensor distribution. The recent lifestyle changes are responsible for the most obstinate, mixed miasmatic skin diseases of the present day. Psoriasis is a chronic painful non contagious condition. It is seen at any age and both the sexes. It involves the skin and nails, and is accompanying with other comorbidities. Skin eruptions are localized or generalized, mostly symmetrical, sharply demarcated, red papules and plaques, and usually covered with white or silver scales. Lesions cause itching, stinging and pain. The dry flakes of skin scales result from the excessively rapid proliferation of skin cells. According to WHO Psoriasis is estimated to affect 2-4% of the population of the western world[3]. Disease according to homeopathy may be psychological, inherited, miasmatic or multifactorial and multifaceted. The miasm is responsible for chronic disease in the body. Psora leads to the inflammation, sycotic leads to the proliferation and syphilitic miasm leads to the destruction in the body[1]. Evaluate the Miasmatic role in treatment of Psoriasis. A 37 years old female, came with complaints of severe itching on dorsal surface of hands with scaling bilaterally, since 1.5 years. The itching was followed by burning and bleeding. For this she had taken many allopathic medicines which had not given any relief. The treatment was started with *constitutional remedy* which gave relief temporarily. After the miasmatic evaluation of case *Anti miasmatic* medicine was given which gave rapid improvement. This study shows significant role of miasm in treatment of psoriasis.

Keywords: Psoriasis, Homoeopathy, Constitutional Remedy, Miasm

INTRODUCTION

The current lifestyle changes may land up into the most obstinate, mixed miasmatic skin diseases of the present day. Psoriasis is a non-contagious chronic condition. It is seen at any age and both the sexes. It involves the skin and nails, and is accompanying with other comorbidities. This skin eruptions are mostly symmetrical, sharply demarcated, red papules and plaques. They generally covered with white or silver scales. Lesions cause itching, stinging and pain. The dry scales result from the excessively quick proliferation of skin cells. According to WHO Psoriasis is estimated to affect 2-4% of the population of the western world. Disease according to homeopathy may be psychological, inherited, miasmatic or multifactorial and multifaceted. The miasm is responsible for chronic disease in the body. Psora leads to the inflammation, sycotic leads to the proliferation and syphilitic miasm leads to the destruction in the body[1].

Objective
To evaluate the Miasmatic role in treatment of Psoriasis

Case History
A 37 years old female, came with complaints of severe itching on dorsal surface of hands with scaling bilaterally, since 1.5 years. The itching was followed by burning and bleeding. For this, she had taken many allopathic medicines which had not given any relief.
Occupation: House maid
P/H: Malaria before 7 years
Obs and gynae History:
Menarche: 13years
Complaints related to Menarche, if any: no
Last Menstrual Period: 12/6/2024
Details of Menstrual cycle: regular, partial clotted and liquid, sometime mild lower abdominal pain
OBG: G5P3L3A2
History of abortion is there, having two daughters healthy, and one son after 2 abortions. Very precious child for her,
Miasm:
Dominant miasm: syco syphilitic
Fundamental miasm: syphilitic

Miasmatic analysis(Table: 1)

General Examination(Table: 2)

TOS
Unbearable itching,
Itching < at night
Eruptions on dorsal surface of hands bilaterally,
Itching till bleeding

RESULT

The treatment was started with constitutional remedy which gave relief temporarily. After the miasmatic evaluation of case Anti miasmatic medicine was given which improvement.(Fig: 1)

Prescription(Table:3)
Pasi Score (Table: 4)

Evidence of case
(Fig.2), (Fig.3), (Fig.4), (Fig.5), (Fig.6)
The treatment was started with constitutional remedy which gave relief temporarily. After the miasmatic evaluation of case Anti miasmatic medicine was given as this case was trimiasmatic representation so I have to select that medicine which cover all three miasm and which gave rapid improvement. Here sulphur act as ant miasmatic remedy. This sulphur is one of the deep acting polycrest remedies in homoeopathy.

CONCLUSION AND DISCUSSION

According to Dr. Hahnemann diseases are dynamic in origin and these dynamic diseases are classified in to acute and chronic[3]. Chronic diseases are due to chronic miasms. These miasms are inherited in our body, when these chronic diseases are treated with Antimiasmatic remedies it acts on the vital force and improves the immunity of the patient[2].Whenever obstacle occur in cure we have to think on miasmatic side in chronic cases. Inner miasm may have major impact on disease and because of it this disease persists.In base of pathology we can say inflammation represent psora, proliferation depict sycotic miasm whereas destruction depict syphilitic miasm. People having psoriasis are frequently defamed and excluded from normal social group. They commonly experience the loneliness, isolation and feelings of being ugly. This may impact them mentally and physically.[4] Here, on miasmatic relation the patient in this instance is more susceptible to syco-syphilitic miasm, which can cause skin damage. This Antimiasmatic treatment treats the patient holistically and produces superior outcomes. PASI score is 0 after the treatment before treatment it was 6.We can conclude that Homoeopathy is effective in reducing the severity of case and it improves the quality of life.

Conflict of Interest

The author declares no conflict of interest.

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Table. 1: Miasmatic analysis

Sr. no.	Symptoms	Analysis and evaluation	Miasm
1.	Unbearable itching	Physical particular	Psoric
2.	Itching at night	Physical particular	Syphilitic
3.	Eruptions on dorsal surface of hands	Physical particular	Sycotic
4.	Itching till bleeding	Physical particular	Syphilitic

Table. 2: General Examination

Appetite: satisfactory, 2 times meal/ day	**Sweat: In summer under the axilla**
Taste: Not specific	Sleep: 7-8 hrs, sound sleep
Thirst: Approx. 2 liters /day, matka water	Dreams: Not specific
Food (foods, drinks & others) Ailments from: - Aggravation: Sour Amelioration: Not specific Aversion: Nothing Craving: Nothing	Habits: Not specific
Urine: 6-7 times/ day	Addiction: No
Stool: Regular, once day	Tendencies/Recurrent complaints: skin disease
Thermal history: Hot patient	

Table. 3: Prescription

Date	Symptoms	Medicines	inference
9/7/2024	Unbearable itching, itching at night	Natrum carb 200 /1 dose, Sac. Lac. /7 days	Waiting for action of drug
17/8/2024	Itching is still there, white scaling, spreading more	Sac. Lac. /15 days	Partially acting drug
6/9/2024	Itching still present and <at night	Sulph 200/ 1 dose state, followed by SL/15 days	Waiting for action of drug
6/10/2024	Itching reduced, patient feels better no bleeding	Sac. Lac. 30/15 days	Medicine is acted well
20/10/2024	Itching is there, no bleeding	Sulph 200/ 1 dose state, followed by SL/15 days	Medicine is acted well
4/11/2024	Itching is decreased, no bleeding	Sac. Lac. 30/15 days	Medicine is acted well

Table. 4: PASI Score

Before treatment	After treatment
Redness -1	Redness -0
Thickness- 2	Thickness- 0
Scaling -3	Scaling -0
Total score- 6	Total score- 0

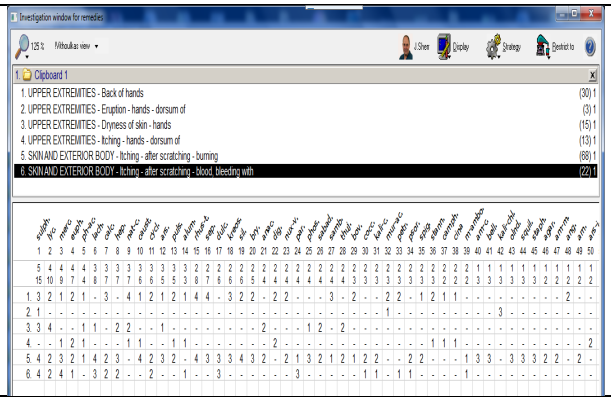


Fig: 1



Fig. 2: 9/07/2024



Fig. 3: 17/08/2024



Fig. 4: 6/10/2024



Fig. 5: 20/10/2024



Fig. 6: 4/11/2024

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RESEARCH ARTICLE

Survey on Multi-Modal Sentiment Analysis in Tamil and Malayalam

Sumy. T .O[1]* and Vinoth A[2]

[1]Ph.D Scholar, Department of Computer Science, Sri Krishna Adithya College of Arts and Science (Co-Education), Kovaipudur, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Computer Science, Sri Krishna Adithya College of Arts and Science (Co-Education), Kovaipudur, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
Sumy. T .O
Ph.D Scholar,
Department of Computer Science,
Sri Krishna Adithya College of Arts and Science (Co-Education), Kovaipudur,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: sumyto84@gmail.com

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ABSTRACT

Sentiment Analysis (SA) is the analysis or computable derivation of sentiment and opinions held by individuals or groups. Sentiment analysis helps to determine positive, negative and neutral sentiments. Thereby, it helps to understand public opinion or emotional tone. The previous works on sentimental analysis from 2019 to 2024 are surveyed in this paper. This survey utilizes the multi-modals for sentimental analysis in Tamil and Malayalam. For this paper, 31 journal articles are examined for sentimental analysis in Tamil and Malayalam. It aids in the integral of underutilized modalities such as gesture or cultural expression into SA for Tamil and Malayalam in future and works with larger annotated datasets for Tamil and Malayalam sentiment analysis. This paper analyzes the accurate SA in Tamil and Malayalam, based on the findings. Earlier researches use two most used approaches, that are Convolutional Neural Networks (CNNs) and Recurrent Neural Networks (RNNs). This survey assesses sentiments such as positive, negative and neutral in Tamil and Malayalam. These sentiments are analyzed with culturally specific expressions including idioms and nuances showing its regional and linguistic characteristics. It includes local context bound unique emotion categories like reverence, community pride and sorrow in poetry and traditional media. This paper concludes with the future research for multimodal sentimental analysis in Tamil and Malayalam.

Keywords: Malayalam**,** Negative, Neutral Sentiment, Positive, Sentiment analysis, Tamil

INTRODUCTION

Sentiment Analysis (SA) refers to the method of categorizing regards defined within a piece of text data as favorable, adverse, and optionally moderate. It is a valuable tool in numerous domains, including Social Media (SM) monitoring and consumer opinion evaluation used in research on markets. Traditionally, the sentiment analysis is applicable to text. Although it is no longer really cutting edge, multi modal sentiment analysis, integrating text, audio and images has greatly increased the precision and granularity of sentiment interpretation. Depending on the combination of these modalities, researchers and practitioners pick up a broader palette of emotional expressions, where language is not offered enough contexts. The languages such as Tamil and Malayalam both spoken in Tamil Nadu and Kerala states respectively are filled with deep cultural and historical connotations and are specked with their own linguistic features. These languages are the big challenge for conducting sentiment analysis as each of them uses different idioms, expressions and has its own regions variations. Further, this also complicated the analysis of sentiment in Tamil and Malayalam since cultural and contextual factors are used to represent sentiments. For instance, if it is humor, sarcasm or quoting something using the regional slang, those things are not translated well and the standard sentiment analysis often misses those out. In recent years, the rise of Tamil and Malayalam content on social media in these non textual formats like memes, video and voice recordings is exponential. Sentiment analysis on these types of content is actually quite complex as they're combined with images, audio and video along with the written text. For example, video contains tone of voice and the visual elements in a meme give more emotional context to the meme than text alone. Therefore, multi-modal approach is needed for sentiment analysis of such mixed media content. Tamil and Malayalam sentiment analysis from multi-modal signals faces at least 3 challenges; the difficulty of inferring non verbal cues such as facial expressions or tone of voice. However, Deep Learning (DL), Natural Language Processing (NLP), and Computer Vision (CV) approaches enable sentiment identification. New methodologies are being explored by researchers on combining data from different modalities so that nuances in sentiment are detected when other methods fail to do so. Additionally, it presented an overview of the existing work on research for sentiment analysis for these regional languages, along with recommendations for future directions and improvements based on existing studies and methodologies. The flowchart depicted in figure 1 represents the reasons for the sentimental analysis in languages, especially Tamil and Malayalam. It includes the complexity, dialects, shuffling, idiomatic expressions, phonetic variation and cultural contents present in media. Thus, sentimental analysis is carried over the languages.

LITERATURE REVIEW

Sentiment analysis used multiple modalities or data modalities like text, audio and images to better understand sentiments. The problem of NLP in Tamil and Malayalam, which were two widely spoken languages in southern India, lies in their linguistic structures, cultural peculiarities and regional variations. Sentiment analysis across these modalities provided much finer grained and accurate sense of sentiment in an application like social media monitoring or for understanding customer comments. However, with rise of regional language content there was an increasing need for effective tools that were capable of capturing the subtleness of sentiment in such languages. Anierudh *et al*. (2024) utilized Machine Learning (ML) approaches for integrating multimodal data analysis for the Dravidian languages. Thus, Dravidian languages, mainly spoken in southern India presented a set of challenges because the languages were linguistically complex and typically had not provided the annotated data necessary for computational processing. This existed huge amount of multimodal data (text, images, videos) generated by social media platforms that required sophisticated analytical approaches to infer context, sentiment among other useful user behavior. This research augmented progress in ML in order to tackle these challenges and it contributed to the area of language technology for underrepresented languages. Table 1 compares the various author works that are relevant to the multi-modal sentimental analysis. The critical problem of building NLP solutions for low resource languages that lacked annotated data and linguistic resources was prevalent as addressed by Antony (2020). The focus of this research was to discover language agnostic features in order to build NLP models that were robust to

diverse linguistic systems. This study aimed to overcome resource constrains by utilizing multilingual datasets and Transfer Learning (TL) techniques. Thus, field of NLP was pushed for lesser studied languages.

In addition to foundational for making language technologies accessible and effective for underrepresented linguistic communities, this work raised core questions about the human language data, included the design of appropriate models to learn. Banavatu and Parthasarathy (2024) presented an adaptive DL framework to recognize handwritten characters from South Indian languages that tend to have intricate scripts and unique structural patterns. Character recognition of handwritten characters in these languages was complicated by high variability in writing styles and complex alphabets. Advanced adaptive DL techniques were utilized in this study to overcome these challenges when improving accuracy and efficiency. The results of this research enabled to digitize regional scripts. Further, computational linguistics were progressed for South Indian languages. Table 2 contrasted various works of multi modal sentimental analysis from 2019 to 2024. Bhaskar and Madathil (2022) overviewed audio-visual speech recognition (AVSR) technologies that were developed. Traditional speech recognition systems failed in noisy environment, needing to compartmentalize visual cues such as lip movements and facial expressions. First, this study addressed the problem of AVSR and second, it identified the challenges in developing robust multimodal AVSR systems. This work of Chakravarthi *et al*. (2022) highlighted the linguistic and contextual difficulties in recognizing prejudiced language, especially in under resourced languages. This research was built on the progress in NLP for creating tools to detect and counter harmful content. This research helped to create a more inclusive and diverse web. Hence, safer online environments were enabled. Comparison of the works relevant to sentimental analysis is carried out in table 3. In Chakravarthi *et al*. (2024), a shared multi-task classification task aimed to detect misogyny and trolling in online memes while solving challenges in that detection. As content that combined image and text, memes presented specific challenges for understanding the acceptance of harmful implicit and explicit messages. This study proposed the importance of solving such toxic online behavior using advanced ML techniques. This research built robust content moderation tools by promoting equality and inclusivity in digital communication. Dar and Delhibabu (2024) investigated audio emotion detection by integration technique of CNN, LSTM and RNN. While exploring the complexities of the Kashmiri language, this work aimed to understand specific speech patterns and what impact they had on the model's performance. The detection of emotion from audio data was extremely necessary for applications such as sentiment analysis and human computer interaction. This research increased the understanding in computational methods for taking care of neighborhood and low asset language.

Existing Methodologies

Multilingual - Bidirectional Encoder Representation in Transformers

Multilingual BERT creates deep bidirectional representations by jointly conditioning on bi-directional contexts in every layer by pre-training on unlabeled texts. A simple classifier for emotional analysis was constructed by simply adding one extra result layer in previous-instruct BERT model. It typically grasp in two training goals, such as, Masked Language Modeling (MLM): With some of the input tokens made at random, the model will predict which one should be placed in that mask. This enables the method to operate in both end phase settings. Next sentence prediction (NSP): The intension is to previous-trained text pair representations for subsequent question responding where; the task is to determine if two phrases appear afterwards. This model in multilingual version works on 104 languages. As a first token for all sequences, there is a special classification token (CLS). Lastly, these two tasks such as aggregation sequence and classification are done on the last hidden layer for this token (Kapil, P., *et al*. (2023)). The flow chart in figure 2 describes the Multi-lingual BERT model. Here, the model works by loading, feeding input, preprocessing it, which is further fed into the model and trained with the labeled sentimental data for the repetition of this process. Finally, if the results are satisfactory, the process is ended.

Modern models for Sentiment Analysis

In research community, sentiment analysis is an area which is already well studied. The major aim of this is to find out the sentiment or the opinion of language. The models classify each of sentiments through positives, negatives or neutral feelings. In the earlier times, sentiment analysis is performed using lexicon based methods in which the sentiment polarity of words is assigned using predefined sentiment lexicons or dictionaries. Some of the modern

models used for sentiment analysis are RNNs, CNNs and LSTM networks, made possible with the advance of DL techniques. So far, these models have the ability to demonstrate good patterns of sentiment contained with text. Also, transformer based language models such as BERT is currently popularly used in many tasks, and sentiment analysis is among them due to the good performance and effectiveness of BERT. Sentiment analysis has also used other modalities like images, audio and video. It has resulted in this extension becoming a multimodal sentiment analysis algorithm, which aims to analyze the sentiment across multiple modalities to understand the sentiment of different languages. More comprehensive sentiment analysis is done by capitalizing on multiple modalities (Das, M., *et al*. (2023))

Monolingual Models

For these experiments, six monolingual models are used: the one task fine tuned model, along with five general models. DistilBERT and HindiBERT are trained in Hindi only, BanglaBERT roBERTa is instruct on Bangla and DistilBERT and BERT are trained in English only. Last but not least, the English task is used to fine tune emoBERTa model Bilingual models, BanglishBERT and HingBERT, are adopted for bilingual models which are instruct on both Bangla-English and Hindi-English. In additional, emoBERTa is the fine tuned English task model used. Bilingual models like BanglishBERT is used for Bangla and HingBERT for Hindi, as the models are trained on Bangla-English and Hindi-English respectively. Later, multilingual models, mBERT and XLM-R pre-trained on 104 and 100 languages are utilized. It also includes Bangla, English and Hindi among those. This work describes IndicBERT which is used for 12 Indic languages and 17 languages for Bangla-English and Hindi respectively. Raihan, N., *et al*. (2024)

Training and Testing in Cross-Lingual

The main contribution to the cross lingual approach is to train the few shot model so that it generalizes over different languages. In training, the model is given data from all languages and learns about speech by capturing the nuances in which various context and language carry through the advantage of pre trained representations. Method is trained in a cross lingual way which allows it to learn the recognized sentiments in Indian languages, which is necessary for powerful production in minimum way outline where data is limited for individual languages. The model is made better able to generalize and detect sentiments such as positive, negative or neutral even without specific language data. In the testing phase, the model performance is tested in individual languages and tests the model ability to adapt and classify audio samples in language-specific setting regardless of model training with a diverse set of languages. This setup presents a scenario similar to the one seen in practice, where a model trained on data across multiple languages is able to rapidly adapt and perform well on new languages with limited labeled data. This approach is effective, and the model is able to perform well across the number of shot settings. Sankaran, A. N., *et al*. (2024)

Deep Learning Models

Data is transformed using word2vec and GloVe embeddings models. Once transformed, the layers of data are fed into applied ML models for classification. Data is used to apply the DL model. According to this work, the sentiments in corresponding pre trained word vectors and equate the word to vector. To convert Word2vec model into the vector of length 300 for instance, make it into vector of length 200 for GloVe model. One embedding layer, one LSTM layer and one dense layer are the part of DL model. Input dimension of embedding layer is the number of particular words found in corpus. Data is taken and processed via LSTM layer, propagated forward, passes information. It too is connected deep for implementing action, but get their input from the neurons of earlier layer. In this fully connected DL model which is classifying data into part of list like negative, neutral, activation layer is the sum of weighted input changed into output. Priyadarshini, I., Sahu, S., and Kumar, R. (2023).

Bilingual Translation Pairs

The translated corpus is obtained using the bitext mining system in exact way as it is used for getting the translations. Text corpora from 16 Indian languages are used for training of a multilingual language model for Indian languages. Training objectives are MLM and Translated Language Models (TLM) in special cases. The latter trains the model

using pairs of translated and transliterated documents and the former one works only on monolingual text, batch size of global batch is 4096, maximum sequence length is 512 and model is trained for 1M steps. Adam optimizer is used with learning rate set to 5e 4 and we have 236M learned parameters in total. As a multilingual model, IndicBERT is developed on extensive corpora consisting of 12 important Indian languages, using ALBERT model. Both Multilingual-BERT and Cross Lingual Roberta (XLM-R) are significantly larger and yet achieve equally high state of art performance in classification task. Based on take advantages of the benefit of relatedness in Indian languages, the model basically learns joint training of all the input languages in the fashion of a single shared model. Kapil, P., *et al*. (2023)

DISCUSSION

The use of DL models and multilingual techniques in field of sentiment analysis has been discussed in this paper. This work shows the transformation from conventional lexicon based sentiment analysis approaches to the current RNNs, CNNs and LSTMs. Thus, these models have been capable of reproducing complex patterns and relationships of textual data and achieved significant performance improvements for the task of sentiment detection. Furthermore, this paper shows the importance of transformer based model like BERT which beat previous methods in many sentiment analysis task. This research also includes multilingual and cross lingual angle, especially for the Indian languages like Bangla, Hindi and English. Indian languages with already trained multilingual methods such as mBERT, XLM R, IndicBERT tuned for sentiment analysis tasks are presented. Utilizing these models is useful for dealing with the challenges like low resource data scenarios and improving sentiment detection as well across the diverse linguistic and cultural landscapes. Furthermore, this paper surveys about the pre trained word embeddings like Word2Vec or GloVe and convert each word to a vector representation. Therefore, those word embeddings are input into a DL model such as LSTM network to classify sentiment within classes such as positive, negative and neutral. Deep Learning architectures examined here admits high accuracy for the model to effectively model sentimental analysis in data. Lastly, Bilingual Translation Pairs are presented to train multiple languages for multilingual sentiment analysis and explain the shared training of adjacent languages for improve efficiency and accuracy. This overall framework integrates the benefits of DL, multilingual models and transfer learning to become a valuable sentiment analysis framework spanning across multiple languages, even when the language is of lower resources. Such a multi faceted approach has tremendous potential for research in future on the area of NLP for the applications such as sentiment analysis with relevance to the Indian context.

CONCLUSION

This survey has examined 31 journal articles, which are relevant to the multimodal sentimental analysis in Tamil and Malayalam. The surveyed papers are taken between the year duration of 2019 and 2024. After the study of various previous works, the sentimental analysis such as positive, negative or neutral is recognized. Among those sentiments, neutral and negative are majorly assessed. The exploitation of existing methodologies has significantly analyzed the sentiments. This survey provides a way using which it is possible to point out the challenges, techniques and resources for multi modal SA in Tamil and Malayalam. In addition, the present survey examines the effectiveness of combining text, speech and visual data for sentiment detection in these languages. This paves the way for future research to build more robust multi-modal models, with the capacity to incorporate regional linguistic nuances and various data sources, to perform sentiment analysis on Tamil and Malayalam.

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Table 1: Related study comparison from 2022 to 2024

Author(s) and Year	Title	Key Focus	Methods	Application
Das, M., Barman, S., and Chatterjee, S., (2023)	Hope Speech Detection (HSD) utilizing Transformer-deployed Methods	Detecting hope speech from texts related diversity and inclusion	Transformer-based models (e.g., BERT, RoBERTa)	Language technology for equality
Divija, S. G., and Surya, R. M., (2023)	Deep Reinforcement Learning (DRL)based method regarding Bridge Health Maintenance	Health administering using reinforcement learning	Deep Reinforcement Learning	Bridge maintenance
Ghous, H., *et al*. (2024)	Navigating Sarcasm in Multilingual Text	Sarcasm detection in multilingual texts	NLP techniques, DL models	Sarcasm detection in multilingual texts
Gupta, V., *et al*. (2022)	HSD for fairness, unfairness, and incorporation	HSD in multilingual contexts	BERT-based transformer models	Equality and diversity in language

Jain, D. *et al.* (2024)	Knowledge-deployed Data Processing (KDP) for Multilingual Natural Language Analysis (MNLA)	Multilingual text processing for knowledge extraction	Rule-based systems, NLP models	Multilingual natural language analysis

Table 2: Analyzing relevant study comparison for 2019 to 2024

Author(s) and Year	Title	Methodology	Focus Area	Application Context
Kumar Jain, D., *et al.* (2023)	KDP regarding MNLA	Rule-based systems, NLP models	Natural language analysis	Multilingual context analysis
Lavanya, S. K., and Navaneethakrishnan, S. C., (2022)	Developing a Tamil text data record on LGBTQIA and dialect identifying using multilingual BERT	Multilingual BERT, text classification	language detection, LGBTQIA	Dataset development for language detection
Madhu, H., *et al.* (2023)	Detecting Speech in Conversational Code-mixed Dialogue on SM	Contingent data record, study	Code-mixed SM speech observation	SM, offensive speech detection
Philip, J., *et al.* (2019)	Baseline Neural Machine Translation System for Indian Languages	Neural Machine Translation (NMT)	Indian languages translation	Multilingual text translation
Rahman, M., *et al.* (2024)	Multimodal Language Detection in Tamil	Fused ML along with DL	Abusive language detection in Tamil	Tamil language offensive speech detection

Table 3: Sentimental analysis studies related work comparison from 2020 to 2024

Author(s) and Year	Title	Methodology/Model	Application
Raihan, N., *et al.* (2024)	A Code-diverse Data record for Bangla-English-Hindi reaction observation	Code-diverse data record, emotion detection techniques	Multilingual emotion detection
Rudrappa, N. T., and Reddy, M. V., (2023)	HiTEK Multilingual Speech Identification Using Combinatorial Model	Combinatorial model for multilingual speech recognition	Speech identification in multilingual contexts
Sankaran, A. N., *et al.* (2024)	Approaching Cross-Lingual Acoustic Misuse Observation for -Resource Conditions via Minimum-Shot Knowing	Few-shot learning techniques	Cross-lingual audio abuse detection
Suryawanshi, S., *et al.* (2020)	Multifaceted Meme Data record (MultiOFF) for recognizing rude the contents of images and texts	Data record of images and text for detecting offensive content	Trolling, violent, and cyber harassment
Suryawanshi, S., *et al.* (2020)	Troll data record Classification of Tamil Memes	Data record for meme analysis and troll classification	SM content classification
Vaidhegi, D., *et al.* (2023)	Homophobia/Transphobia Detection in SM Comments Using Linear Classification (LC) methods	LC methods	SM comment analysis

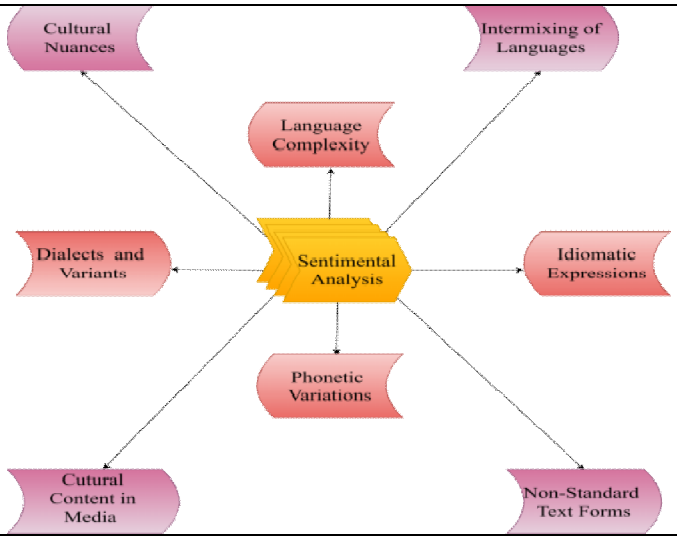


Figure 1: Causes for Sentimental Analysis

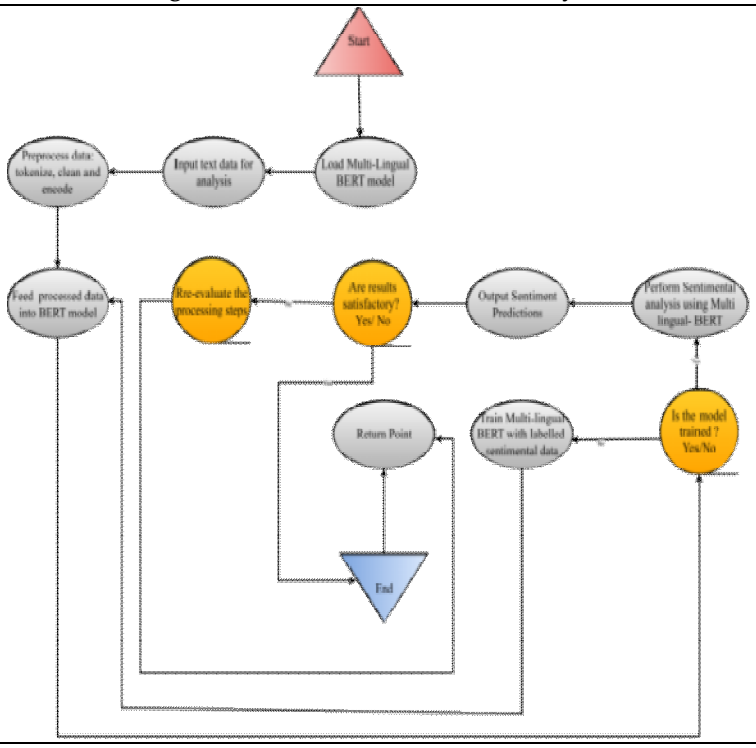


Figure 2: Flow chart of Multi-lingual BERT

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RESEARCH ARTICLE

Study the Dynamic Behavior of the Enzyme - Substrate Reaction for an Un-Competitive Inhibitors using Mathematical Modeling

Jyoti Kumawat[1]* and Kaushal Patel[2]

[1]Research Scholar, Department of Mathematics, Veer Narmad South Gujarat University Surat, Gujarat, India.
[2]Professor, Department of Mathematics, Veer Narmad South Gujarat University Surat, Gujarat, India.

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***Address for Correspondence**
Jyoti Kumawat
Research Scholar,
Department of Mathematics,
Veer Narmad South Gujarat University Surat,
Gujarat, India.
E.Mail: jyotikmwt13@gmail.com

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ABSTRACT

Biological reactions in anthropology are multifaceted study. These reactions are analysed through Enzyme kinetics using Mathematical modelling, which help predicting enzyme behaviour in living organisms. Enzyme inhibitors are compounds that modify the catalytic properties of the enzyme and, therefore, slow down the reaction rate, or even stop the catalysis. Such inhibitors work by blocking or distorting the active site. In particular, Un-competitive inhibitors impair reaction progress by binding to the enzyme-substrate complex. At any given time, the Un-competitive inhibitor can bound to the complex generated by enzyme -substrate reversibly other than the active site. That is, the inhibitor and substrate both combines to the enzyme at different sites. In order to cope with the exact theatrical solution for the enzymatic reaction having analytical solutions till date. The perseverance of this work is to derive the numerical solution of the system of mathematical model of enzyme reaction in the influence of Un-competitive inhibitor with convergence criteria along with the stability analysis. The use of the Next Generation Matrix method and calculation of the reproduction number for the stability of our model is proceeded by the existence of possible equilibrium points with their restrictions, followed by sensitive analysis. We derive asymptotically stable solutions to the model with numerical simulation.

Keywords: Kinesiology of Un-Competitive inhibitor on Enzymes; System of Non-linear equations for reaction; Next Generation Matrix Technique (R_0); Stability Analysis; Asymptotic Behaviour; Sensitive Analysis.

INTRODUCTION

Biological reactions in anthropology are multifaceted study. To fully understand such intricate reactions, one must explore beyond the confines of chemical and biological tools and look towards mathematical modelling and model reduction techniques to provide a vast array of analysis tools for such models. Majority of physiological reactions are analysed through Enzyme kinetics using Mathematical modelling, which help predicting enzyme behaviour in living organisms. The Protein molecules Enzyme increase the rate of chemical reactions or chemical process in human body. These BIOCATALYSTS act upon molecules called Substrates to generate products rapidly, deprived of being absorbed during the procedure. Enzymes are precise by their kinetic performance of binding with the substrate for product formation, which is much more enticing. These enzymes bind with Enzyme Activators or Enzyme Inhibitors and bestow or deprive the working speed of reactions respectively. Enzyme inhibitors have several categories among which one is reversible inhibition which again has three types as competitive, Non-Competitive and Un- competitive inhibition. We can cure number of pathological conditions by using enzyme inhibitors. Sometimes receptor blocking is not getting net effect what we want, at that stage we can inhibit target enzyme which give proper and perfect result[1,2]. Binding of Uncompetitive inhibitors are restricted to enzyme-substrate complex and hence it cannot bind to free enzyme. Their working mechanism is through structural distortion of the active site, followed by the binding of inhibitor to the enzyme-substrate complex. Binding of the inhibitor does not prevent substrate binding, but may inactivate the enzyme so that no products are formed[3]. The enzyme inhibitor is judged by its specificity and potency and their high value ensure its less side effect and toxicity. Concentration of enzyme inhibitors increased; activity of inhibitors decreased. Enzyme inhibitors are not only used for human being but also used for other purposed like control pests in farm as a 'pesticides'[4,5]. They impair enzymes catalytic activity through steric hindrance, aggregation, and/or conformational alterations. Their mechanism of action varies depending on the enzyme and, in most cases, leads to the neutralization of enzyme activity[6]. Enzyme inhibitors have been demonstrated to have a significant impact in many biological and beneficial fields, such as fertility control,[7] neurological disorders,[8] skin diseases,[9] or lysosomal storage diseases, [10,11]to name a few. Several mathematical works have explored models of biochemistry using differential equation systems. Biochemical reaction networks, such as model reductions, Quasi-equilibrium manifold approximations, total-quasi-steady-state approximations, model reductions in chemical dynamics, slow invariant manifolds, singular perturbations, thermodynamic estimates, reaction graph analysis by smearing mathematical modelling techniques have been applied to enzyme inhibitors with slow and fast subsystems[12]. Also a theoretical study of a mathematical model of the amperometric biosensors in uncompetitive inhibition developed analytical solutions to the mathematical model[13]. In order to cope with the exact theatrical solution for the enzymatic reaction having analytical solutions till date. The perseverance of this work is to Formulate the normalized model as a system of nonlinear differential equations and derive the numerical solution of the system of mathematical model of enzyme reaction in the influence of Un-competitive inhibitor to attain convergence criteria along with the stability analysis. The use of the Next Generation Matrix method to compute threshold value and calculation of the reproduction number for the stability of our model is proceeded by the existence of possible equilibrium points with their restrictions, followed by sensitive analysis using all possible parametric values. We derive asymptotically stable solutions to the model with numerical simulation.

METHODOLOGY

The mathematical formulation of the enzyme kinematics problem with Un-competitive inhibitors in biochemical systems has been modeled using ordinary differential equations. The enzyme binds with substrate to form an enzyme-substrate complex. This complex either binds with Uncompetitive inhibitor at a site different from the substrate active site to generates Enzyme – substrate - Inhibitor complex or the complex enzyme substrate releases the product and free enzyme. The free enzyme is then available for another reaction to produce a new reactant. Here the system is written as

The fundamental enzymatic reaction for Un-competitive inhibitor

$$e + s \underset{r_2}{\overset{r_1}{\rightleftharpoons}} es \xrightarrow[r_3]{} e + p\,, \qquad es + i \underset{r_5}{\overset{r_4}{\rightleftharpoons}} esi \tag{1}$$

Where$i$ is the inhibitor, es, and esi are complex intermediate components. The model has five parameters$r_1, r_2, r_3, r_4,$ and r_5. Model variables are $s = [s], e = [e], c_1 = [es], c_2 = [esi]$and $p = [p]$in their normalized concentrations to be 1 mg.

This mechanism illustrates the binding of substrate s and the release of product p in the presence of an Un-competitive inhibitor"i". "e" is the free enzyme, "c_1" is the enzyme-substrate complex and "c_2" is the enzyme-substrate-inhibitor complex. $r_1, r_2, r_3, r_4\ and\ r_5$ denote the measure of the rate of reaction of all processes. Note that substrate binding and inhibitor binding are reversible but product release is not. This chemical reaction, can be converted to a mathematical model using the law of mass action and favorable analysis, the system of following non-linear reaction equations as follows:[14]

$$\frac{ds}{dt} = -r_1 es + r_2 c_1 \tag{2a}$$

$$\frac{de}{dt} = -r_1 es + r_2 c_1 + r_3 c_1 \tag{2b}$$

$$\frac{di}{dt} = -r_4 c_1 i + r_5 c_2 \tag{2c}$$

$$\frac{dc_1}{dt} = r_1 es - r_2 c_1 - r_3 c_1 - r_4 c_1 i + r_5 c_2 \tag{2d}$$

$$\frac{dp}{dt} = r_3 c_1 \tag{2e}$$

$$\frac{dc_2}{dt} = r_4 c_1 i - r_5 c_2 \tag{2f}$$

Where $r_1$is the measure of the forward rate of complex formationc_1, r_2 is the measure of the backward rate constant, r_3 is the rate of product formation and enzyme release,$r_4$is the measure of the forward rate of complex formationc_2, r_5 is the measure of the backward rate constantand $c_1, c_2$are the complex intermediate components.

Consider the normalized initial conditions for substrate s, enzyme e, complex c, and product p as follows

$$s(0) = s_0, e(0) = e_0,\ i(0) = i_0,\ c_1(0) = c_2(0) = p(0) = 0. \tag{3}$$

Where, equation (2e) is directly integrable, and equations (2c, 2f) are reciprocal of each other.

So, adding equations (2c) and (2f),

$$\frac{di}{dt} + \frac{dc_2}{dt} = 0 \tag{4}$$

Also, the initial conditions in equation (3) states that,

$$e(t) + c_1(t) + c_2(t) = e_0,\ c_2(t) + i(t) = i_0 \text{and} c_1(t) + s(t) + p(t) + c_2(t) = s_0 \tag{5}$$

hence, the system of ordinary differential equations reduces, for s, i and $c_1$as

$$\frac{ds}{dt} = -r_1 s(e_0 - i_0 + i - c_1) + r_2 c_1 \quad (6a)$$

$$\frac{di}{dt} = -r_4 c_1 i + r_5(i_0 - i) \quad (6b)$$

$$\frac{dc_1}{dt} = r_1 s(e_0 - i_0 + i - c_1) - (r_2 + r_3)c_1 \quad (6c)$$

Where substrate s, i and complex c_1 has normalized initial conditions $s(0) = s_0, i(0) = i_0$ *and* $c_1(0) = 0$.

The stability of this System

Theorem: *A system of a differential equation is asymptotically stable if and only if its basic reproduction number* (R_0) *is less than 1.*

To determine basic reproduction number using Next Generation Matrix. $FV^{-1}$is next generation matrix, where F and V both are Jacobian of Σ and Λ evaluated for Enzyme, Substrate, Un-competitive inhibitor, productand both Complexes at an equilibrium point[15,16].

So, $\Sigma(X) = \begin{bmatrix} r_1 s(i_0 + c_1) \\ 0 \\ 0 \end{bmatrix}$ and $\Lambda(X) = \begin{bmatrix} -(r_1 s(e_0 + i) + r_2 c_1 \\ -(r_4 c_1 i) + r_5(i_0 - i) \\ r_1 s(e_0 - i_0 + i - c_1) - (r_2 + r_3)c_1 \end{bmatrix}$

Where, $X = (s, c)^t, \Sigma(X)$ denotes the entrance to the compartment and $\Lambda(X)$ denotes transferring to another compartment or leaving the system. Then the Jacobian at the equilibrium point is given by $F = \left[\frac{\partial \Sigma}{\partial X_i}\right]$ and $V = \left[\frac{\partial \Lambda}{\partial X_i}\right]$, *where* $i = 1,2,3$.

As a result,

$$F = \begin{bmatrix} r_1(i_0 + c_1) & 0 & r_1 s \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix} \text{ and}$$

$$V = \begin{bmatrix} -r_1(e_0 + i) & -r_1 s & r_2 \\ 0 & -r_4 c_1 - r_5 & -r_4 i \\ r_1(e_0 - i_0 + i - c_1) & r_1 s & -(r_1 s + r_2 + r_3) \end{bmatrix}$$

Theorem: *If the determinant of a matrix is non-zero then its inverse does exist.*

Accordingly for our model we have, $|V| =$

$$\{-(i_0 + c_1)r_1 r_2 r_5 - r_1(e_0 + i)(r_3 r_5 + s r_1 r_5 + c_1 r_3 r_4) - ({c_1}^2 + i_0 c_1) r_1 r_2 r_4 - (c_1 e_0 + i_0 i + 2c_1 i) s {r_1}^2 r_4\} \neq 0$$

Since determinant of V is non-zero: $V^{-1} = \begin{bmatrix} V_{11} & V_{12} & V_{13} \\ V_{21} & V_{22} & V_{23} \\ V_{31} & V_{32} & V_{33} \end{bmatrix}$

Where;

$$V_{11} = -\frac{r_5(r_2 + r_3) + c_1 r_4(r_2 + r_3) + s r_1(r_5 + c_1 r_4 + i r_4)}{r_1 r_2(i_0 + c_1)(r_5 + c_1 r_4) + (e_0 + i)(r_1 r_3 r_5 + s {r_1}^2 r_5 + c_1 r_1 r_3 r_4) + c_1 s {r_1}^2 r_4(e_0 + 2i) + i_0 i s {r_1}^2 r_4}$$

$$V_{12} = \frac{(r_1 s + r_3)s}{r_2(c_1 + i_0)(c_1 r_4 + r_5) + (e_0 + i)(r_3 r_5 + c_1 r_3 r_4 + s r_1 r_5) + (c_1 e_0 + i_0 i + 2c_1 i) s r_1 r_4}$$

$$V_{13} = -\frac{r_2(r_5+c_1r_4)+isr_1r_4}{r_1r_2(i_0+c_1)(r_5+c_1r_4)+(e_0+i)(r_1r_3r_5+sr_1{}^2r_5+c_1r_1r_3r_4)+c_1sr_1{}^2r_4(e_0+2i)+i_0isr_1{}^2r_4}$$

$$V_{21} = -\frac{ir_4(i_0+c_1-e_0-i)}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$V_{22} = -\frac{i_0r_2+c_1r_2+e_0r_3+ir_3+e_0sr_1+isr_1}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$V_{23} = \frac{ir_4(e_0+i)}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$V_{31} = \frac{(r_5+c_1r_4)(i_0-c_1-e_0-i)}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$V_{32} = -\frac{(i_0+c_1)sr_1}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$V_{33} = -\frac{(r_5+c_1r_4)(e_0+i)}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

As a result, $K = FV^{-1} = \begin{bmatrix} a_{11} & a_{12} & a_{13} \\ 0 & 0 & 0 \\ 0 & 0 & 0 \end{bmatrix}$

Where;

$$A_{11} = -\frac{c_1{}^2r_4(r_2+r_3)+(i_0+c_1)(r_2r_5+r_3r_5)+c_1i_0(r_2+r_3)+(e_0+i)sr_1r_5+(c_1e_0+i_0i+2c_1i)sr_1r_4}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$A_{12} = \frac{sr_1r_3(i_0+c_1)}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

$$A_{13} = -\frac{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)sr_1r_5+(c_1e_0+i_0i+2c_1i)sr_1r_4}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}$$

The basic reproduction number is defined to be the spectral radius which is the magnitude of the largest eigenvalue of the matrix K.

So, $Spectral\ Radius\ \lambda =$

$$-\left\{\frac{c_1{}^2r_4(r_2+r_3)+(i_0+c_1)(r_2r_5+r_3r_5)+c_1i_0(r_2+r_3)+(e_0+i)sr_1r_5+(c_1e_0+i_0i+2c_1i)sr_1r_4}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}\right\}$$

Therefore, $R_0 =$

$$-\left\{\frac{c_1{}^2r_4(r_2+r_3)+(i_0+c_1)(r_2r_5+r_3r_5)+c_1i_0(r_2+r_3)+(e_0+i)sr_1r_5+(c_1e_0+i_0i+2c_1i)sr_1r_4}{r_2(c_1+i_0)(c_1r_4+r_5)+(e_0+i)(r_3r_5+c_1r_3r_4+sr_1r_5)+(c_1e_0+i_0i+2c_1i)sr_1r_4}\right\}$$

Consequently, for all possible positive parametric values, the basic Reproduction number (R_0) of our system of differential equation is less than 1. Hence the system is asymptotically Stable.

Boundedness / Existence of solution

Theorem: *Let* $D \subseteq \mathbb{R} \times \mathbb{R}^n$ *be a closed rectangle with* $(t_0, y_0) \in D$. *Let* $f: D \to \mathbb{R}^n$ *be a function that is continuous in t and Lipchitz Continuous in y. Then, there exists some* $\varepsilon > 0$ *such that the initial value problem* $y'(t) = f(t, y(t)), y(t_0) = y_0$ *has a unique solution* $y(t)$ *on the interval* $[t_0 - \varepsilon, t_0 + \varepsilon]$.

The mathematical model of our system is well-posed. In actuality, Picard's theorem states that its unique solution exist and is constantly dependent on the initial data. Because we are dealing with the human population, we must guarantee that our solutions are positive and bound[17].

Equilibrium Point and their existence

solving the algebraic equations(7a), (7b) and (7c),

$$\frac{ds}{dt} = -r_1 s(e_0 - i_0 + i - c_1) + r_2 c_1 \quad (7a)$$

$$\frac{di}{dt} = -r_4 c_1 i + r_5(i_0 - i) \quad (7b)$$

$$\frac{dc_1}{dt} = r_1 s(e_0 - i_0 + i - c_1) - (r_2 + r_3)c_1 \quad (7c)$$

The equilibrium points of System are defined to be

There are four equilibrium points$E_i(s, i, c_1)$ as

1. $E_1\left(\frac{r_2 c_1}{r_1(e_0 - i_0 - c_1)}, 0, \frac{r_1 s + r_2 + r_3}{r_1 s(e_0 - i_0)}\right)$exists under the condition,

$i_0 = -(s^2 r_1^2 + 2r_2 r_3 - (r_2 + r_3 - c_1 r_1 + e_0 r_1 + s r_1)^2 + r_2^2 + r_3^2 + c_1^2 r_1^2 + e_0^2 r_1^2 - 2c_1 s r_1^2 + 2e_0 s r_1^2 - 2c_1 r_1 r_2 + 2c_1 r_1 r_3 + 2e_0 r_1 r_2 - 2e_0 r_1 r_3 + 2s r_1 r_2 + 2s r_1 r_3 - 2c_1 e_0 r_1^2)/(4r_1 r_3)$.

2. $E_2\left(0, \frac{r_5 i_0}{r_4 c_1 + r_5}, 0\right)$ exists if $i_0 - e_0 = i$.

3. $E_3(0, i_0, 0)$ exists under the condition, $i_0 = e_0 + i$.

4. $E_4\left(\frac{r_2 c_1}{r_1(e_0 - i_0 + i - c_1)}, i_0, 0\right)$ exists for $e_0 + i = i_0$.

Stability Analysis

The local/global stability behaviour of equilibrium points is determined by eigenvalues of corresponding Jacobian Matrix[18-21].

Theorem: *The equilibrium point is asymptotically stable if all the Eigenvalues of a* $n \times n$ *matrix must be negative, provided they are real.*

Theorem: *The equilibrium point is asymptotically stable if the real part of each complex Eigenvalues of a* $n \times n$ *matrix is negative.*

The general Jacobian Matrix M for our model (7a), (7b) and (7c) is given as

$$M = \begin{bmatrix} -r_1(e_0 - i_0 + i - c_1) & -r_1 s & r_1 s + r_2 \\ 0 & -(r_4 c_1 + r_5) & -r_4 i \\ r_1(e_0 - i_0 + i - c_1) & r_1 s & -(r_1 s + r_2 + r_3) \end{bmatrix}$$

Let M_i, Where $i = 1,2,3$ *and* 4 be the matrix evaluated at the equilibrium point $E_i$where $i = 1,2,3$and4.

Then the matrix M_1 evaluated at the equilibrium E_1 is represented as follows

$$M_1 = \begin{bmatrix} -r_1(e_0 - i_0 - c_1) & -r_1 s & r_1 s + r_2 \\ 0 & -(r_4 c_1 + r_5) & 0 \\ r_1(e_0 - i_0 - c_1) & r_1 s & -(r_1 s + r_2 + r_3) \end{bmatrix}$$

Where; $s = \frac{r_2 c_1}{r_1(e_0 - i_0 - c_1)}$ *and* $c_1 = \frac{r_1 s + r_2 + r_3}{r_1 s(e_0 - i_0)}$

Since, the matrix M_1 has negative eigen values under the condition:

$i_0 = -(s^2 r_1^2 + 2r_2 r_3 - (r_2 + r_3 - c_1 r_1 + e_0 r_1 + sr_1)^2 + r_2^2 + r_3^2 + c_1^2 r_1^2 + e_0^2 r_1^2 - 2c_1 s r_1^2 + 2e_0 s r_1^2 - 2c_1 r_1 r_2 + 2c_1 r_1 r_3 + 2e_0 r_1 r_2 - 2e_0 r_1 r_3 + 2s r_1 r_2 + 2s r_1 r_3 - 2c_1 e_0 r_1^2)/(4r_1 r_3)$,

$E_1$is a stable equilibrium point.

Now, M_2 be the matrix evaluated at the equilibrium E_2 is symbolized as follows

$$M_2 = \begin{bmatrix} -r_1(e_0 - i_0 + i) & 0 & r_2 \\ 0 & -r_5 & -r_4 i \\ r_1(e_0 - i_0 + i) & 0 & -(r_2 + r_3) \end{bmatrix} \quad \text{Where; } i = \frac{r_5 i_0}{r_4 c_1 + r_5}$$

As the matrix M_2 contributes the negativeeigen values under the condition:$i_0 - e_0 = i$, E_2 is a stable equilibrium point.

Again, the matrix M_3 calculated at the equilibrium E_3 is characterized as follows

$$M_3 = \begin{bmatrix} -r_1 e_0 & 0 & r_2 \\ 0 & -(r_4 c_1 + r_5) & -r_4 i_0 \\ r_1 e_0 & 0 & -(r_2 + r_3) \end{bmatrix}$$

Since, the matrix M_3 contributes negative Eigen values under the condition: $i_0 = e_0 + i$, E_3 is a stable equilibrium point.

Similarly, the matrix M_4 calculated at the equilibrium E_4 is characterized as follows

$$M_4 = \begin{bmatrix} -r_1 e_0 & -r_1\left(\frac{r_2 c_1}{r_1(e_0 - i_0 + i - c_1)}\right) & r_1\left(\frac{r_2 c_1}{r_1(e_0 - i_0 + i - c_1)}\right) + r_2 \\ 0 & -r_5 & -r_4 i_0 \\ r_1 e_0 & r_1\left(\frac{r_2 c_1}{r_1(e_0 - i_0 + i - c_1)}\right) & -(r_1\left(\frac{r_2 c_1}{r_1(e_0 - i_0 + i - c_1)}\right) + r_2 + r_3) \end{bmatrix}$$

Since, the matrix M_4 contributes negative Eigen values under the condition: $e_0 + i = i_0$.E_4 is a stable equilibrium point.

Sensitive Analysis

On considering the initial conditions to be equal for all quantities. (as$e(0) = s(0) = i(0) = 1\ mg.$) The corresponding parametric values can be measured one by one keeping any one of them fixed. As we keep parameter r_1 fixed, 24 different possibilities of other parameters are analyzed as per their time consumption and we conclude that under the assumption $r_2 < r_4 < r_1 < r_3 < r_5$time consumption is minimum whereas it is maximum in $r_3 < r_5 < r_1 < r_2 < r_4$.

As we keep parameter q_2 fixed, 24 different possibilities of other parameters are analyzed as per their time consumption and we conclude that under the assumption$r_4 < r_5 < r_2 < r_1 < r_3$time consumption is minimum and maximum in $r_3 < r_5 < r_2 < r_1 < r_4$.

As we keep parameter q_3 fixed, 24 different possibilities of other parameters are analyzed as per their time consumption and we conclude that under the assumption $r_4 < r_2 < r_3 < r_5 < r_1$ time consumption is minimum and maximum in$r_1 < r_5 < r_3 < r_4 < r_2$.
As we keep parameter q_4 fixed, 24 different possibilities of other parameters are analyzed as per their time consumption and we conclude that under the assumption $r_2 < r_1 < r_4 < r_5 < r_3$time consumption is minimum and maximum in $r_3 < r_1 < r_4 < r_5 < r_2$.
As we keep parameter q_5 fixed, 24 different possibilities of other parameters are analyzed as per their time consumption and we conclude that under the assumption $r_4 < r_2 < r_5 < r_1 < r_3$time consumption is minimum and maximum in $r_3 < r_1 < r_5 < r_4 < r_2$.
It is important to note down that taking any parametric values to be non – positive, it results to the failure.

RESULT AND DISCUSSION

As the minimum time consumption for product formation is under the assumption$r_4 < r_2 < r_5 < r_1 < r_3$, keeping the parameters to be fixed at $r_1 = 4, r_2 = 2, r_3 = 5, r_4 = 1, r_5 = 3$. The rate of change of reaction for enzyme-substrate in presence of Un-Competitive inhibitor for this hypothesis is studied as follows

Case-I: The initial concentration of enzyme is less than inhibitor is less than that of substrate $(e(0) = 0.8mg, i(0) = 1mg \; and \; s(0) = 1.2mg)$.

Time – Concentration relation for $e(0) < i(0) < s(0)$(Fig. 1)

Case-II: The initial concentration of enzyme is less than substrate is less than that of inhibitor $(e(0) = 0.8mg, s(0) = 1mg \; and \; i(0) = 1.2mg)$.

Time – concentration relation$e(0) < s(0) < i(0)$ (Fig. 2)

Case-III: The initial concentration of substrate is less than inhibitor is less than that of enzyme $(s(0) = 0.8mg, i(0) = 1mg \; and \; e(0) = 1.2\; mg)$.

Time –Concentration relations$(0) < i(0) < e(0)$ (Fig. 3)

Case-IV: The initial concentration of inhibitor is less than substrate is less than that of enzyme $(i(0) = 0.8mg, s(0) = 1mg \; and \; e(0) = 1.2mg)$.

Time –Concentration relation$i(0) < s(0) < e(0)$ (Fig. 4)

Case-V: The initial concentration of inhibitor is less than enzyme is less than that of substrate $(i(0) = 0.8mg, e(0) = 1mg \; and \; s(0) = 1.2mg)$.

Time –Concentration relation$i(0) < e(0) < s(0)$ (Fig. 5)

Case-VI: The initial concentration of substrate is less than enzyme is less than that of inhibitor $(s(0) = 0.8mg, e(0) = 1mg \; and \; i(0) = 1.2mg)$.

Time –Concentration relations$(0) < e(0) < i(0)$ (Fig. 6)

Case-VII: The initial concentration of all enzyme, Inhibitor and substrate is taken to be equal $(e(0) = s(0) = i(0) = 1mg)$.

Time –Concentration relation$e(0) = i(0) = s(0)$ (Fig. 7)

It is noted that, while compelling all the possible ratios of concentration of substrate, enzyme and inhibitor with fixed parametric values In the first and second case, for$e(0) < i(0) < s(0)$and $e(0) < s(0) < i(0)$the constant product formation is after 3.7 seconds. Again in third and fourth case, $s(0) < i(0) < e(0)$ and $i(0) < s(0) < e(0)$respectively, constant product formation is later 3.0 seconds. Whereas for five, six and seven, to be $i(0) < e(0) < s(0), s(0) < e(0) < i(0)$and $e(0) = s(0) = i(0)$respectively, the timing of product formation is measured later 3.2 seconds.

CONCLUSIONS

In this paper, it is revealed that the experimentally observed enzyme–substrate relationship under the influence of un-competitive inhibitor is consistent with theatrical calculations. Using the parametric values from the graph, the Reproduction Number is calculated as -0.8 which is the threshold value for product formation. Further the stability analysis carried out with constant initial conditions stated that, variation in rate of constants r_1, r_2, r_3, r_4andr_5 do affects the time consumption for product formation. It is inferred that $r_4 < r_2 < r_5 < r_1 < r_3$ consumes the minimum time span for product formation whereas $r_3 < r_1 < r_5 < r_4 < r_2$ takes the maximum time for the same. Also, it is observed that taking any of the parametric value to be negative tends to the failure of product formation. On applying restrictions to the initial conditions, Also it is inferred that enzyme concentration must be more than the concentration of substrate and inhibitor whereas the concentration of substrate and inhibitor may be equal or slightly more from each other is acceptable, for ideal product formation. Whereas, the concentration of enzyme to be less than that of substrate or inhibitor will consumes more time for product formation.

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Conflict of interest

The authors declare that there is no conflict of interest involved in this study.

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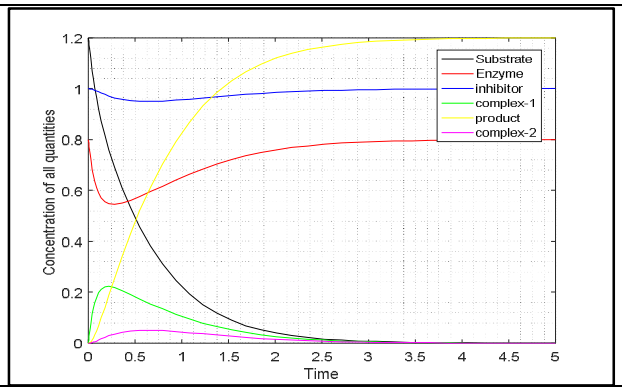


Fig. 1. Time – Concentration relation for $e(0) < i(0) < s(0)$

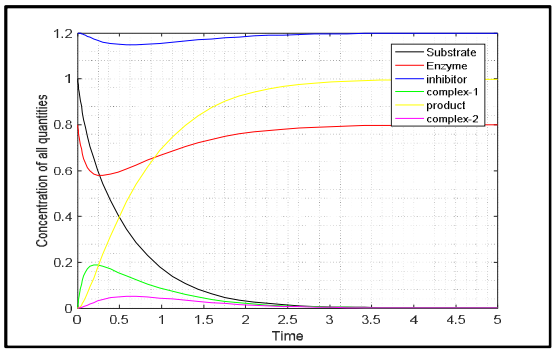


Fig. 2. Time – concentration relation$e(0) < s(0) < i(0)$

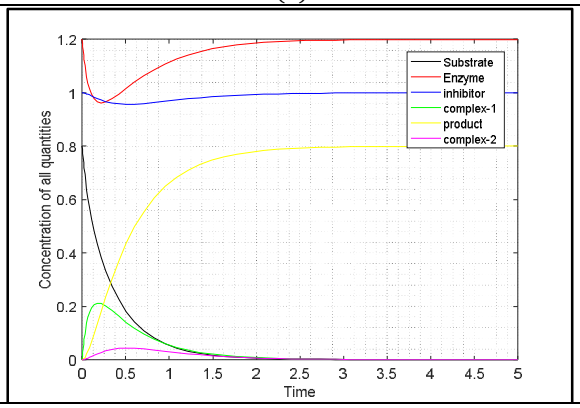


Fig. 3. Time –Concentration relation$s(0) < i(0) < e(0)$

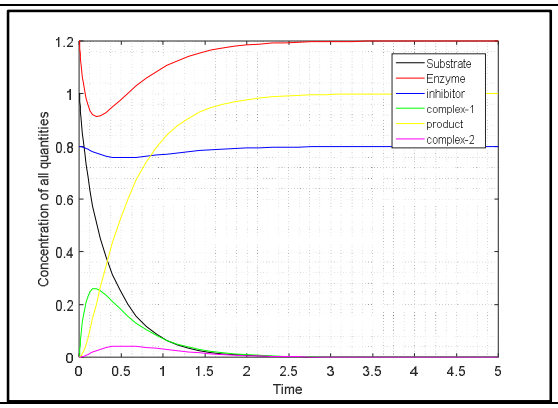


Fig. 4. Time –Concentration relation$i(0) < s(0) < e(0)$

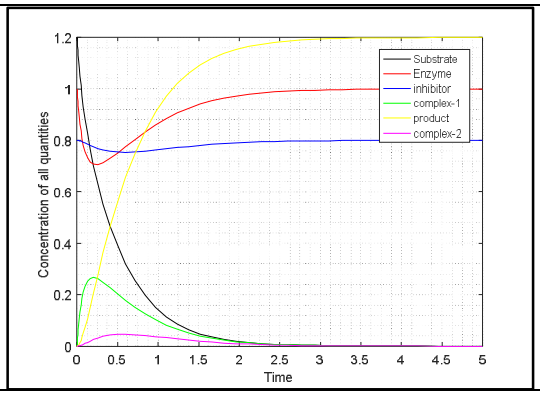


Fig. 5. Time –Concentration relation$i(0) < e(0) < s(0)$

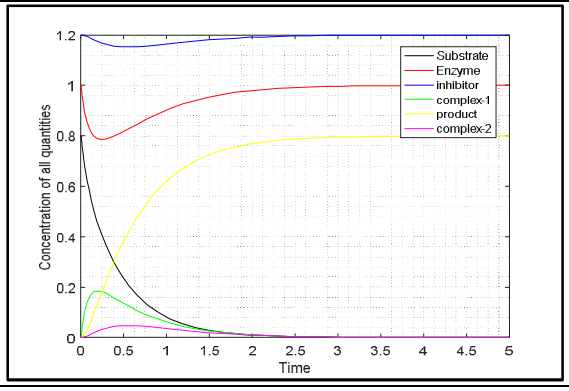


Fig. 6. Time –Concentration relation$s(0) < e(0) < i(0)$

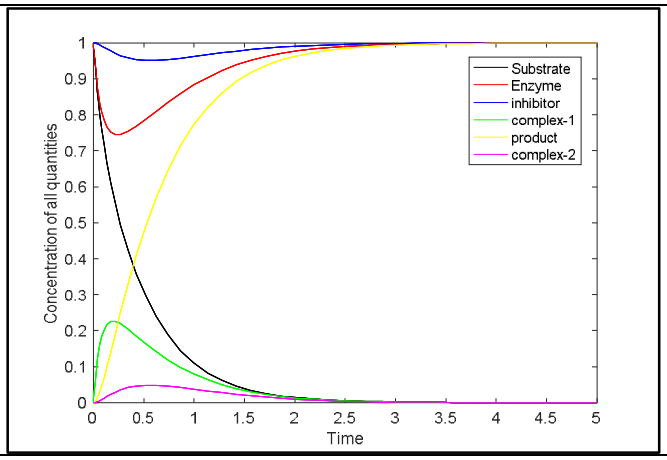


Fig. 7. Time –Concentration relation$e(0) = i(0) = s(0)$

RESEARCH ARTICLE

Evaluating the Antioxidant Activity of *Orilaithamarai Karpam* using *In vitro* Methods

R. Sumithra[1], V. Mahalakshmi[2]*, P. Manivannan[1], M. Keerthi Grace[1] and N.J. Muthukumar[3]

[1]PG Scholar, Department of Siddhar Yoga Maruthuvam, National Institute of Siddha, (Affiliated to The Tamil Nadu Dr. M.G. R Medical University, Guindy), Chennai, Tamil Nadu, India.
[2]Professor, Department of Siddhar Yoga Maruthuvam, National Institute of Siddha, Tambaram Sanatorium (Affiliated to The Tamil Nadu Dr. M.G. R Medical University, Guindy), Chennai, Tamil Nadu, India.
[3]Professor, Director General, Central Council for Research in Siddha, Ministry of Ayush, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
V. Mahalakshmi
Professor,
Department of Siddhar Yoga Maruthuvam,
National Institute of Siddha, Tambaram Sanatorium
(Affiliated to The Tamil Nadu Dr. M.G. R Medical University, Guindy),
Chennai, Tamil Nadu, India.
E.Mail: rvmahalakshmi85@gmail.com

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ABSTRACT

Kayakarpam is a distinctive aspect of the Siddha medical system advocated for rejuvenation and longevity of life."*Kayakarpam*" is given as "Antioxidant" nowadays. Oxidative reactions within the body generate free radicals, which can lead to oxidative stress by damaging vital cellular components such as DNA, proteins, and lipids. This oxidative damage is implicated in the pathogenesis of various chronic and degenerative diseases. The present study investigates the antioxidant potential of *Orilaithamarai Karpam* (OTK) through a series of in vitro assays.To assess its ability to scavenge free radicals, four distinct assays were employed: DPPH, Nitric Oxide, ABTS and Hydrogen Peroxide radical scavenging assays. The findings revealed that OTK exhibited dose-dependent antioxidant activity across all assays. Specifically, OTK demonstrated strong radical scavenging activity in the DPPH assay, with 86.23% inhibition, and in the ABTS assay, with 85.63% inhibition at a concentration of 1 mg/ml- comparable to standard antioxidants such as ascorbic acid and Trolox. Moderate antioxidant effects were observed in the Nitric Oxide and Hydrogen Peroxide assays, with inhibition values of 46.93% and 53.06%, respectively, at the highest concentration tested. These results suggest that OTK possesses significant antioxidant properties, particularly effective against DPPH and ABTS radicals. As such, OTK may serve as a promising natural

source of antioxidants. Further studies are warranted to explore its mechanisms of action and potential therapeutic applications.

Keywords: *Kayakarpam,* Siddha system, Antioxidant, *Orilaithamarai karpam*, Internal medicine.

INTRODUCTION

Siddha system is a traditional system of healing that originated in south India and is considered to be one of the India's oldest systems of medicine[1]**.** Therefore, *kayakarpam* means sturdy as a rock and ageless physical body. While consuming *karpa* medicines, it naturally prevents the body from diseases and deterioration of physical stature[2]. Antioxidants are substances that prevent or slow the oxidation process in other molecules by interrupting the initiation or progression of oxidative chain reactions. Free radicals, which are unstable molecules, pose a threat to health by damaging essential cellular components such as DNA, proteins, and membranes[3]. In plants, adverse environmental factors like extreme temperatures, drought, toxic metals, nutrient shortages, and high salt levels can lead to an overproduction of reactive oxygen species (ROS), resulting in oxidative stress. To combat this, living organisms have developed sophisticated antioxidant defense systems made up of both enzymatic and non-enzymatic components. Non-enzymatic antioxidants work in various ways: by blocking enzyme activity, binding metal ions that promote free radical generation, neutralizing reactive species, and enhancing other antioxidant pathways[4]. As a result, they reduce the chance of developing chronic diseases. Due to their ability to scavenge free radicals at the biochemical level, these chemicals, which include vitamins C and E, beta-carotene, and polyphenols, play an important part in rejuvenating therapies[5-6]. They contribute to the preservation of cellular health and delay the consequences of ageing. One notable *Kayakarpam* is *Orilaithamarai karpam* highlighted in Siddha literature, including in the Bogar *karpam* 300[7], for its health benefits. The human body is constantly working to resist aging, and scientific studies show that free radical damage plays a key role in age-related diseases. Antioxidants help counter this by protecting cells from oxidative damage. The purpose of this study was to evaluate the antioxidant potential of *Orilaithamarai karpam* using an in-vitro model across various assays[8].

MATERIALS AND METHODS

Collection and Authentication of Drug

The whole plant was collected from Thirissur, Kerala, India, and was authenticated by a medicinal botanist at the National Institute of Siddha, Chennai – 47.

Ingredients of *Orilaithamarai karpam*

Orilaithamarai (*Nervilia aragoana* Gaudich.) – Whole plant.

Preparation

The whole plant of *Orilaithamarai* was dried at room temperature in a dark room for 7 days, then powdered and stored in an airtight container.

Indication

Siruneer kulirndhu kan erichchal, kamalai, kadiya pitham.

Adjuvant: Ghee.

Duration: 40 days.

***In Vitro* Antioxidant Activity**

Free radical scavenging ability by the use of a stable DPPH radical (1, 1-diphenyl-2-picrilhydrazyl)

The effect of given samples on DPPH radical was estimated according to the procedure described by Von Gadow *et al.* (1997). Two mL of 6 $\times 10^{-5}$ M methanolic solution of DPPH were added to 50 µl of the sample solution with different concentrations (2 µl extract + 48 µl water, 4 µl extract + 46 µl water, 6 µl extract + 44 µl water, 8 µl extract + 42 µl water, 10 µl extract + 40 µl water). The decrease of absorbance at 515 nm was recorded in a spectrophotometer for 16 min at room temperature. The scavenging effect (decrease of absorbance at 515 nm) was plotted against the time and the percentage of DPPH radical scavenging ability of the sample was calculated from the absorbance value at the end of 16 m in duration as follows

All determinations were performed in triplicate. The percentage inhibition of the DPPH radical by the samples was calculated according to the formula of Yen and Duh (1994).

$IP = [(A_{C(0)} - A_{A(t)} / A_{C(0)})] \times 100$

Where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidants at t = 16 min[9,10].

$$\text{Radical scavenging} = \frac{\text{Absorbance of control} - \text{Absorbance of sample}}{\text{Absorbance of control}} \times 10$$

Nitric oxide scavenging activity

Nitric oxide generated from sodium nitroprusside in an aqueous solution at physiological pH was measured by the Griess reaction (Marcocci *et al.*, 1994). The reaction mixture (3ml) containing sodium nitroprusside (10mm) in phosphate buffer saline and the test extract (10, 25, 50 and 100µg/ml) was incubated at 25^{0}C for 150 min, after incubation 1.5ml of the reaction mixture was removed and 1.5ml of the Griess reagent (1% sulphanilamide, 2% orthophosphoric acid and 0.1% Napthylethyline diamine hydrochloride) was added. The absorbance of the chromophore formed was read at 546 nm. Percent inhibition of nitric oxide scavenging was calculated using the formula.

Percentage Inhibition = (A of Control – A of Sample) /A of Control× 100. A- absorbance[11].

Free radical scavenging ability by the use of a stable ABTS radical cation 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

The free radical scavenging capacity of samples was tested using ABTS radical cation decolorization assay (Re *et al.*, 1999). ABTS dissolved in water to get 7 mM concentration. ABTS radical cation (ABTS*$^+$) was produced by reacting ABTS stock solution with 2.45 mM potassium persulphate (final concentration) and allowing the mixture to stand in the dark room temperature for 12-16 hrs before use. The free radical was stable for more than two days, when stored in the dark room temperature. For the study of the test samples, the ABTS*$^+$ solution was diluted with absolute ethanol to an absorbance of 0.700 (±0.02) at 734 nm and equilibrated at 30°C. Reagent blank reading was taken (A_0). After addition of 2.0 mL of diluted ABTS*$^+$ solution ($A734\ nm$ = 0.700 (±0.02) to 50 µL of test sample (20 mg/mL), the absorbance reading was taken at 30°C exactly 6 min after initial mixing (A_t). Appropriate solvent blanks were run in each assay. All determinations were carried out at least three times. The percentage inhibition of absorbance at 734 nm was calculated using the above formula and decrease of the absorbance between A_0 and A_t.

$PI = [(A_{C(0)} - A_{A(t)}) / A_{C(0)}] \times 100$

where $A_{C(0)}$ is the absorbance of the control at t = 0 min; and $A_{A(t)}$ is the absorbance of the antioxidant at t = 6 min[12].

Hydrogen peroxide scavenging activity

Scavenging activity of hydrogen peroxide by the plant extract was estimated using the method of Ruch *et al*. (1989). Plant extract (4 ml) prepared in distilled water at various concentration was mixed with 0.6 ml of 4 mM H_2O_2 solution prepared in phosphate buffer (0.1 M pH 7.4) and incubated for 10 min. The absorbance of the solution was taken at 230 nm against blank solution containing the plant extract without H_2O_2. The reaction mix containing H_2O_2 without plant extract served as control. The amount of hydrogen peroxide radical inhibited by the extract was calculated using the following equation

H_2O_2 radical scavenging activity = {(Abs control – Abs sample)/ (Abs control)} × 100
Where; Abs control is the absorbance of H_2O_2 radical; Abs sample is the absorbance of H_2O_2 radical + sample extract[13].

RESULT AND DISSCUSION

The antioxidant potential of test drug OTK was evaluated using four different assays: DPPH, Nitric Oxide, ABTS and Hydrogen Peroxide radical scavenging assays. The results were compared with standard antioxidants.

Figure 1: Various assays of OTK demonstrating its antioxidant activity (a) DPPH assay, (b) NO assay, (c) ABTS assay, (d) H_2O_2 assay

DPPH assay

Percentage inhibition of test drug OTK on DPPH radical scavenging assay (Table 2)

The DPPH radical scavenging assay results for OTK demonstrate a clear, concentration-dependent increase in antioxidant activity. The control sample showed an optical density (OD) of 0.61, corresponding to 0% inhibition. At 0.2 mg, the OD was reduced to 0.225, yielding 63.11% inhibition, and at 0.4 mg, the OD further decreased to 0.147, resulting in 75.90% inhibition. At 0.6 mg, the OD dropped to 0.097, with 84.10% inhibition, and at 0.8 mg and 1 mg, the ODs were 0.088 and 0.084, respectively, with inhibitions of 85.57% and 86.23%. The results show a dose-dependent increase in inhibition, meaning that as the concentration of OTK increases, its ability to neutralize DPPH radicals improves. At 1 mg, OTK reaches an inhibition of 86.23%, which indicates strong antioxidant potential, though this is still slightly lower than that of potent antioxidants like ascorbic acid or Trolox. Compared to potent antioxidants like ascorbic acid and Trolox, OTK shows moderate antioxidant activity. While ascorbic acid typically exhibits greater than 90% inhibition at low concentrations, OTK achieves around 85-86% inhibition at 1 mg. Notably, OTK's performance is highly promising when compared to well-established antioxidants. Its inhibition values, consistently close to or exceeding 80%, suggest that its antioxidant efficacy is comparable to or even superior to that of ascorbic acid and Trolox at equivalent concentrations. Overall, OTK demonstrates significant antioxidant activity in the DPPH assay, indicating moderate to strong antioxidant potential, particularly in scavenging lipophilic and hydrophilic radicals. Additionally, OTK exhibited a significant antioxidant effect with an IC50 value of 0.24 mg, which is comparable to ascorbic acid (IC50 = 0.15 mg) and Trolox (IC50 = 0.2 mg). OTK's IC50 value (0.24 mg) is slightly higher than Trolox (0.20 mg) but lower than ascorbic acid (0.15 mg). OTK's inhibition percentage at 1 mg (86.23%) is comparable to Trolox (90.52%) but lower than ascorbic acid (95.12%).

Nitric oxide scavenging assay

Percentage inhibition of test drug OTK on NO assay (Table 3)

The nitric oxide (NO) scavenging assay results for the test sample show a dose-dependent increase in antioxidant activity with increasing concentrations. The control sample had an optical density (OD) of 0.929, corresponding to 0% inhibition. At 0.2 mg, the OD decreased to 0.901, resulting in 3.01% inhibition. At 0.4 mg, the OD further decreased to 0.779, yielding 16.15% inhibition. At 0.6 mg, the OD dropped to 0.643, giving 30.79% inhibition, and at 0.8 mg and 1 mg, the ODs were 0.566 and 0.493, corresponding to 39.07% and 46.93% inhibition, respectively. These results suggest that the sample's ability to scavenge nitric oxide increases with concentration, with the highest inhibition of 46.93% observed at 1 mg. However, the NO radical scavenging activity of OTK is significantly lower compared to its activity in the DPPH and ABTS assays. At 1 mg, OTK achieves only 46.93% inhibition, indicating its relatively weak efficacy in neutralizing nitric oxide radicals. This discrepancy suggests that OTK is less effective at scavenging NO radicals than other types of radicals, likely due to the particular challenges involved in neutralizing nitric oxide. To effectively neutralize NO, OTK may need to be administered at higher concentrations or may require a more potent mechanism of action. The NO assay results indicate that OTK's antioxidant properties are less effective against nitric oxide radicals. This is in line with the understanding that nitric oxide requires distinct mechanisms for effective neutralization. In comparison to established antioxidants like N acetylcysteine (NAC) and alpha-lipoic acid, which have a strong track record in scavenging NO radicals, OTK's lower inhibition in the NO assay suggests a reduced

ability to neutralize nitric oxide effectively. Although OTK demonstrated moderate antioxidant activity, with a maximum inhibition of 46.93% at 1 mg, its effectiveness was significantly lower compared to well-known antioxidants such as ascorbic acid and Trolox, which have demonstrated inhibition rates above 70-80% in various assays. For example, ascorbic acid shows 85.61% inhibition at 1 mg, while Trolox achieves 78.52% inhibition at the same concentration. This highlights that while OTK does exhibit some capacity to scavenge nitric oxide, its potency is relatively weak compared to ascorbic acid and Trolox. Despite this, the dose-dependent increase in inhibition observed in the NO scavenging assay suggests that OTK holds some potential as an NO scavenger. Further studies with higher concentrations and direct comparisons to other established NO scavengers would be essential to fully assess its relative efficacy and explore its potential as an antioxidant agent for nitric oxide. OTK's IC50 value (1.2-1.5 mg) is significantly higher than ascorbic acid (0.50 mg) and Trolox (0.60 mg). OTK's inhibition percentage at 1 mg (46.93%) is substantially lower than ascorbic acid (85.61%) and Trolox (78.52%).

Free radical scavenging ability by the use of a stable ABTS radical cation 2, 2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid)

Percentage inhibition of test drug OTK on ABTS radical scavenging assay (Table 4)

The ABTS radical cation decolorization assay results for the test sample reveal a clear, dose-dependent increase in antioxidant activity with rising concentrations. The control sample showed an optical density (OD) of 0.701, corresponding to 0% inhibition. At 0.2 mg, the OD decreased to 0.477, resulting in 32.15% inhibition, while at 0.4 mg, the OD further decreased to 0.251, yielding 64.30% inhibition. At 0.6 mg, the OD was reduced to 0.178, producing 74.68% inhibition, and at 0.8 mg and 1 mg, the ODs were 0.145 and 0.101, leading to inhibitions of 79.37% and 85.63%, respectively. These results suggest a significant increase in antioxidant activity as the concentration of the sample rises. OTK exhibits a moderate to strong, dose-dependent increase in percentage inhibition in the ABTS assay, reaching a maximum of 85.63% inhibition at 1 mg. The antioxidant activity of OTK is significant in both DPPH and ABTS assays, achieving approximately 85-86% inhibition at 1 mg. These findings suggest that OTK possesses moderate to strong antioxidant potential. In comparison to established antioxidants, OTK's performance is notable but slightly less potent than ascorbic acid, which typically exhibits over 90% inhibition at low concentrations in ABTS assay. Trolox, a Vitamin E analog, demonstrates high efficacy in ABTS assay. OTK shows similar radical-scavenging abilities to Trolox but may require higher concentrations to achieve equivalent effectiveness. OTK exhibited moderate antioxidant activity, with an IC50 value of 0.43 mg, which is slightly higher than that of ascorbic acid (IC50 = 0.35 mg) and Trolox (IC50 = 0.38 mg). OTK's inhibition percentage at 1 mg (85.63%) is lower than ascorbic acid (94.15%) and Trolox (90.17%).

Hydrogen peroxide scavenging activity

Percentage inhibition of test drug OTK on H_2O_2 assay (Table 5)

The hydrogen peroxide (H_2O_2) radical scavenging assay results for the test sample reveal a dose-dependent increase in antioxidant activity with rising concentrations. The control sample had an optical density (OD) of 0.098, corresponding to 0% inhibition. At 0.2 mg, the OD decreased to 0.089, resulting in 9.18% inhibition, and at 0.4 mg, the OD further decreased to 0.083, yielding 15.31% inhibition. At 0.6 mg, the OD dropped to 0.076, resulting in 22.45% inhibition. At 0.8 mg and 1 mg, the ODs were 0.053 and 0.046, corresponding to 45.92% and 53.06% inhibition, respectively. The data shows a gradual increase in the inhibition of hydrogen peroxide as the concentration of OTK increases. Starting from 0% inhibition at the control, the inhibition progressively rises to 53.06% at 1 mg. This dose-dependent trend is a hallmark of antioxidant activity, indicating that OTK is capable of scavenging H_2O_2 radicals in a concentration-dependent manner. When compared to known antioxidants, the inhibition of H_2O_2 radicals by OTK is moderate. For instance, ascorbic acid and Trolox, both well-established antioxidants, typically show inhibition rates above 80% at similar or lower concentrations. In contrast, OTK achieves 53.06% inhibition at 1 mg, suggesting that while it demonstrates significant activity, its scavenging capacity for H_2O_2 is less potent than that of ascorbic acid or Trolox. The scavenging of hydrogen peroxide by OTK is likely through its antioxidant properties, which may involve the neutralization of H_2O_2 molecules either directly or through indirect mechanisms. The increasing inhibition with higher concentrations suggests that OTK may act as a hydrogen donor or facilitate the conversion of H_2O_2 into less reactive species, such as water or oxygen. OTK's ability to neutralize H_2O_2 radicals shows promise, particularly at

higher concentrations. However, its maximum inhibition of 53.06% at 1 mg suggests that OTK might require optimization to improve its effectiveness against hydrogen peroxide. Further studies could focus on increasing the concentration range tested, as well as exploring the underlying mechanisms that contribute to its scavenging activity. OTK's IC50 value (0.73 mg) is higher than ascorbic acid (0.40 mg) and Trolox (0.50 mg). OTK's inhibition percentage at 1 mg (53.06%) is lower than ascorbic acid (90.19%) and Trolox (85.95%).

CONCLUSION

The study demonstrates the antioxidant potential of the test drug OTK. It exhibits moderate to strong activity in scavenging lipophilic radicals, such as DPPH and ABTS, but shows relatively weaker activity against hydrophilic radicals, including nitric oxide (NO) and hydrogen peroxide (H_2O_2). Further optimization or the use of higher concentrations may enhance its efficacy, particularly in scavenging NO and H_2O_2. Despite its lower effectiveness in certain assays, OTK shows promise as a potential antioxidant agent, especially in conditions involving radical-mediated damage, and warrants further investigation in alternative models or formulations to improve its activity.

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Author contribution

Conceptualization: MV; Data collection and compilation: MV and MP; Manuscript Writing: MV, MP and KG; Proofreading and editing: MV, MP, KG and NJMK.

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Table: 1 Percentage inhibition of test drug *Brammathandu karpam* on DPPH radical scavenging assay

SL.NO.	Sample Concentration (mg)	METHOD	Optical Density	RESULTS (% inhibition)
1	Control	DPPH assay Von Gadow *et al*. (1997)	0.61	0.00
2	0.2		0.086	85.90
3	0.4		0.075	87.70
4	0.6		0.55	9.84
5	0.8		0.054	91.15
6	1		0.052	91.48

Table: 2 Percentage inhibition of test drug *Brammathandu karpam* on NO assay

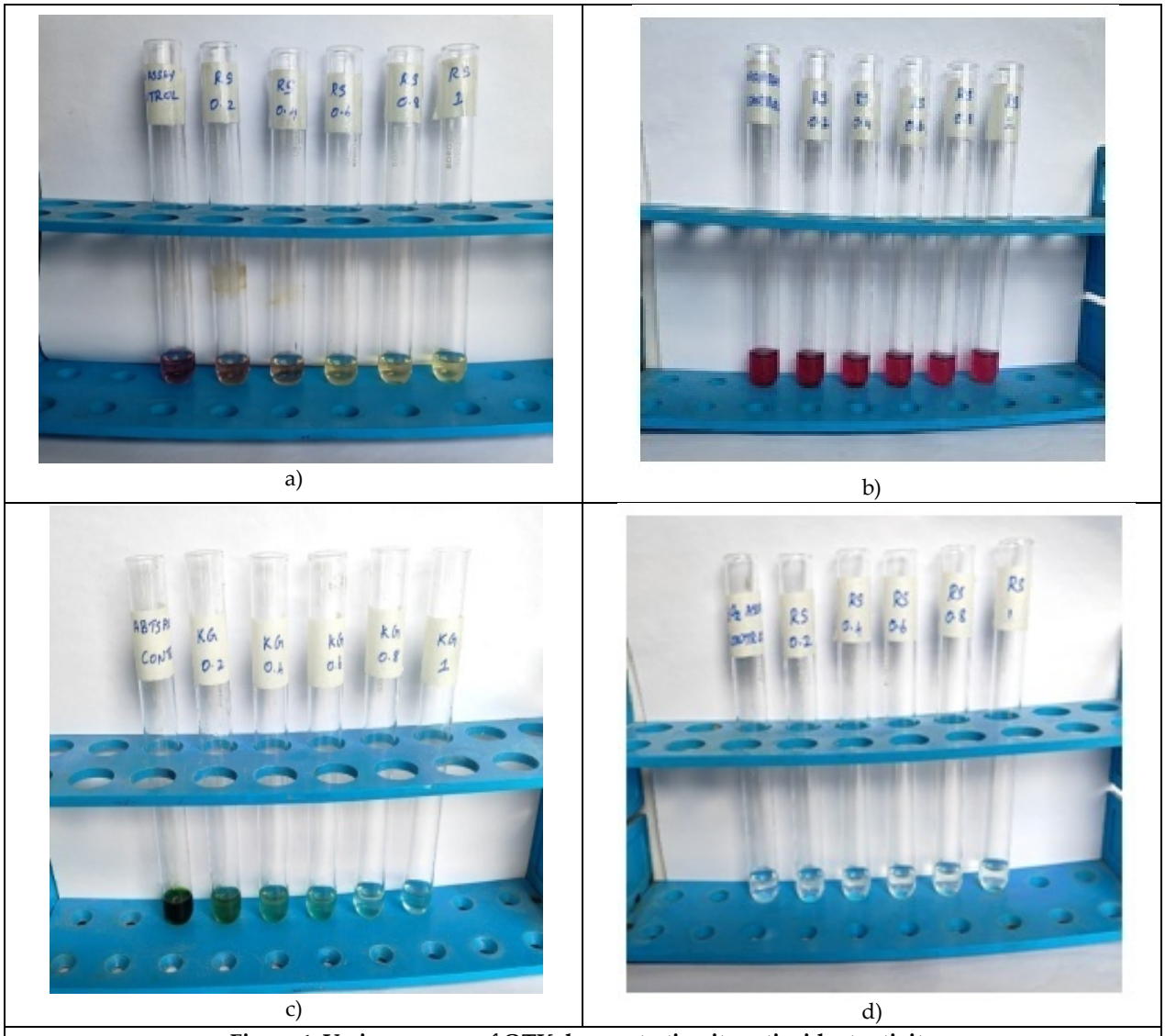
SL.NO.	Sample Concentration (mg)	METHOD	Optical Density	RESULTS (% inhibition)
1	Control	NO assay Marcoci *et al* (1994)	0.768	0.00
2	0.2		0.558	27.34
3	0.4		0.542	29.43
4	0.6		0.493	35.81
5	0.8		0.441	42.58
6	1		0.404	47.40

Table: 3 Percentage inhibition of test drug *Bramathandu karpam* on ABTS radical scavenging assay

SL.NO.	Sample Concentration (mg)	METHOD	Optical Density	RESULTS (% inhibition)
1	Control	ABTS radical cation decolorization assay (Re *et al*., 1999)	0.701	0.00
2	0.2		0.324	53.91
3	0.4		0.214	69.56
4	0.6		0.141	79.94
5	0.8		0.104	85.21
6	1		0.078	88.90

Table: 4 Percentage inhibition of test drug *Brammathandu karpam* on H202assay

SL.NO.	Sample Concentration (mg)	METHOD	Optical Density	RESULTS (% inhibition)
1	Control	H_2O_2 radical inhibition assay (Elizabeth and Rao, 1990)	0.093	0.00
2	0.2		0.089	4.30
3	0.4		0.074	20.43
4	0.6		0.072	22.58
5	0.8		0.069	25.81
6	1		0.067	27.96



a) b) c) d)

Figure 1: Various assays of OTK demonstrating its antioxidant activity
(a) DPPH assay, (b) NO assay, (c) ABTS assay, (d) H_2O_2 assay

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RESEARCH ARTICLE

Formulation and Evaluation of Losartan Potassium Bilayer Floating Tablet

S. Rohini Reddy[1], K. Jyothi[2], K. Supriya[2] and Ch. Shanthi Priya[3]*

[1]Associate Professor, Department of Regulatory Affairs, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Secunderabad (Affiliated to Osmania University, Hyderabad), Telangana, India.
[2]Student, Department of Pharamaceutics, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Secunderabad (Affiliated to Osmania University, Hyderabad), Telangana, India.
[3]Associate Professor, Department of Pharamaceutics, Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Secunderabad, (Affiliated to Osmania University, Hyderabad) Telangana, India.

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***Address for Correspondence**
Ch. Shanthi Priya
Associate Professor,
Department of Pharamaceutics,
Sarojini Naidu Vanita Pharmacy Maha Vidyalaya, Secunderabad,
(Affiliated to Osmania University, Hyderabad)
Telangana, India.
E.Mail: shanthipriyapharma@gmail.com

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ABSTRACT

This study is done to prepare and characterize Losartan potassium bilayer floating tablets where HPMC E15 is used as a polymer. Eight sets of Losartan potassium bilayer tablets are manufactured through direct compression method. The physicochemical characteristics of the compressed tablets, including thickness, weight variation, friability, hardness, drug content determination, were then assessed. An *In-vitro* floating studies were carried out. Physiochemical characteristics such as hardness (5.0 ± 0.1 to 5.5 ± 0.7 kg), weight variation (222± 1.2 to 225 ± 1.3 mg), friability (0.52 to 0.54 %), thickness test (5.0± 0.03 to 7.0± 0.03 mm), and drug content (90.4 to 96.6%) were within limits. The results of floating lag time of (63 sec to98sec) and total floating time is >10 hrs. Floating Bilayer tablets are with (immediate and controlled layer) where each formulation with dissolution, 98.26% highest release (F5) to lowest release of 86.53% (in F7) 98.27% highest release, (F5 at 12 hrs) 85.15% lowest release (F7 at 12hr) in controlled release layer. The FTIR spectrum shows that the drug and excipients have zero interaction with each other. Results led to the conclusion that Losartan potassium bilayer floating tablets may be developed.

Keywords: Losartan potassium, bilayer tablets.

INTRODUCTION

Gastro-Retentive Drug Delivery System (GRDDS)

Gastro-Retentive delivery systems are meant to be retained in the stomach for a prolonged period. Thus, they provide sustained and prolonged drug release to the upper part of the gastrointestinal (GI) tract. Certain types of drugs have benefited by using gastric retentive devices. These include; Acting locally in the stomach. Primarily absorbed in the stomach. Poorly soluble at an alkaline pH. Narrow window of absorption. Absorbed rapidly from the GI tract. Degrade in the colon[1,2]. Floating Drug Delivery System: Floating drug delivery Systems are low-density systems with a bulk density less than gastric fluids. Because of its low density, it remains buoyant in the stomach for a prolonged period. The system floats on the gastric contents and releases the drug slowly at the desired rate. Once the drug is released completely, the stomach empties the residual system. This results in increased gastric residence time and better control of the fluctuations in plasma drug concentration with a low risk of toxicity. Drugs that are locally active in the stomach, drugs with narrow absorption windows, and unstable in the intestine and colonic environment are the potential drug candidates for FDDS. This system improves drug absorption and also minimizes the mucosal irritation of drugs. FDDS requires a high fluid level in the stomach to float and work efficiently, which limits the approach to some extent[3,4]. The need of bilayer floating is to control the delivery rate of single or two active pharmaceutical ingredients. For the administration of fixed-dose drug combinations, prolong the product life cycle, buccal or mucoadhesive delivery systems, and fabricate novel drug delivery systems such as chewing devices and floating tablets for GRDDS. To separate incompatible active pharmaceutical ingredients from each other, to control the release of API from one layer by utilizing the functional property of another layer. To modify the total surface area available for the active pharmaceutical ingredient layer by sandwiching with one or two inactive layers to achieve swellable /erodible form for modified release[5,6]. A bilayer tablet contains two layers; one layer is immediate release layer, where the initial dose from the system is released, and the second layer is sustained release layer, which absorbs gastric fluid, which forms an impenetrable colloidal gel barrier on its surface and maintains a bulk density of less than unity and thereby it remains buoyant in the stomach. Bilayer floating tablet is the new era for the successful development of controlled release formulation. Bilayer tablet is better than the traditionally used other dosage forms. A bilayer tablet is suitable for sequentially synthesizing two drugs in combination. It's also able to separate two incompatible substances. The delivery rate of either single or two API"S can be controlled in bilayer tablets[7,8].

MATERIALS AND METHODS

Losartan potassium, HPMC E15, Ethyl cellulose, Sodium bicarbonate, Microcrystalline cellulose, Magnesium stearate, Colloidal silicon, Crospovidone, Micro crystalline cellulose, Magnesium stearate, Methylene red. All the materials are procured from S.D Fine Chemicals Ltd, Mumbai, India and Molychem, Mumbai, India.

Experimental Methodology

Formulation of Bilayer Floating Tablets of Losartan Potassium

Formulation of Immediate Release Layer

Direct compression method is used to prepare Losartan potassium bilayer floating tablets. Required powders were weighed. Active pharmaceutical ingredient was blended with crospovidone, magnesium stearate, colloidal silicon dioxide, micro crystalline cellulose, methylene red. 40-mesh sieve is used and the given ingredients were blended. F1 to F8 groups was prepared.

Formulation of Controlled Release Layer

Required powders were weighed. Active pharmaceutical ingredient was taken and blended with HPMC E15, Sodium bicarbonate, Microcrystalline cellulose, magnesium stearate, Ethyl cellulose, colloidal silicon dioxide. 40-mesh sieve is used and these ingredients are blended to form F1 to F8 batches.

Formulation of Losartan Potassium Bilayer Floating Tablets

Firstly, pass the blended layer formulation for compression with low speed. Then the controlled release layer formulation was passed for compression on the immediate release layer with high speed to form bilayer floating tablets of losartan potassium. Eight groups developed(F1 to F8).

RESULTS AND DISCUSSIONS

Melting Point Determination: Capillary method is used to determine melting point to be around 181°C. The purity of the drug substance is confirms based on the literature.

Solubility: PH 6.8 phosphate buffer and 0.1N HCL is used.

Standard graph of Losartan potassium

UV spectrophotometer is used produce standard curve and lambda max 232 nm is used to detect absorption. In Losartan potassium standard calibration curve concentration (X axis) and absorbance (Y axis) was plotted against each other. Losartan potassium standard calibration curve having an R2 of 0.9996 which shows good linearity. Table: 3 and Figure1. In Losartan potassium standard calibration curve concentration (X axis) and absorbance (Y axis) was plotted against each other. Losartan potassium standard calibration curve having an R2 of 0.9998 which shows good linearity. Table 4 and Figure 2.

FTIR (Drug -Excipient compatibility) studies

For the analysing drug-excipient interactions FTIR spectroscopy is used. FTIR spectrum of Losartan potassium and losartan potassium + excipients are observed in FTIR spectra and there is zero interaction between the drug and the excipient according to the peaks observed in the FTIR spectrum. This shows the stability of the drug (losartan potassium) in physical mixture. Table 5, Figure 3 andFigure 4.

Precompression Studies: (Preformulation parameters of powder mixture)

Preformulated parameters like Angle of repose, Bulk density, tapped density, Carr's index and Hausner ratio was performed to see the powders satisfactory flow properties. According the observer results of the formulation the angle of repose values range from 24.27 ± 0.28 to 26.78 ± 0.34, bulk densities(gm/ml) show range of 0.454 and 0.564, tapped densities(gm/ml) shows range 0.558 and 0.564, Hausner's ratio shows range 1.21 and 1.24, Carr's index shows range from 17.12 to 17.97. This shows the flow of powders were satisfactory.(Table: 6)

Evaluation of Bilayer Floating Tablets of Losartan Potassium

The prepared formulation was evaluated for their wt. variation hardness, Friability, thickness and Drug content using standard procedure.

***In-vitro* quality control parameters**

Evaluation tests for Immediate release tablets of losartan potassium and Controlled Floating Layer

Evaluation tests were done and the values of Weight variation is between 72± 1.2 to 75± 1.4, friability values between 0.40± 0.5 to 0.44± 0.7, hardness values between 3.2±0.1 to 3.7±0.6and thickness values between 2.27±0.01to 2.77± 0.01 were found within limits. (Table: 7). Evaluation tests were done and the values of Weight variation is between 149± 1.2 to 151± 1.2, friability values between 0.52± 0.5 to 0.54± 0.7, hardness values between 5.0±0.1 to 5.5±0.7and thickness values between 5.4± 0.03to 7.0± 0.03 were found within limits. (Table: 8). Evaluation of Immediate release tablets of losartan potassium (Table: 9). Evaluation of Bilayer floating tablets of losartan potassium (Table: 10)

***In-Vitro* floating studies**

Bilayer floating tablets of losartan potassium were evaluated for their time of floating and their total floating lag time using standard procedures. In-vitro floating studies were done and the values of floating time was between 63sec to

98 sec and the Values of total floating time was >10 was found. Evaluation of Bilayer floating tablets of losartan potassium (Table: 11)

***In vitro* drug release**

Dissolution studies showed that 98.27% of cumulative drug is released for 10 hours with excellent release rate for F5 formulation, which is considered as optimized formulation. In-vitro cummulative studies of losartan potassium immediate release tablets (Table 12). In-vitro cummulative release of controlled release tablets of losartan potassium (Table 13)

CONCLUSION

From this study of bilayer floating tablets of losartan potassium shows the anti-hypertensive effects of losartan potassium (250mg/table) formulated by direct compression method. The polymer used was HPMC E15, Crospovidone as super disintegrant, microcrystalline cellulose as binder, magnesium stearate as lubricant. The powder blend's physicochemical properties were examined by using different criteria ,which shows satisfactory results. After that, developed formulations were assessed for characterization. The results of drug content were good for F5 formulation batch, which was found to be 96% among the different formulations. The results of floating lag time of F5 formulation 63(sec) and total floating time are >10 hrs. In this formulation there are 2 layers (immediate layer and controlled release layer) where every layer has different dissolution profile for prepared formulations, for immediate release layer the invitro cumulative release of drug is 98.27% (in F5) and in controlled release layer the invitro cumulative release of drug for 12 hrs is 98.26% in F5 formulation. The optimized formulation F5 containing HPMC E15. FTIR study shows zero interaction between drug and excipients. From all the prepared (F1- F8) formulations it is concluded that F5 formulation is taken as an optimize formulation.

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Table 1: Formulation of immediate release layer

Sno	Requirements (mg)	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)	F6(mg)	F7(mg)	F8(mg)
1	Losartan potassium	50	50	50	50	50	50	50	50
2	Crospovidone	0.5	1.0	1.5	2.0	2.5	3.0	3.5	4
3	Micro crystalline cellulose	21.05	20.55	20.05	19.55	19.05	18.55	18.05	17.55
4	Magnesium Stearate	3	3	3	3	3	3	3	3
5	Colloidal silicon dioxide	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25

6	Methylene red	0.2	0.2	0.2	0.2	0.2	0.2	0.2	0.2
Total (mg)		75	75	75	75	75	75	75	75

Table 2: Formulation of controlled release layer

Sno	Requirements (mg)	F1(mg)	F2(mg)	F3(mg)	F4(mg)	F5(mg)	F6(mg)	F7(mg)	F8(mg)
1	Losartan potassium	50mg	50mg	50mg	50mg	50mg	50mg	50mg	50mg
2	HPMC E15	10	15	20	25	27	35	37	39
3	Ethyl cellulose	30	28	26	24	22	20	15	12
4	Sodium bicarbonate	20	25	20	25	20	25	20	25
5	Microcrystalline cellulose	36.75	28.7	30.7	22.7	27.7	16.7	24.7	20.75
6	Magnesium stearate	3	3	3	3	3	3	3	3
7	Colloidal silicon	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Total (mg)		150	150	150	150	150	150	150	150

Table 3: Melting Point of Losartan Potassium

Drug Trials	Melting point (^{0}c)	Melting point (Average)	Melting point (Reference)
1	181		
2	180	181^{0}c	178-184^{0}c
3	182		

Table 4: Solubility of losartan potassium

Sno	Solvents used	Observation
1	0.1N HCL	Freely solubilized
2	Phosphate Buffer pH 6.8	Freely solubilized

Table 5: Standard Graph of Losartan potassium using 0.1N HCL

Conc. (µg/mL)	Absorbances (nm)
0	0.0
2.01	0.152
4.01	0.319
6.01	0.488
8.01	0.640
10.01	0.825
12.0	0.969

Table 6: Standard Graph of Losartan potassium in Ph 6.8 buffer (232nm)

Conc. (µg/mL)	Absorbance (nm)
0	0
2	0.046
4	0.092
6	0.135
8	0.185
10	0.229

Table 7: Losartan potassium shows the principal bands at a wave number in the FTIR spectra

Functional groups	Wave numbers (cm^{-1})
Cyclic amines	1256.53cm^{-1}
C-H stretches	2870.26cm^{-1}

O-H bending	3172.30cm^{-1}
Chlorine	762.24cm^{-1}

Table 8: Preformulation parameters of powder mixture

F code	Angle of repose (θ)	Bulk density (gm/cm^3)	Tapped density (gm/cm^3)	Carr's index (I)	Hausner's ratio
F1	24.47±0.32	0.541	0.563	17.12	1.24
F2	25.78±0.50	0.456	0.559	17.78	1.21
F3	25.85±0.32	0.459	0.564	17.97	1.22
F4	24.55±0.98	0.433	0.558	17.64	1.22
F5	24.27±0.28	0.460	0.564	17.62	1.22
F6	25.47±0.23	0.455	0.563	17.97	1.24
F7	26.47±0.25	0.558	0.559	17.64	1.21
F8	24.27±0.25	0.556	0.564	17.62	1.22

Table 9: Evaluation of Immediate release tablets of losartan potassium

F code	Wt. Variation (mg ±SD)	Hardness (Kg ±SD)	Friability (%)	Thickness (mm ±SD)	Drug Content (%)	Disintegration Time (sec)
F1	74 ± 1.3	3.5± 0.6	0.43± 0.7	2.77± 0.01	97.1	22.45 ± 1.59
F2	75± 1.4	3.5±0.7	0.41± 0.2	2.75± 0.04	96.4	23.54 ± 1.45
F3	75± 1.0	3.4±0.6	0.42± 0.3	2.67± 0.02	96.4	21.85 ± 1.86
F4	72± 1.2	3.6±0.1	0.40± 0.5	2.66± 0.03	96.5	26.54 ± 1.33
F5	74± 1.2	3.7±0.6	0.44± 0.7	2.57± 0.04	97.6	24.16 ± 1.57
F6	75 ± 1.1	3.4± 0.6	0.43± 0.5	2.37± 0.03	94.1	25.85 ± 1.67
F7	75± 1.2	3.5±0.7	0.40± 0.6	2.57± 0.02	94.5	26.57 ± 1.73
F8	75±1.0	3.2±0.1	0.44± 0.5	2.27±0.01	95.5	20.56 ± 1.55

Table 10: Evaluation of Bilayer floating tablets of losartan potassium

F code	Wt. Variation (mg ±SD)	Hardness (Kg ±SD)	Friability (%)	Thickness (mm ±SD)	Drug Content (%)
F1	150± 1.9	5.0± 0.6	0.53	7.0± 0.03	92.1
F2	149± 1.2	5.5±0.7	0.51	5.4± 0.03	90.4
F3	151± 1.2	5.2±0.6	0.52	6.1± 0.03	91.4
F4	150± 1.3	5.0±0.1	0.50	5.0± 0.03	90.5
F5	151 ± 1.2	5.5±0.6	0.54	6.0± 0.03	96.6
F6	150 ± 1.2	5.0± 0.6	0.53	7.0± 0.03	92.1
F7	151± 1.3	5.5±0.7	0.50	5.4± 0.03	90.5
F8	150±1.3	5.0±0.1	0.54	5.0±0.03	95.5

Table 11: Evaluation of Bilayer floating tablets of losartan potassium

F code	lag time(sec)	Tot. floating time (hrs)	Swelling Index (%)
F1	75	>10	62.56±0.56
F2	75	>10	65.66±0.23
F3	95	>10	61.16±0.15
F4	70	>10	66.89±0.55
F5	63	>10	67.56±0.23
F6	92	>10	58.36±0.87

F7	91	>10	59.55±0.76
F8	98	>10	60.55±0.88

Table 12*: In-vitro* cummulative studies of losartan potassium immediate release tablets

F Code	Time (min)					
	0	**10**	**20**	**30**	**45**	**60**
F1	0	12.87±0.29	21.25±0.25	42.47±0.17	67.89±0.29	92.01±0.18
F2	0	13.76±0.57	23.26±0.17	43.72±0.12	69.56±0.36	93.62±0.14
F3	0	11.19±0.16	21.43±0.16	41.48±0.13	68.75±0.33	91.23±0.15
F4	0	14.55±0.29	25.53±0.18	46.18±0.19	72.35±0.30	95.34±0.23
F5	0	16.79±0.73	26.87±0.19	55.53±0.19	78.76±0.45	98.26±0.21
F6	0	14.83±0.17	24.29±0.18	44.47±0.27	71.34±0.35	94.54±0.36
F7	0	11.18±0.88	20.46±0.54	41.46±0.18	67.76±0.46	89.15±0.46
F8	0	10.80±0.59	16.44±0.26	41.36±0.16	65.32±0.58	86.53±0.59

Table 13: *In-vitro* cummulative release of controlled release tablets of losartan potassium

Fcode	Time(hrs)							
	0	**1**	**2**	**4**	**6**	**8**	**10**	**12**
F1	0	10.49±0.2	15.87±0.24	23.29±0.21	29.43±0.33	47.46±0.18	75.35±0.26	94.05±0.17
F2	0	9.52±0.19	14.70±0.51	22.23±0.16	27.21±0.34	45.27±0.15	74.33±0.39	92.62±0.14
F3	0	8.19±0.53	13.19±0.14	21.44±0.14	26.37±0.32	43.41±0.19	71.76±0.39	91.21±0.19
F4	0	7.68±0.19	12.48±0.23	19.56±0.18	24.43±0.32	42.15±0.13	70.35±0.27	90.36±0.26
F5	0	11.53±0.14	16.78±0.74	26.88±0.10	35.78±0.18	55.58±0.13	78.23±0.44	98.27±0.20
F6	0	8.94±0.18	14.84±0.13	20.27±0.15	27.46±0.14	44.49±0.28	73.27±0.39	93.56±0.30
F7	0	6.68±0.29	10.18±0.84	16.43±0.59	18.29±0.57	40.46±0.17	65.72±0.56	85.15±0.49
F8	0	7.41±0.26	11.82±0.58	18.42±0.20	20.17±0.69	42.33±0.18	68.34±0.56	89.53±0.54

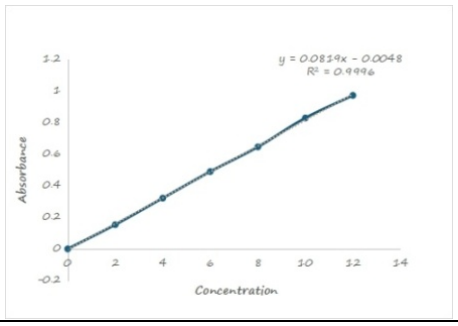


Figure 1: Standard graph of Losartan potassium using 0.1N HCL (232nm)

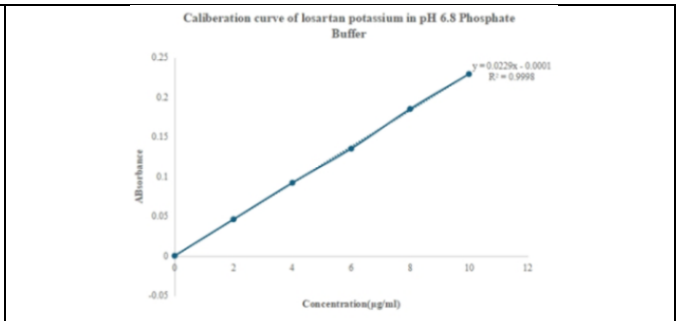


Figure 2: Standard Calibration curve of Losartan potassium in pH 6.8 buffer(232nm)

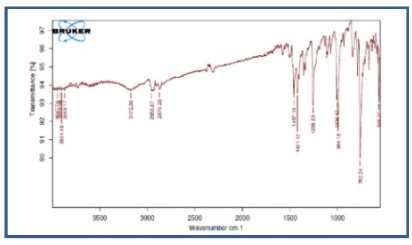


Figure 3: FTIR Spectra of pure losartan potassium

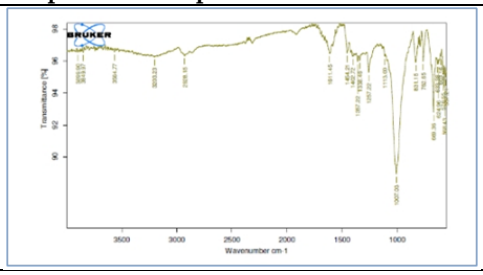


Figure 4: FTIR Spectra of pure losartan potassium with excipients

RESEARCH ARTICLE

Effects of Dry Needling Technique Versus Muscle Energy Technique in Improving Range of Motion and Functional Activity in Subjects with Shoulder Impingement Syndrome -A Comparative Study

Nimalan P[1], Hemalatha S[1] and K .Ramanan[2]*

[1]Student (BPT), Department of Physiotherapy, Saveetha Medical College, Saveetha Institute of Medical and Technical Sciences (Deemed-to-be University), Chennai, Tamil Nadu, India.
[2]Assistant Professor, Department of Physiotherapy, Saveetha Medical College, Saveetha Nagar, Saveetha University (Deemed-to-be University), Thandalam, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
K .Ramanan
Assistant Professor,
Department of Physiotherapy,
Saveetha Medical College, Saveetha Nagar,
Saveetha University (Deemed-to-be University),
Thandalam, Chennai, Tamil Nadu, India.
E.Mail: ramana.sports@gmail.com

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ABSTRACT

The purpose of the study is to examine the effects of dry needling and muscular energy treatments on the improvement of shoulder range of motion and functional activities in patients with shoulder impingement syndrome. Dry needling promotes range of motion, flexibility, and the release of tense muscles. Muscle Energy Technique (MET) stimulates the body's natural healing mechanisms, enhancing muscular function and range of motion. The study aims to compare how well these methods work in helping patients with shoulder impingement syndrome improve their functional activities and range of motion in the shoulder. The study aims to examine how dry needling and muscle energy treatments increase shoulder range of motion and functional activities in individuals with shoulder impingement syndrome**.** 40 patients with SIS were selected and randomly assigned into two groups: The Dry Needling Technique (DNT) group (n=20) and the Muscle Energy Technique (MET) group (n=20). Shoulder Pain and Disability Index (SPADI) the intervention lasted 4 weeks, with both groups having 3 sessions per week. Pre and post-test values were recorded and analyzed. Both groups showed improvement in shoulder function and range of motion. But DNT group showed higher improvement than the MET group with a p-value <0.0001. Both dry needling and muscle energy techniques improve shoulder range

of motion and functional activities in SIS, but dry needling is better. So dry needling can be considered a treatment approach for shoulder impingement syndrome.

Keywords: Shoulder Impingement, Shoulder Mobility, Functional Activity

Abbreviations Muscle energy technique (MET), Dry Needling technique (DNT), Shoulder Impingement Syndrome (SIS), Functional status.

INTRODUCTION

The painful ailment known as shoulder impingement syndrome is brought on by irritation or inflammation of the bursa, a fluid-filled sac, and the tendons in the shoulder joint. This irritation typically occurs when these structures get pinched between the acromion (bony projection on the shoulder blade) and the humeral head during overhead movements. Shoulder pain stands as the third most common musculoskeletal condition. According to reports, 14.7 out of 1000 primary care subjects experience shoulder pain each year, and up to 70% of subjects will experience it in their entire life. Adults report experiencing shoulder symptoms up to 20% of the time, and the number of complaints is increasing [1]. Millions of people worldwide suffer from shoulder impingement syndrome (SIS), a common musculoskeletal ailment. Understanding its prevalence and impact across different populations is crucial for improving diagnosis, treatment, and prevention strategies [2]. In general, 18–26% of individuals report having had shoulder discomfort at some time in their life. Shoulder impingement syndrome is a primary cause of this pain, and 7–34% of people who have shoulder complaints are diagnosed with SIS. In addition, SIS is responsible for 44–65% of all occurrences of shoulder pain worldwide. In India, Research indicates that an individual's incidence of shoulder impingement syndrome ranges from 6.7% to 66.7% [3]. Certain groups like adults with diabetes or those involved in occupations with repetitive overhead movements might be at higher risk[4]. SIS is most common in middle-aged adults (40-60 years old), Men are generally more affected than women, with a 2:1 to 4:1 male-to-female ratio[5]. Professions involving repetitive overhead movements (athletes, construction workers, painters) have a higher risk, and Certain sports like swimming, baseball, and tennis increase the risk. Individuals with rotator cuff injuries, osteoarthritis, or other shoulder problems are more susceptible. Because it is a joint made up of a ball and socket, the shoulder joint offers a broad range of motion[6]. The humeral head inside the glenoid cavity is stabilized by the four muscles that make up the rotator cuff: the supraspinatus, infraspinatus, teres minor, and subscapularis.

Flexion, extension, abduction, adduction, internal rotation, and external rotation are the primary shoulder motions[7][8]. The muscles that comprise the rotator cuff—the supraspinatus, infraspinatus, teres minor, and subscapularis—are crucial for supporting the joint and regulating these motions. Because of its unusual form, which permits a broad range of motion, the shoulder is vulnerable to several biomechanical issues, such as shoulder impingement syndrome. Understanding the biomechanics of the shoulder is crucial for diagnosing and managing conditions affecting this complex joint. The biomechanical Factors in Impingement occur when the subacromial space has a narrow gap between the underside of the acromion and the top of the humerus[9]. The humeral head approaches the acromion when moving overhead, potentially compressing the rotator cuff tendons and bursa within this space and when the scapula upwardly rotates and tilts posteriorly during overhead movements to provide adequate clearance for the rotator cuff tendons[10]. However, in the scapular muscles, poor coordination can lead to inadequate scapular motion, further reducing the subacromial space and increasing impingement risk. Rotator cuff weakness, tears, or tendinitis can further compromise the stability of the humeral head, making it more prone to impingement during overhead movements[11]. A shoulder impingement syndrome clinical diagnosis was made during a physical and orthopedic examination. Tightened myofascial tissues can weaken the surrounding muscles, particularly the rotator cuff muscles responsible for shoulder stability. This weakness can further contribute to impingement by allowing the humeral head to move more freely and impinge on the surrounding structures[12]. Diagnosing shoulder impingement syndrome (SIS) includes a physical examination, some tests, and a clinical history.

The patient-reported outcome measures are how shoulder impingement syndrome is detected. People with shoulder issues can have their pain, function, and quality of life evaluated with the help of questionnaires like the Shoulder Pain and Disability Index (SPADI).

Functional tests Measuring specific tasks like throwing, reaching overhead, or lifting weights can evaluate improvements in shoulder function following treatment[13][14]. Pain management and mild range-of-motion exercises were the main focus of early physical therapy. Injections of corticosteroids, anesthetics, or other pain-relieving medications were used to directly address inflammation and pain. In severe cases or when conservative treatments failed, surgery was considered. Open surgeries were more common in the past, involving larger incisions and longer recovery times. It's crucial to remember that these earlier therapies' degrees of efficacy varied widely, and some even posed dangers for consequences, with advancements in medical technology and understanding of shoulder biomechanics, treatment options for shoulder impingement syndrome have evolved significantly. While past physiotherapy interventions offered some relief for shoulder impingement syndrome, they often lacked specificity and effectiveness. Modern approaches have evolved significantly, focusing on targeted interventions, specific exercises, and subject education, leading to better functional outcomes and improved quality of life for individuals suffering from this condition. This study mainly focuses on muscle energy technique and dry needling technique for subjects with shoulder impingement syndrome. "Muscle energy techniques" are a type of soft tissue osteopathic manipulation techniques that combine carefully planned and regulated subject-initiated isometric and/or isotonic contractions to improve musculoskeletal function and reduce pain.[15]For the management of musculoskeletal pain, physical therapists use dry needling as a therapy method. Using a small needle, it penetrates the muscles, subcutaneous tissues, and skin to manually destabilize the interior tissues[16] and the other one is the Muscle Energy Technique. The main objective of direct, non-invasive manual therapy is known as the Muscle Energy Technique (MET). Non-invasive therapies like the muscle energy method (MET) and dry needling (DN) are becoming more popular for treating shoulder impingement syndrome. However, understanding their effectiveness and gaps in knowledge is crucial for optimal treatment. In the past manual therapy and modalities were given for the shoulder impingement syndrome. It addresses the pain but not the ROM and causes.

Mechanical Effects of Dry Needling Technique are the insertion of thin filiform needles into trigger points and other myofascial structures, which has gained popularity for its potential pain-relieving effects. The exact mechanical effect is Trigger Point Depolarization in which insertion of the needle is thought to mechanically disrupt the trigger point's hypertonic muscle fibers, leading to depolarization and a local twitch response. This technique can also help to release Myofascial restrictions, which restrict ROM and contribute to pain and dysfunction this helps to improve joint range of motion[17]. Also, MET can stimulate the Mechanoreceptors, which can help to reduce pain and improve proprioception. While these are the proposed mechanisms, the evidence supporting them is still evolving. Studies show that dry needling and MET can improve the range of motion, decrease discomfort, and improve muscle function in some disorders, including shoulder impingement syndrome[18]. However, the precise mechanisms remain unclear, and the long-term effects of dry needling and MET require further investigation. The effectiveness of treatment might also depend on factors like needle type, depth, duration of treatment, and individual patient factors[19]. Thus, this study's goal is to assess the impact of muscular energy strategy versus dry needling for patients suffering from shoulder impingement syndrome and improve their ROM and functional activities. Imaging techniques. Were not always needed for initial diagnosis but imaging tests provide valuable insights into the underlying structures and confirm the presence of impingement. Commonly used techniques for shoulder impingement syndrome X-rays were useful for detecting bone abnormalities like bone spurs or calcifications under the acromion that contribute to impingement[20].

MATERIALS AND METHODS

It was a comparative study done with 40 subjects with shoulder impingement syndrome aged between 35-55 years both men and women. Convenient sampling is used in the study.

Selection criteria
Inclusion criteria: subjects diagnosed with shoulder impingement
Age: 34-55
Gender: male, female

Exclusion criteria
Subjects other than shoulder impingement such as rotator cuff tear, adhesive capsulitis, and shoulder tendinitis. This research followsevery procedure carried out during the study complied with the institutions' ethical standards. The study was approved by the Saveetha College of Physiotherapy Institutional Scientific Review Board[ISRB] [04/020/2023/ISRB/SR/SCPT]

PROCEDURE

A total of 120 participants were invited for the study and in this 70 were selected according to the inclusion and exclusion criteria. Of these 70 participants, 30 participants were dropped due to lack of follow-up and personal inconvenience. Finally, 40 subjects were selected. The selected participants were informed about the procedures of the study and got a consent form. The selected participants were assigned into 2 groups Dry needling group (n=20) and the muscle energy group (n=20). Pre-analysis is carried out using a goniometer and SPADI. The participants underwent intervention for 4 weeks. Both groups underwent 3 sessions per week The participants were gathered in Chennai, and a total of (n=40)patients were recruited as a sample size. Data from the pre-tests and post-tests were collected to compare the results before and following the implementation of the rehabilitation strategy. Four weeks of Dry needling and Muscle energy techniques were included in the rehabilitation program. To collect patient input both before and after sessions, a survey form was also sent.

Clinical guidelines for dry needling technique
Materials required
1. Dry needles: single-use needles made of surgical-grade stainless steel.
(0.22-0.35mm diameter)
2. Alcohol wipes and gloves.
The groups were treated with a dry needle that weighed 0.25 grams and measured 25 mm. An acupuncture needle was never used more than once. The therapist cleaned the area where the needle was inserted with an alcohol swab and wore surgical gloves the entire time. The latent MTP was located by pincher palpation.

Dry NeedlingLevator scapula MTrP was identified by flat or deep palpation from the position in which one is side-lying, with the affected side at the top. The therapist will palpate your neck and shoulder area to locate trigger points in the levator scapulae muscle. These are often tender and taut bands of muscle fibers. The therapist will use thin, sterile needles to insert into the identified trigger points. The needles are not injected with any medication. Insertion depth can vary depending on the trigger point's location.

Dry Needling for Supraspinatus The patient is usually positioned in a way that allows easy access to the Supraspinatus muscle. This may involve sitting or lying on the side, depending on the practitioner's preference. A thin, sterile needle is gently inserted into specific points within the supraspinatus muscle. The practitioner will use palpation and anatomical knowledge to locate trigger points or areas of muscle tension. Once the needle is in place, the practitioner may manipulate it by applying subtle movements, such as twirling or piston-like motions. This helps stimulate the trigger points and release tension within the muscle.

Dry Needling for Infraspinatus The patient is usually positioned in a way that allows easy access to the infraspinatus muscle. This may involve lying on the side or in a prone position. The practitioner will ensure that the skin and the area around the infraspinatus muscle are properly sterilized to reduce the risk of infection. A thin, sterile

needle is gently inserted into specific points within the infraspinatus muscle. The needles are typically left in place for a short duration, and then they are carefully removed.

Clinical guidelines for muscle energy technique (MET)

Muscle Energy Technique (MET) is used to address shoulder impingement by influencing muscle tone and joint mobility. The therapist guides the patient to perform an isometric contraction of the agonist muscle (muscle causing impingement) against gentle resistance from the therapist. Then the therapist gently stretches the antagonist muscle to a new barrier to motion, capitalizing on the post-isometric relaxation effect. For ten seconds, the stretch was held. The procedure was carried out three times. This increases the range of motion for internal rotation by lengthening the external rotators.

MET treatment for Supraspinatus With one hand supporting the shoulder on the side which requires to be treated and the other reaching out in front of the patient to support the ß flexed elbow and forearm, the therapist stands behind the seated patient. After the patient's upper arm had been brought to its easy barrier, the patient tried to bring the arm back with 20% of their strength, but the therapist resisted. The patient takes a deep breath during this procedure. The patient exhales air following an isometric contraction lasting 10 seconds. With the patient's help, the arm is gently moved toward its new resistance barrier to achieve greater adduction. Repeat multiple times, allowing at holding each painless stretch for at least 20 seconds.

MET Treatment for Infraspinatus The patient was positioned in a supine posture on the examination table, with their shoulders abducted and elbows flexed to a 90-degree angle, respectively. The therapist will gently rotate the shoulder internally until the first mobility barrier is overcome. Subsequently, the patient will be instructed to maintain an isometric posture in the direction of external rotation for five seconds at a 25% maximum voluntary contraction. To assist the patient in completing this exercise, the examiner will provide an opposing force at the distal forearm. Throughout this procedure, the patient inhales. After five seconds of isometric contraction, the patient releases air. The procedure is done in the same way. The patient is instructed to unwind following the contraction. After that, the therapist applies 30 seconds, shifts the shoulder into a new internal rotation range, and then repeats the procedure.

MET Levator Scapulae Therapy The patient lies face down on the treatment table with their head turned slightly to the opposite side of the levator scapulae being treated. The therapist supports the patient's head with their hand. The therapist asks the patient to gently push their head down against the therapist's resistance for a few seconds. This isometric contraction activates the antagonist muscle (depressor scapulae) and helps to relax the levator scapulae. After the contraction, the therapist asks the patient to relax their head and gently turns it further towards the treated side, stretching the levator scapulae. For ten seconds, the stretch was held. The procedure was carried out three times. This increases the range of motion for internal rotation by lengthening the external rotators.

OUTCOME MEASURES

Range of motion (ROM) The goniometer is composed of two arms: the movable arm and the stationary arm. Every arm is positioned at particular points on the body, and the joint that needs to be measured is where the goniometer center is aligned.

Shoulder Pain and Disability Index (SPADI) To assess shoulder pain and disability in an outpatient context, the Shoulder Pain and Disability Index (SPADI) was created. The 13 items that comprise the two domain-evaluating SPADI are an 8-item component for disability and a 5-item sub-scale for pain.

ILLUSTRATIONS

A comparison of the pre-test and post-test values of the dry needling technique using a goniometer reveals that the mean value increased from 70.75 (SD = 10.22) in the pre-test to 142.50 (SD = 15.52) in the post-test. The paired t-test yielded a t-value of 37.46, with a p-value of <0.0001, indicating that the findings are extremely statistically significant

(Table:1). A comparison of the pre-test and post-test values of the dry needling technique using the SPADI scale shows a significant reduction in scores, with the mean decreasing from 67.30 (SD = 4.97) in the pre-test to 34.30 (SD = 4.21) in the post-test. The paired t-test yielded a t-value of 22.65, and the p-value was <0.0001, indicating the results are extremely statistically significant (Table:2). Comparison of the pre-test and post-test values of the Muscle Energy Technique (MET) using a goniometer indicates a notable improvement, with the mean increasing from 71.15 (SD = 10.67) to 116.75 (SD = 9.36). The paired t-test produced a t-value of 36.38, and the p-value was <0.0001, demonstrating extremely statistically significant results(Table: 3). A comparison of the pre-test and post-test values of the Muscle Energy Technique (MET) using the SPADI scale revealed a significant reduction in scores, with the mean decreasing from 67.80 (SD = 5.51) to 39.05 (SD = 4.89). The paired t-test resulted in a t-value of 17.44, and the p-value was <0.0001, indicating the results are extremely statistically significant(Table: 4). Comparing the post-test values of the Dry Needling Technique (DNT) and Muscle Energy Technique (MET) using a goniometer showed that the DNT group had a higher mean post-test value (142.50, SD = 15.52) compared to the MET group (116.75, SD = 9.36). The t-value was 6.35, with a p-value of <0.0001, indicating an extremely statistically significant difference between the two groups(Table: 5). Compares the post-test SPADI scores between the Dry Needling Technique (DNT) group and the Muscle Energy Technique (MET) group (Group B). The DNT group had a mean score of 34.30 (SD = 4.21), while the MET group had a higher mean score of 39.05 (SD = 4.89). The t-value was 3.29, with a p-value of <0.0022, indicating a statistically significant difference in SPADI scores between the two groups(Table:6) The therapist performed the Dry Needling Technique with the participant in a comfortable prone or side-lying position. After locating the trigger point in the supraspinatus muscle, a sterile, disposable filiform needle was inserted perpendicularly into the identified taut band. The needle was manipulated using pistoning or fanning techniques to elicit a local twitch response. The needle was retained in place for approximately 10 minutes, with periodic gentle stimulation.

Throughout the procedure, the participant was instructed to remain relaxed and breathe normally. After needle removal, gentle passive range of motion was performed to enhance muscle relaxation, followed by a rest interval of approximately 5 seconds(Fig: 1). The therapist administered the Dry Needling Technique with the participant in a side-lying or prone position to allow optimal access to the infraspinatus muscle. After identifying the myofascial trigger point within the muscle belly, a sterile, disposable filiform needle was inserted perpendicular to the skin or angled slightly, depending on anatomical landmarks. The needle was advanced carefully into the trigger point until a local twitch response was elicited. Pistoning or gentle rotation techniques were applied for effective stimulation. The needle was retained for approximately 10 minutes, with intermittent manual stimulation. The participant was instructed to remain relaxed and breathe deeply to aid in muscle release. Following needle removal, a gentle passive stretch was provided, and a brief rest period of 5 seconds was given(Fig: 2). The therapist administered the Muscle Energy Technique with the participant in a supine or side-lying position, depending on the specific rotator cuff muscle targeted. The technique involved positioning the shoulder near the restriction barrier relevant to the involved muscle. The participant was instructed to perform a sub-maximal isometric contraction (30–40% of their maximum effort) against the therapist's resistance, held for approximately 10 seconds. Following the contraction, a relaxation phase was initiated, during which the therapist gently stretched the muscle to a new barrier for about 30 seconds. This procedure was repeated three times. During the final relaxation phase, participants were encouraged to perform deep breathing to facilitate neuromuscular relaxation. A rest interval of 5 seconds was provided between each repetition. This post-isometric relaxation approach helped reduce muscle tightness and improve functional range of motion in the glenohumeral joint(Fig: 3).

RESULT

When compared to the pre-assessment and the post assessment shows that there is a significant decrease in shoulder pain and increase in quality of life using Goniometer and SPADI scales. The statistical mean value for goniometer DNT pre intervention was 70.75± and standard deviation was 10.22± post intervention value was 142.50± and 15.52±.Hence the post intervention mean value shows higher significant value than the pre intervention. The statistical mean value for goniometer of group B pre intervention was 71.15± and standard deviation was 10.67± and

post intervention value was 116.75± and 9.36±.Hence the post intervention mean value shows higher significant value than the pre intervention. The statistical mean value for SPADI of DNT pre intervention was 67.30± and standard deviation was 4.97± post intervention value was 34.30± and 4.21±. Hence the post intervention mean value shows higher significant value than the pre-intervention. The statistical mean value for SPADI of group B pre intervention was 67.80± and standard deviation was 5.51± post intervention value was 39.05± and 4.89±. Hence the post intervention mean value shows higher significant value than the pre-intervention. The paired t-test and unpaired t-test was used to statistically analyze the values. A statistically significant difference was found between DNT (Dry Needling) and GROUP B (Muscle Energy Technique) as well as within the group, according to the statistical analysis performed on the quantitative data. .In DNT the post mean value of Goniometer and SPADI is 142.50± and 34.30± whereas in group B it is 116.75± and 39.05±. This demonstrates that the Dry Needling group final results shows a significant reduction in pain.

DISCUSSION

The goal the present research is to compare the effectiveness of Dry needling and MET to reduce pain in shoulder impingement syndrome and also to assess the the effectiveness in terms reducing pain and disability. The comparison is demonstrated with a duration of 4 weeks. The results were measure using the goniometer and SPADI questionnaire before and after the treatment. Beneficial effect were significantly greater in DNT (Dry Needling) than the group B (MET).When the responses were compared between both groups, the result showed a significant difference in Dry needling than the MET. In the study Naveetbadoni.*et al*., (2023) states that in the fourth and eighth weeks of the treatment program, patients with diabetic frozen shoulder who received Muscle Energy Technique (MET), Stabilization Exercise, and Moist Heat Therapy (MHT) significantly improved in terms of pain, range of motion, muscular strength, and joint position sense. MET works well to extend the glenohumeral joint's range of motion. The physiological mechanisms underlying the MET-induced changes in muscle extensibility include relaxation of the reflex, changes in viscoelastic or muscle properties, and modifications in stretch tolerance. The body of scientific literature primarily supports the modification in stretch tolerance. Increased range of motion (ROM) at the joint results from these mechanisms, which also alter the physiology of the muscles[11]. Hardy C *et.al*.,(1986) shows that the deviation from the subacromial soft-tissue structures' normal relationships is the first sign of shoulder impingement syndrome. Prolonged illness results in secondary skeletal changes. In comparison to patients with clearly defined bony constriction treatment with medications intended to reduce swelling and inflammatory changes in the subacromial soft tissues may be more effective in patients with less advanced growth and degenerative bone changes demonstrates, however, that there was no relationship at all between the frequency or severity of the radiographic abnormalities linked to impingement syndrome and the immediate response to medical therapy[2]. KUMARAN N.*et.al*.,(2023) showed that the centrally mediated pain inhibitory mechanism and the neuronal mechanism in the dorsal Horn are caused by tissue and neurological factors. These include stimulation of low-threshold mechanoreceptors, which may have gating effects, and the effect of rhythmic muscular contractions. A study by Blanco diaz.*et.al*.,(2022)showed the goal of this study is to evaluate the efficacy of dry needling (DN) and conventional physiotherapy in promoting the recovery of patients with subacromial syndrome (SAS).DN has been shown in several studies to be as effective as applying a placebo to minimize pain in four weeks for various pathologies, particularly those involving the upper limb.

A single DN session has also been shown to be effective. While a reduction in functional limitations is implied by the effectiveness of the interventions, this has nothing to do with how effective the technique is in terms of work capacity or sick leave duration. It is advised to look into the effectiveness of DN in other diseases to validate its value and then incorporate it with other treatments that are administered in clinical settings[8]. In the study Flannigan et al.,(1990) compares MR arthrography with conventional MR imaging in patients with suspected labral or rotator cuff abnormalities, providing valuable insight into the relative accuracy of each technique. Images were evaluated independently and blindly by two radiologists, reducing bias and strengthening the validity of the findings[19]. A study by Ludewig and Braman *et.al*., (2011)study showed the Shoulder impingement Biomechanical considerations

in rehabilitation provides a valuable in-depth look at the biomechanical factors contributing to shoulder impingement, moving beyond the traditional focus on rotator cuff tears. The article provides a comprehensive review of relevant literature, supporting the arguments presented and offering context for further research. The discussion mainly centers on subacromial impingement, neglecting other types of shoulder impingement that may have different biomechanical considerations[17]. Bhawna and Kundu *et.al*., (2016) states that the study shows 22.9% prevalence of shoulder pain among adults aged 30-70 years in Northern India. Obesity, left-hand dominance, and diabetes were identified as significant risk factors for shoulder pain. Reliance on self-reported pain, potentially introducing bias.Limited information on the specific characteristics and severity of shoulder pain. Study exploring the effectiveness of different treatment interventions for shoulder pain in Northern India, considering cultural and socioeconomic factors[16]. A studyby Clark *et.al*., (2001) shows that the Muscle Energy Techniques (METs) are manual therapy techniques that utilize the patient's own muscle energy to achieve various therapeutic goals like Stretching and lengthening shortened muscles, Strengthening weak muscles, Improving joint mobility and range of motion. Provides a concise and clear overview of the principles and applications of METs, Describes different types of METs and their specific techniques. Discusses the potential benefits and indications for using MET. Primarily focuses on the theoretical underpinnings and techniques of METs, with limited discussion on research evidence supporting their efficacy[7]. In the study Lucado *et.al*., (2011) demonstrate that the literature reviewing this paper connects abnormal scapulothoracic muscle function to shoulder joint pathology and pain. The findings of the reviewed studies suggest that scapular muscle imbalance, specifically weakness of the serratus anterior and upper trapezius, is linked to shoulder pain conditions such as rotator cuff impingement, superior labrum anterior to posterior (SLAP) lesions, and multidirectional instability. For patients with glenohumeral joint disease, proper assessment of the pectoralis minor, serratus anterior, and upper trapezius length, strength, and activation are essential to their rehabilitation. Consequently, the current study set out to investigate the effects of dry needling, which helps improve shoulder functions. The study's conclusions have shown that dry needling is a useful treatment for shoulder impingement syndrome, and it can help patients heal.

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Table 1: Comparison of pre and post value of dry needling technique using goniometer by paired t-test

Statistical Analysis	Pre test	Post test	T value	P value
MEAN	70.75	142.50	37.46	<0.0001
SD	10.22	15.52		
SEM	2.28	3.47		

A comparison of the pre-test and post-test values of the dry needling technique using a goniometer reveals that the mean value increased from 70.75 (SD = 10.22) in the pre-test to 142.50 (SD = 15.52) in the post-test. The paired t-test yielded a t-value of 37.46, with a p-value of <0.0001, indicating that the findings are extremely statistically significant.

Table 2: Comparison of pre and post-values of DNT using spadi scale by paired t-test

Statistical analysis	Pre test	Post test	T value	P value
MEAN	67.30	34.30	22.65	<0.0001
SD	4.97	4.21		
SEM	1.11	0.94		

A comparison of the pre-test and post-test values of the dry needling technique using the SPADI scale shows a significant reduction in scores, with the mean decreasing from 67.30 (SD = 4.97) in the pre-test to 34.30 (SD = 4.21) in the post-test. The paired t-test yielded a t-value of 22.65, and the p-value was <0.0001, indicating the results are extremely statistically significant.

Table 3: Comparison of pre- and post-values of MET using a goniometer by paired t-test

Statistical analysis	Pre test	Post test	T value	P value
MEAN	71.15	116.75	36.38	<0.0001
SD	10.67	9.36		
SEM	2.39	2.09		

comparison of the pre-test and post-test values of the Muscle Energy Technique (MET) using a goniometer indicates a notable improvement, with the mean increasing from 71.15 (SD = 10.67) to 116.75 (SD = 9.36). The paired t-test produced a t-value of 36.38, and the p-value was <0.0001, demonstrating extremely statistically significant results.

Table 4: Comparison of pre- and post-values of met using the SPADI scaleby paired t-test

Statistical analysis	Pre test	Post test	T value	P value
MEAN	67.80	39.05	17.44	<0.0001
SD	5.51	4.89		
SEM	1.23	1.09		

A comparison of the pre-test and post-test values of the Muscle Energy Technique (MET) using the SPADI scale revealed a significant reduction in scores, with the mean decreasing from 67.80 (SD = 5.51) to 39.05 (SD = 4.89). The paired t-test resulted in a t-value of 17.44, and the p-value was <0.0001, indicating the results are extremely statistically significant.

Table 5 : Comparison of post value of DNT and MET of the goniometer by using an unpairedt-test

Statistical analysis	Dnt	met	T value	P value
MEAN	142.50	116.75	6.35	<0.0001
SD	15.52	9.36		
SEM	3.47	2.09		

comparing the post-test values of the Dry Needling Technique (DNT) and Muscle Energy Technique (MET) using a goniometer showed that the DNT group had a higher mean post-test value (142.50, SD = 15.52) compared to the MET group (116.75, SD = 9.36). The t-value was 6.35, with a p-value of <0.0001, indicating an extremely statistically significant difference between the two groups.

Table 6: Comparison of post value of DNT and MET of SPADI by using the unpaired t-test

Statistical analysis	Dnt	Group B	T value	P value
MEAN	34.30	39.05	3.29	<0.0022
SD	4.21	4.89		
SEM	0.94	1.09		

Compares the post-test SPADI scores between the Dry Needling Technique (DNT) group and the Muscle Energy Technique (MET) group (Group B). The DNT group had a mean score of 34.30 (SD = 4.21), while the MET group had a higher mean score of 39.05 (SD = 4.89). The t-value was 3.29, with a p-value of <0.0022, indicating a statistically significant difference in SPADI scores between the two groups.

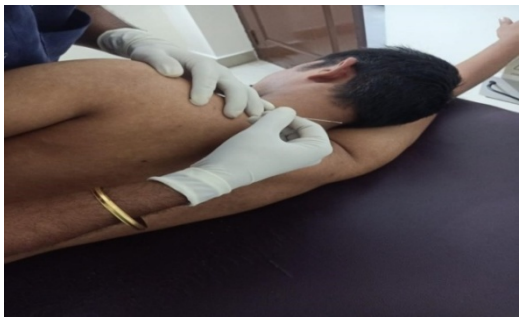


Fig 1. Dry needling for supraspinatus

The therapist performed the Dry Needling Technique with the participant in a comfortable prone or side-lying position. After locating the trigger point in the supraspinatus muscle, a sterile, disposable filiform needle was inserted perpendicularly into the identified taut band. The needle was manipulated using pistoning or fanning techniques to elicit a local twitch response. The needle was retained in place for approximately 10 minutes, with periodic gentle stimulation. Throughout the procedure, the participant was instructed to remain relaxed and breathe normally. After needle removal, gentle passive range of motion was performed to enhance muscle relaxation, followed by a rest interval of approximately 5 seconds.



Fig 2. Dry needling for the infraspinatus

The therapist administered the Dry Needling Technique with the participant in a side-lying or prone position to allow optimal access to the infraspinatus muscle. After identifying the myofascial trigger point within the muscle belly, a sterile, disposable filiform needle was inserted perpendicular to the skin or angled slightly, depending on anatomical landmarks. The needle was advanced carefully into the trigger point until a local twitch response was elicited. Pistoning or gentle rotation techniques were applied for effective stimulation. The needle was retained for approximately 10 minutes, with intermittent manual stimulation. The participant was instructed to remain relaxed and breathe deeply to aid in muscle release. Following needle removal, a gentle passive stretch was provided, and a brief rest period of 5 seconds was given.



Fig 3. Muscle energy technique for rotator cuff muscles

The therapist administered the Muscle Energy Technique with the participant in a supine or side-lying position, depending on the specific rotator cuff muscle targeted. The technique involved positioning the shoulder near the restriction barrier relevant to the involved muscle. The participant was instructed to perform a sub-maximal isometric contraction (30–40% of their maximum effort) against the therapist's resistance, held for approximately 10 seconds. Following the contraction, a relaxation phase was initiated, during which the therapist gently stretched the muscle to a new barrier for about 30 seconds. This procedure was repeated three times. During the final relaxation phase, participants were encouraged to perform deep breathing to facilitate neuromuscular relaxation. A rest interval of 5 seconds was provided between each repetition. This post-isometric relaxation approach helped reduce muscle tightness and improve functional range of motion in the glenohumeral joint.

RESEARCH ARTICLE

Assessing Digital Literacy Skills of Undergraduate Students at Government Degree College, Ramachandrapuram, Andhra Pradesh

K.V.Sobha Rani*

Lecturer, Department of Computer Applications, Government Degree College, Ramachandrapuram, (Affiliated to Adikavi Nannaya University, Rajahmundry), Andhra Pradesh, India.

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***Address for Correspondence**
K.V.Sobha Rani
Lecturer, Department of Computer Applications,
Government Degree College, Ramachandrapuram,
(Affiliated to Adikavi Nannaya University, Rajahmundry),
Andhra Pradesh, India.
E.Mail: vsobharanikadumu@gmail.com

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ABSTRACT

Digital Literacy no longer simply means knowing how to turn on a computer, open a document or send an email. In today's world, digital literacy entails the ability to understand and use digital technologies in a functional, secure, and context-appropriate way for everyday and professional needs. The primary goal of this paper is to examine and evaluate the levels of digital literacy skills among the students at Government Degree College, Ramachandrapuram. The results indicate that 81 students, representing 24% of the examined population, use computers daily, while 141 students or 42% use them weekly. This suggests a significant portion of the students utilize computers weekly, with a substantial number using them daily. A very large majority of the surveyed students, specifically 333 out of 338, prefer Google Chrome as their primary web browser. This indicates a strong preference for Chrome among this student group. The data further indicates that students have foundational internet skills and are familiar with database concepts.

Keywords: Digital Literacy, Internet, Online Learning, Under Graduate Students, Web Browser.

INTRODUCTION

Digital literacy encompasses the skills required to use technology safely, effectively and responsibly. As technology continues to become more and more ingrained in daily life, the importance of learning digital literacy skills is becoming increasingly apparent. Digital literacy is indeed crucial for developing nations aiming for social and economic progress. It empowers individuals, fosters innovation and promotes inclusive growth by enabling access to information, education and economic opportunities. Digital literacy is widely recognized as a crucial and valuable tool for lifelong learning in our increasingly technology-driven world. In the modern world, digital literacy has

become a fundamental skill not just for students but for everyone, including those in the workforce and job seekers. A number of companies are actively looking for individuals with strong digital skills due to the increasing digitalization of the workplace. Numerous studies have explored digital literacy, encompassing its definition, impact on learning, and the factors that influence an individual's level of digital literacy. Sinha and Andimuthu studied the digital literacy skills of students at central university and they specified that the concept of digital literacy has reached both rural and urban students of Tamil Nadu [1]. Digital literacy among female postgraduate students of Karnataka University, Dharwad, Karnataka, India had been studied by Shettappanavar and Krishnamurthy [2]. The consequences of digital literacy and academic integrity and digital literacy influences on digital environment and current education have studied by Paul, Kerkhoff and Spires [3]. The digital literacy skills among library professionals had discussed by Natarajan and Mulat [4]. The digital literacy of Mangalore University students was focused by Kumari and Lokesha [5]. Shuhidan, Tohara, Nordin and Bahry's work in Malaysia highlights how digital literacy skills can enhance teaching and learning practices for special needy students [6]. Internet, computer and database literacy skills of the university students had explored by Prakash Bhairu Bilawar [7]. In 21st century India, digital literacy among teachers and students was stated by Pawar [8].

Purposes of the study

The purposes of this study are

- To assess undergraduate students' internet and computer skills, including their ability to use web browsers effectively.
- To evaluate the use of smart phones for internet accessing by the undergraduate students in order to meet their information needs.
- To effectively assess undergraduate students' understanding of sharing of data.
- To study computer and internet usage patterns among undergraduate students.

METHODOLOGY

The present study is to examine the digital literacy skills of students at Government Degree College, Ramachandrapuram, Dr. B.R. Ambedkar Konaseema district, Andhra Pradesh, India. The study used a purposive online survey to collect data from students enrolled in a college. A questionnaire was created using Google Forms and shared with students to gather their opinions. Google Form was shared with students via WhatsApp. A total of 338 students provided complete responses to the survey questions. Charts, tables and explanatory statements are used for presenting the students data.

RESULTS AND DISCUSSIONS

Stream-wise respondents

Table 1 and Figure 1 show the distribution of respondents of the questionnaire from different streams.

The survey data indicates that out of the students who responded, 11 students (3%) were from the Arts stream, 214 students (63%) were from the Commerce stream, and 113 students (34%) were from the Science stream. It indicates that the largest proportion of respondents belongs to the Commerce stream.

Year-wise respondents

Table 2 and Figure 2 show the respondents of the questionnaire from different years. The data shows that the majority of respondents are from the first year, with 233 (69%) students responding, compared to 23 (7%) from the second year and 82 (24%) from the third year.

Regularity and access points in the use of computer and internet

It is concluded from Figure 3 that the majority of students use computers on a weekly basis (41%), followed by "rarely" (32%), "daily" (24%), and "monthly" (3%). Table 3 shows the venues/places to use and access the internet and

computer. The provided data indicates that classrooms/laboratories are the most preferred locations for computer and internet access (95%), while libraries are the least preferred (47%). The other places identified are KRC (77%), at internet café (63%), at hostel/home (94%).

Computer Literacy Skills

The computer literacy skills of GDC (Government Degree College) students at Ramachandrapuram are specified in Table 4. It indicates a high level of awareness (94%) among students regarding computer and laptop types and specifications. This suggests a good understanding of the technical aspects of these devices. It also indicates that 93% of students having knowledge about hardware, software, operating systems, and programming languages. Further 95% students have better knowledge about different office software like Excel, Word, Power Point etc. 91% of students got awareness on different file formats and multimedia components. 33% of students have familiar with storage system with cloud applications. 96% of students have knowledge on installing the system, uploading, downloading and printing the resources. 95% of students know how to share the devices for quick retrieval of data or information. Hence, the college students have better computer literacy skills as out of 8 skill elements, 7 skill elements ratio is above 50%.

Internet Literacy Skills

The Internet literacy of GDC students at Ramachandrapuram is specified in Table 5. It specifies that 85% of the college students at GDC, Ramachandrapuram have awareness to setup internet connection, manage its settings and use of bookmarks. 95% of the college students have knowledge on WWW, URL, IP addresses, Web Browsers and techniques of navigation. 85% of the college students are familiar with webpage designing, database, data leakage and its security. 97% of the students are using mobile/internet for academics or training tasks, online learning etc. However, it is noticed from the Figure 4 that 98% of students prefer Google Chrome as their web browser, with Opera and Internet Explorer at 1% each, and Safari at 0%.

Usage of smart phones/mobiles for internet access

Table 6 shows the usage of smart phones/mobiles for accessing internet by the college students. 99% of the students use smart phones specifically for E-mail checking purposes. 98% are for chatting (Whatsapp / Facebook). 97% are using for entertainment, 96% for online shopping and 97% for E-learning tasks. Further it is noticed that 99% of the students have knowledge about sharing of data.

CONCLUSION

Digital literacy skills empower students in changing world by equipping them with the knowledge and tools to navigate the digital landscape efficiently. From the present study, it can be concluded that a significant portion of students possess computer literacy, internet usage and basic database skills. It is further noticed that 67% of the students lack knowledge about cloud storage and applications. Based on the data, interactive sessions and awareness programs are needed to educate students at Government Degree College, Ramachandrapuram about cloud storage systems and applications. Furthermore, with students possessing high digital literacy skills, college can leverage this by enhancing online learning platforms, incorporating digital tools into coursework, and focusing on digital citizenship education. This includes developing personalized learning paths, promoting ethical digital practices and fostering collaborative online projects.

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Table 1: Stream-wise respondents

S.No.	Stream	No. of Respondents	Percentage
1	Arts	11	3%
2	Science	113	34%
3	Commerce	214	63%

Table 2: Year-wise respondents

S.No.	Year of Study	No. of Respondents	Percentage
1	I	233	69%
2	II	23	7%
3	III	82	24%

Table 3: Places to access Computer/Internet

Places to use and access Computer/Internet	No. of students	Percentage (%)
Classroom/Laboratory	322	95
Library	160	47
Knowledge Resource Centre (KRC)	260	77
Home/hostel	317	94
Internet cafe	213	63

Table 4: Computer Literacy Skills

Sl. No.	Elements of Computer Literacy Skills	No. of Students	Percentage
1	Knowledge about specification/types of laptop/computer	317	94%
2	Awareness on hardware /software /Operating System /programming languages	314	93%
3	Being familiar with office software's like Word, PowerPoint, Excel etc.	322	95%
4	Having knowledge of different file formats (pdf, doc/docs, HTML, text, jpeg etc.) Media Players etc.	306	91%
5	Being familiar with system installations, uploading, downloading and printing of the resources	324	96%
6	Knowledge about storage system with cloud applications	112	33%

7	Familiarization with sharing the devices for quick retrieval of information/data	322	95%
8	Undergone computer course/training to get knowledge in computers	293	87%

Table 5: Internet Literacy Skills

Sl. No.	Internet Literacy Skills	No. of Students	Percentage
1	Knowledge about setup of internet connection, manage settings and use of bookmarks	287	85%
2	Awareness on WWW, IP addresses, URL, Web Browsers and techniques of navigation	322	95%
3	Being familiar with webpage designing, database, data leakage and its security	288	85%
4	Use of mobiles/internet for academic/ training tasks, online learning etc.	329	97%

Table 6: Use of Smart phone/Mobiles

Sl. No.	Use of Smart phone/Mobiles	No. of Students	Percentage
1	For E-mail checking	333	99%
2	For Chatting (Whatsapp/Facebook)	332	98%
3	For Entertainment	327	97%
4	For Online shopping	326	96%
5	For E-learning	327	97%

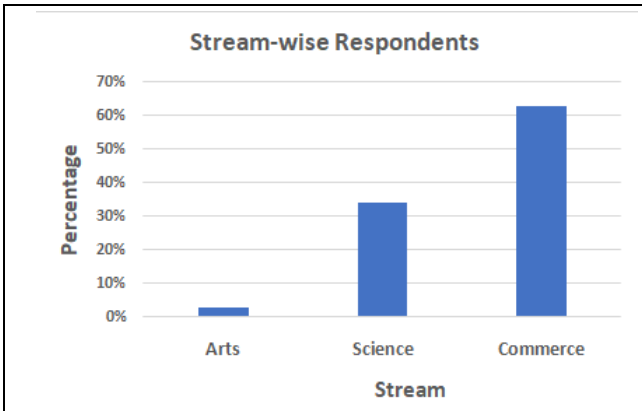


Figure 1: Graphical representation of Stream-wise respondents

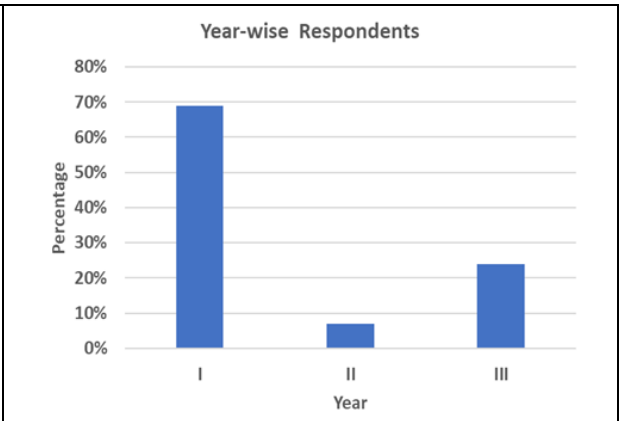


Figure 2: Graphical representation of Year-wise respondents

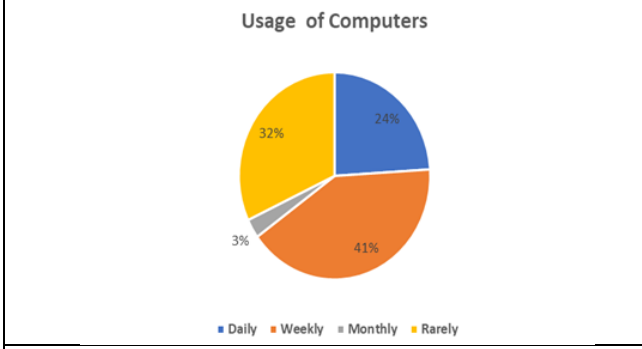


Figure 3: Regularity in the use of computers

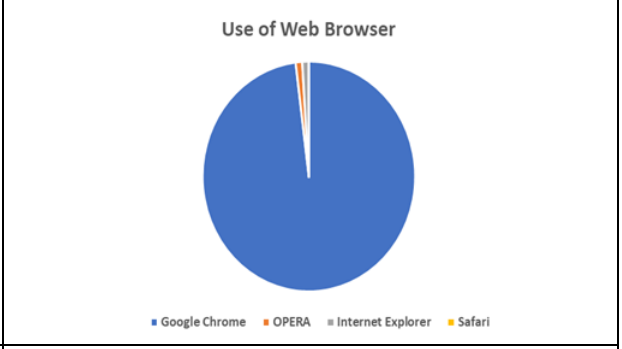


Figure 4: Use of Web browser software

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RESEARCH ARTICLE

Isolation, Identification and Antimicrobial Resistance Pattern of ESKAPEE Isolated from Clinical Samples

Vasvi Patel[1] and Meghna Diarsa[2*]

[1]M.Sc Student, Department of Medical Laboratory Technology, Bapubhai Desaibhai Patel Institute of Paramedical Sciences, (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.
[2]Assistant Professor, Department of Medical Laboratory Technology, Bapubhai Desaibhai Patel Institute of Paramedical Sciences , (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.

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***Address for Correspondence**
Meghna Diarsa
Assistant Professor,
Department of Medical Laboratory Technology,
Bapubhai Desaibhai Patel Institute of Paramedical Sciences,
(Affiliated to Charotar University of Science and Technology),
Anand, Gujarat, India.
E.Mail: meghnadiarsa.bdips@charusat.ac.in

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ABSTRACT

ESKAPEE pathogens Multi-drug resistant (MDR) bacteria include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* spp., and *Escherichia coli*. These bacteria provide a growing treatment challenge to healthcare facilities and public health globally. During the study between March 2024 to May 2024, a total of 51 clinical samples were processed and out of 51 samples 33 isolates of ESKAPEE were found positive. ESKAPEE species were obtained which were *E. coli*, *Klebsiella spp*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. ESKAPEE species were found in a urine specimen, followed by a Pus sample. *E. coli* highest isolated from urine specimens. AMR study shows the highest resistance towards Cefazolin (72.72%), Cefepime (57.57%), and (54.54%) respectively and ESKAPEE strains were sensitive towards Gentamycin and Intermediate towards Tobramycin.

Keywords: ESKAPEE, Antimicrobial resistance, Antimicrobial susceptibility test.

INTRODUCTION

The ability of microorganisms to resist and remain viable in the presence of antimicrobial drugs is known as antimicrobial resistance (AMR) [1]. Antibiotics, which are believed to be an essential part of modern clinical care, are seriously at risk from antimicrobial resistance, which is becoming increasingly common in bacteria from nosocomial and community origins. This affects people's health significantly [2]. The indiscriminate, improper, and insufficient use of antibiotics is the main cause of the global increase in AMR [3]. The major global problem of antimicrobial

resistance is putting the health of people, animals, and the environment at risk due to the emergence, spread, and persistence of multidrug-resistant (MDR) bacteria, commonly referred to as "superbugs" [4]. The global rise of multidrug resistance poses a problem to the treatment of infections and may need the use of other medicines with lower safety profiles and higher cost-effectiveness [5]. Due to their MDR nature and increased resistance to last-resort drugs, the ESKAPEE pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter spp, Escherichia coli*) are listed as organisms of critical importance, requiring the urgent development of new antibiotics [6]. ESKAPEE is an acronym given to a group of multidrug-resistant Gram-positive and Gram-negative bacterial pathogens that exhibit improved transmission, a variety of pathogenicity mechanisms, and the ability to resist the biocidal actions of antibiotics [7]. The mechanisms of multidrug resistance exhibited by ESKAPEE are broadly classified into several categories, including drug inactivation, which is typically caused by an irreversible cleavage catalysed by an enzyme, modification of drug binding sites/targets, reduced drug accumulation caused by either increased drug efflux or reduced permeability, and biofilm formation [8]. ESKAPEE pathogens are included on the World Health Organization's (WHO) list of pathogens. Pathogens are classified as critical, high, or medium priority, depending on the urgency of developing new antibiotics to fight against them. Microbes with multiple resistance to drugs that are a threat to hospital patients are among the pathogens in the most critical group. *Pseudomonas*, *Acinetobacter*, and several *Enterobacteriaceae*, including *K. pneumoniae, E. coli, and Enterobacter spp.*, are among the critical-priority microorganisms. Bacteria like *Enterococcus faecium* and *Staphylococcus aureus* are included in the high-priority category [9].

MATERIALS AND METHODS

Sample collection

The study was conducted in the Infection Control Microbiology Laboratory at Charotar University of Science and Technology during the study period of March 2024 to May 2024. The samples were collected from the Charusat Hospital and Lifeline Laboratory. A total of 51 samples were collected from different clinical specimens (Urine, Blood, Pus and Sputum) of both sexes with different ages.

Ethical Approval

The study was approved by the Institutional Ethics Committee of Charusat University.

Isolation and Identification

Collected samples were cultured on Nutrient agar, MacConkey agar, Blood agar, CLED (Cystine Lactose Electrolyte Deficient) agar, and Chocolate agar and the plates were incubated at 37^0 C overnight. After overnight incubation, Culture plates were examined for Colonial characteristics, Gram Staining and Motility tests were performed and a set of Biochemical tests were inoculated for its final identification.

Antimicrobial Susceptibility Testing

The Antimicrobial Susceptibility Test (AST) was performed by the Kirby-Bauer Disc Diffusion Method [10-12]. Prepare 0.5 Mc Farland inoculum by suspending 2-3 well-isolated colonies of the test organism in peptone water. In the Kirby-Bauer Method, the suspension was inoculated on the Muller Hinton Agar (MHA) plates and covered with different antibiotic discs including Gentamycin (10µg), Tobramycin (10µg), Imipenem (10µg), Meropenem (10µg), Doripenem (10µg), Chloramphenicol (30µg), Ceftazidime (30µg), Cefepime (30µg), Cefazolin(30µg), Cefoxitin (30µg), Ertapenem (10µg), Cefotaxime (30µg), Cefazolin (30µg), Tetracycline (30µg), Trimethoprim (5µg), Azteronem (30µg), Ciprofloxacin (5µg), Levofloxacin (5µg), Cefuroxime (30µg), Ampicillin-Sulbactum (10/10µg), Ampicillin (10µg), Amikacin (30µg), Pipercicillin (100µg), Ofloxacin(5µg), Linezolid (30µg), Penicillin (10 units), Rifampin (5µg), Vancomycin (30µg), Clindamycin (2µg), Oxacillin (1µg), Doxycycline (30µg). The plates were incubated overnight at 37°C. The Clinical and Laboratory Standard Institute (CLSI) guideline and standard zone size chart were used to interpret the results (Sensitive, Intermediate, Resistance).

RESULT AND DISCUSSION

A Total of 51 clinical specimens were processed in the Infection Control Microbiology Laboratory at CHARUSAT during the study period of 03 months (March 2024 to May 2024. Among the 51 samples, 33 (64.70%) ESKAPEE species were isolates were obtained. Table 1 shows the result among the gender distribution. The maximum ESKAPEE were isolated from the female (63.63%) as compared to the male (36.36%). However, the result is contradictory to the finding of Ngoi *et al.*, 2021 [6]. They found that ESKAPEE infections were maximum found in males (60.31%) as compared to females (39.68%). Our study shows, that in both males and females, the infection of ESKAPEE was mostly found in the age group of 16-75 years. The highest prevalence of ESKAPEE was observed among the female age group of 16-30 (21.21%), followed by the 46-60 age group (15.15%), 31-45 age group (12.12%), 61-75 age group (9.09%) and the lowest prevalence among age group 76-90 (6.06%). However, in the male the highest prevalence of ESKAPEE was observed among the age group of 61-75 (12.12%), followed by the 46-60 age group (9.09%) and, the 16-30 age group (6.06%). The age group of 0-15 and 76-90 years had the lowest proportion of patients (3.03%). The study also shows that the prevalence of ESKAPEE was absent in the female age group of 0-15. Figure_1 illustrates the distribution of ESKAPEE isolates in different clinical samples. During the study, 04 different samples (Urine, Pus, Blood, and Sputum) were collected for the isolation of ESKAPEE. This result indicates that ESKAPEE isolates were most frequently found in the urine sample (31, 93.93%), followed by Pus samples (02, 6%). ESKAPEE species were absent in the blood and sputum samples. The sources of ESAKPEE species are shown in Table 2. *Escherichia coli* was counted for the highest percentage of isolates from the urine sample (54.54%) followed by *Klebsiella* spp. (33.33%) and *Klebsiella pneumoniae* (6.06%). Many authors reported that the highest prevalence of *Escherichia coli* from urine samples in females [13-16]. On the other hand, *Pseudomonas aeruginosa* and *Staphylococcus aureus* were counted for the lowest frequency of isolates from the Pus sample (3.03%).

ESKAPEE species were absent in the blood and sputum samples. Table 3 represents the data for all the species of ESKAPEE that exhibited Resistant, Intermediate and Sensitive responses against antibiotics. Based on Clinical and Laboratory Standards Institute (CLSI) guidelines, the results revealed that the ESKAPEE species exhibited the highest resistance towards the Cefazolin (72.72%), Cefepime (57.57%), and (54.54%) for the Imipenem, Ampicillin/Sulbactam, Ceftazidime respectively. The strains were sensitive against Gentamycin (45.45%). ESKAPEE shows (27.27%) intermediate for Tobramycin. According to Table 4, *E. coli* shows the highest resistance (39.39%) and (33.33%) towards the Cefazolin and Levofloxacin respectively. Ngoi *et al.* (2021) [6] reported that resistance towards ampicillin (87.8%), cefepime (61.5%), and ceftriaxone (61.0%). *Klebsiella spp.* recorded the highest resistance to Ampicillin/Sulbatum (27.27%), Ceftazidime (33.33%), Meropenem (18%), 24% to Imipenem and Cefazolin respectively. Namratha *et al.* (2015) [17] reported that the *Klebsiella spp.* shows the highest resistance for ampicillin 89%, cefotaxime 65%, followed by Ceftriaxone 52%, Ceftazidime 57%, and Meropenem 48%. The prevalence of antibiotic sensitivity in different ESKAPEE isolates is shown in Table 5. Out of five species of ESKAPEE, *E.coli* is found to be more sensitive toward Gentamycin 18.18%, Imipenem and Meropenem 12.12%. Ruekit *et al.* (2022) [18] reported that *E.coli* species showed 42% sensitivity to Gentamycin, Amikacin 96% and tobramycin 59%. *Klebsiella spp* shows sensitivity towards Doripenem and Gentamycin 24%. Samah *et al.* (2017) [19] also stated similar sensitivity towards Gentamycin 87.3%, Imipenem 99.5% and Amikacin 86.6%. Table 6 represents information about the prevalence of antibiotic intermediate in different ESKAPEE isolates. *E. coli* and *Klebsiella spp* are found more intermediate (12.12%) to Tobramycin and Chloramphenicol.

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Table 1: Demographic profile (n=33)

Age groups (year)	Male	Female	Total
0-15	1(3.03%)	0	1(3.03%)
16-30	2(6.06%)	7(21.21%)	9(27.27%)
31-45	1(3.03%)	4(12.12%)	5(15.15%)
46-60	3(9.09%)	5(15.15%)	8(24.24%)
61-75	4(12.12%)	3(9.09%)	7(21.21%)
76-90	1(3.03%)	2(6.06%)	3(9.09%)
Total	12(36.36%)	21(63.63%)	33

Table 2: The source of ESKAPEE species according to specimen (n=33)

Sample	*Klebsiella spp* (%)	*Escherichia coli* (%)	*Pseudomonas aeruginosa* (%)	*Klebsiella pneumonia* (%)	*Staphylococcus aureus* (%)
Urine	11 (33.33%)	18 (54.54%)	0	2(6.06%)	0
Pus	0	0	0	0	0
Pus swab	0	0	1(3.03%)	0	1(3.03%)
sputum	0	0	0	0	0
Blood	0	0	0	0	0
Total (%)	11 (33.33%)	18 (54.54%)	1(3.03%)	2(6.06%)	1(3.03%)

Table 3: Antibiotic susceptibility patterns if the total ESKAPEE isolates (n=33)

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant(%)
Amikacin	0	0	5(15.15%)
Ampicillin	0	1(3.03%)	8(24.24%)
Aztreonem	3(9.09%)	0	5(15.15%)
Ampicillin/Sulbactum	0	1(3.03%)	18(54.54%)
Ceftazidime	2(6.06%)	1(3.03%)	18(54.54%)
Ciprofloxacin	0	1(3.03%)	3(9.09%)
Cefazolin	1(3.03%)	0	24(72.72%)
Cefepime	1(3.03%)	0	19(57.57%)
Cefotaxime	0	3(9.09%)	8(24.24%)
Chloramphenicol	10(30.30%)	2(6.06%)	5(15.15%)
Cefoxitin	1(3.03%)	1(3.03%)	2(6.06%)
Doripenem	13(39.39%)	2(6.06%)	10(30.30%)
Ertapenem	1(3.03%)	0	8(24.24%)
Gentamycin	15(45.45%)	1(3.03%)	5(15.15%)
Imipenem	5(15.15%)	4(12.12%)	18(54.54%)
Meropenem	6(18.18%)	4(12.12%)	16(48.48%)
Levofloxacin	5(15.15%)	2(6.06%)	16(48.48%)
Tetracycline	5(15.15%)	2(6.06%)	7(21.21%)
Trimethoprim	3(9.09%)	4(12.12%)	15(45.45%)
Tobramycin	5(15.15%)	9(27.27%)	8(24.24%)
Pipercicillin	0	0	1(3.03%)
Ofloxacin	0	1(3.03%)	0
Linezolid	1(3.03%)	0	0
Penicillin	0	0	1(3.03%)

Rifampin	1(3.03%)	0	0
Vancomycin	0	0	1(3.03%)
Clindamycin	1(3.03%)	0	0
Oxacillin	0	0	1(3.03%)
Doxycycline	1(3.03%)	0	0

Table 4: Prevalence of antibiotic resistance in different ESKAPEE isolates (n=33)

Antibiotic	***E.coli* (n=18)**	***Klebsiella spp* (n=11)**	***P. aeruginosa* (n=1)**	***K. pneumniae* (n=2)**	***S. aureus* (n=1)**
Amikacin	3(9.09%)	1(3.03%)	1(3.03%)	0	0
Ampicillin	6(18.18%)	1(3.03%)	0	1(3.03%)	0
Aztreonem	4(12.12%)	1(3.03%)	0	1(3.03%)	0
Ampicillin/ Sulbactm	8(24.24%)	9(27.27%)	0	1(3.03%)	0
Ceftazidime	6(18.18%)	11(33.33%)	0	1(3.03%)	0
Ciprofloxacin	0	1(3.03%)	1(3.03%)	0	1(3.03%)
Cefazolin	13(39.39%)	8(24.24%)	0	1(3.03%)	1(3.03%)
Cefepime	7(21.21%)	9(27.27%)	0	1(3.03%)	0
Cefotaxime	5(15.15%)	2(6.06%)	0	0	0
Chloramphenicol	3(9.09%)	2(6.06%)	0	0	0
Cefoxitin	1(3.03%)	1(3.03%)	0	0	0
Doripenem	9(27.27%)	2(6.06%)	0	0	0
Ertapenem	4(12.12%)	4(12.12%)	0	0	0
Gentamycin	5(15.15%)	0	0	0	0
Imipenem	10(30.30%)	8(24.24%)	0	0	0
Meropenem	8(24.24%)	6(18.18%)	0	1(3.03%)	0
Levofloxacin	**11(33.33%)**	3(9.09%)	1(3.03%)	2(6.06%)	1(3.03%)
Tetracycline	5(15.15%)	1(3.03%)	0	1(3.03%)	0
Trimethoprim	4(12.12%)	7(21.21%)	0	1(3.03%)	0
Tobramycin	5(15.15%)	5(15.15%)	0	0	0
Pipercicillin	0	0	1(3.03%)	0	0
Ofloxacin	0	0	1(3.03%)	0	0
Linezolid	0	0	0	0	0
Penicillin	0	0	0	0	1(3.03%)
Rifampin	0	0	0	0	0
Vancomycin	0	0	0	0	0
Clindamycin	0	0	0	0	0
Oxacillin	0	0	0	0	1(3.03%)
Doxycycline	0	0	0	0	0

Table 5: Prevalence of antibiotic sensitivity in different ESKAPEE isolates (n=33)

Antibiotic	***E. coli (n=18)***	***Klebsiella spp (n=11)***	***P.aeruginosa (n=1)***	***K.pneumoniae (n=2)***	***S. aureus (n=1)***
Amikacin	0	0	0	0	0
Ampicillin	0	0	0	0	0
Aztreonem	2(6.06%)	0	0	0	0
Ampicillin/ Sulbactum	0	0	0	0	0

Ceftazidime	2(6.06%)	0	0	0	0
Ciprofloxacin	0	0	0	0	0
Cefazolin	0	0	0	1(3.03%)	0
Cefepime	1(3.03%)	0	0	0	0
Cefotaxime	0	0	0	0	0
Chloramphenicol	3(9.09%)	6(18.18%)	0	0	1(3.03%)
Cefoxitin	1(3.03%)	0	0	0	0
Doripenem	3(9.09%)	8(24.24%)	1(3.03%)	0	0
Ertapenem	0	0	0	0	0
Gentamycin	6(18.18%)	8(24.24%)	0	1(3.03%)	1(3.03%)
Imipenem	4(12.12%)	0	1(3.03%)	0	0
Meropenem	4(12.12%)	2(6.06%)	0	0	0
Levofloxacin	0	6(18.18%)	0	0	0
Tetracycline	4(12.12%)	0	0	0	1(3.03%)
Trimethoprim	2(6.06%)	1(3.03%)	0	0	1(3.03%)
Tobramycin	3(9.09%)	1(3.03%)	1(3.03%)	0	0
Pipercicillin	0	0	0	0	0
Ofloxacin	0	0	0	0	0
Linezolid	0	0	0	0	1(3.03%)
Penicillin	0	0	0	0	0
Rifampin	0	0	0	0	1(3.03%)
Vancomycin	0	0	0	0	0
Clindamycin	0	0	0	0	1(3.03%)
Oxacillin	0	0	0	0	0
Doxycycline	0	0	0	0	1(3.03%)

Table 6: Prevalence of antibiotic Intermediate in different ESKAPEE isolates (n=33).

Antibiotic	***E.coli* (n=18)**	***Klebsiella spp* (n=11)**	***P. aeruginosa* (n=1)**	***k. pneumoniae* (n=2)**	***S. aureus* (n=1)**
Amikacin	0	0	0	0	0
Ampicillin	1(3.03%)	0	0	0	0
Aztreonem	0	0	0	0	0
Ampicillin/ Sulbactum	0	1(3.03%)	0	0	0
Ceftazidime	0	0	1(3.03%)	0	0
Ciprofloxacin	1(3.03%)	0	0	0	0
Cefazolin	0	0	0	0	0
Cefepime	0	0	0	0	0
Cefotaxime	1(3.03%)	1(3.03%)	0	1(3.03%)	0
Chloramphenicol	4(12.12%)	0	0	0	0
Cefoxitin	1(3.03%)	0	0	0	0
Doripenem	0	2(6.06%)	0	0	0
Ertapenem	0	0	0	0	0
Gentamycin	1(3.03%)	0	0	0	0
Imipenem	0	3(9.09%)	0	1(3.03%)	0
Meropenem	1(3.03%)	1(3.03%)	0	0	0
Levofloxacin	0	2(6.06%)	0	0	0
Tetracycline	2(6.06%)	0	0	0	0

Trimethoprim	3(9.09%)	1(3.03%)	0	0	0
Tobramycin	2(6.06%)	4(12.12%)	0	2(6.06%)	0
Pipercicillin	0	0	0	0	0
Ofloxacin	0	0	1(3.03%)	0	0
Linezolid	0	0	0	0	0
Penicillin	0	0	0	0	0
Rifampin	0	0	0	0	0
Vancomycin	0	0	0	0	0
Clindamycin	0	0	0	0	0
Oxacillin	0	0	0	0	0
Doxycycline	0	0	0	0	0



Figure 1: Distribution of ESKAPEE species in various clinical specimens (n=33)

RESEARCH ARTICLE

Determination of Antibiotic Susceptibility of Probiotic Bacteria by using Dairy and Pharmaceutical Products

Sahana Nagarakere Chandranna*, Shyni Daniel and Bharath M

Research Scholar, Department of Studies in Environmental Science, University of Mysore, Manasagangotri, Mysore, Karnataka, India.

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***Address for Correspondence**
Sahana Nagarakere Chandranna
Research Scholar,
Department of Studies in Environmental Science,
University of Mysore, Manasagangotri,
Mysore, Karnataka, India.
E.Mail: sahana@envsci.uni-mysore.ac.in

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ABSTRACT

Probiotics, meaning "for life," have been used for thousands of years in fermented foods like yogurt, cheese, and kefir for their nutritional and therapeutic benefits. These beneficial bacteria, including *Lactobacillus*, *Streptococcus*, and *Bifidobacterium*, naturally exist in the human gut and play a key role in digestion, immune support, and disease prevention. They help maintain gut microbial balance, reduce allergies, prevent antibiotic-associated diarrhea, and support cardiovascular and mental health. Antibiotics, on the other hand, are used to treat bacterial infections but do not affect viruses. However, excessive and improper use of antibiotics has led to bacterial resistance, resulting in the emergence of "superbugs." This resistance can spread through plasmid exchange, transformation, and the food chain, especially through fermented dairy and meat products consumed without heat treatment. Certain lactic acid bacteria, such as *Enterococci*, have been linked to antibiotic resistance, raising concerns about the safety of commercial probiotics. This study aims to screen probiotic bacterial isolates from dairy and pharmaceutical products to evaluate their antibiotic resistance frequency. Understanding the extent of resistance in probiotics will help ensure their safe use in food and pharmaceutical applications.

Keywords: Antibiotic, Dairy, LAB bacteria, Pathogen, and Probiotic.

INTRODUCTION

The Latin term "probiotic" means "for life"[1]. Fermented foods including beer, bread, wine, kefir, kumis, and cheese were widely used for nutritional and medicinal benefits long before probiotic microbes were known about [2]. It is generally accepted that fermented items were most likely found—or, more accurately, found on their own.

According to mythology, yogurt most likely originated from a fermenting process in the animal skin pouches that were used to transport milk and water in areas with high temperatures and low humidity (Middle Asia and the Middle East)[3]. Probiotics have a long history that parallels the growth of the human race and, according to modern, advanced tools, can be traced back to around 10,000 years ago[1]. Food products, particularly dairy products like yoghurt, cheese, and milk, as well as medicinal treatments, include probiotic microorganisms. Lactic acid bacteria (LAB) species such as *Lactobacillus, Streptococcus, Bifidobacterium, Enterococcus, Pediococcus,* and *Bacillus* are examples of probiotic bacteria [4].A diverse collection of microorganisms found in nature, LABs are extensively dispersed. Both human and animal gastrointestinal (GI) and urogenital tracts contain these bacteria, which are also found on plant matter, in milk and meat, and in a variety of fermented foods [5]. Because lactic acid bacteria naturally cause spontaneous fermentation, they have been linked to traditional dairy products, cereals, and fermented vegetables and meats[6]. In addition to their potential health benefits, they are also employed as starter cultures in the development of probiotic products and industrial foods. The most researched dietary system for delivering probiotic bacteria to the human stomach is milk and dairy products [7]. The idea of probiotics has developed and is currently the subject of various studies. The process of choosing, characterizing, and using novel cultures in food production has advanced significantly[8].

Benefits of Probiotics

Probiotics helps in the balance of good bacteria in our digestive system. It has the ability to restore the natural balance of gut bacteria. Probiotics can help to prevent and to treat diarrhea. Most of the studies suggested that use of the probiotics to reduce the risk of antibiotic associated diarrhea [9]. Probiotics supplements helps in the improving in some mental health conditions and also in increasing gut health to improve mental health. Some of the probiotic strains are helpful to keep our heart healthy i.e. by breaking down bile. Probiotics can also prevent it from being reabsorbed in the gut. It can enter the blood as Cholesterol It also reduces the blood pressure[10]. Probiotics may reduce the severity of certain allergies; it may also reduce inflammatory response in people with milk or dairy allergies. Probiotics also helps in boosting of our Immune system by inhibiting the growth of harmful bacteria. The probiotic *Lactobacillus crispatus*has also shown to reduce the risk of urinary tract infections in women's by 50%[11].

Antibiotics

It is a drug which inhibits the growth of harmful microorganisms. Antibiotic are used to treat bacterial infections but there is no effects on viral infections[12].

Antibiotic resistance

Antibiotic resistance results in reducing the effectiveness of the bacteria using drugs (it is a natural phenomenon)[13]. (Fig: 1)

Development of antibiotic resistance in probiotic bacteria

Antibiotic resistance can be developed in probiotic bacteria naturally via natural selection, but also be engineered by applying evolutionary stress. If a bacterium develops resistance via such mode, then it is genetically transfer resistance gene in a horizontal fashion by plasmid exchange[14]. Bacteria containing several resistance genes within its DNA, then it is said to be multi resistance or a super bug, it can also be introduced into microorganisms through transformation processes and incorrect diagnosis unnecessary description.

Effects on environment

The spread of antibiotic resistance from animals to humans is largely influenced by the food chain. More precisely, fermented meat and dairy products that are not heated before consumption serve as a conduit for bacteria resistant to antibiotics and provide a direct connection between the human gastrointestinal system and the native microbiota of animals. Since these bacterial strains cause a wide range of dangerous infectious diseases, there has been a lot of research done on antibiotic resistance in LAB [15]. Lactic starter cultures, which are used in fermented milk products, are bacteria that enter our intestines in high quantities and interact with the intestinal microbiota[16]. Negative effects

could potentially result from the commercial introduction of probiotics including strains resistant to antibiotics. Given all of this, the current study was conducted to identify the prevalence of antibiotic resistance in dairy and pharmaceutical goods and screen for probiotic bacterial isolates from them [17].

Experimental Methods

Chemicals and media

Sodium chloride (NaCl), ethanol, alpha-napthol(3%), KOH(40%), double distilled water , 3% H_2O_2 solution, sulphanilic acid, N,N-dimethyl-1-naphylamine, Baritt reagent A, Baritt reagent B, MRS agar (de Man Rogosa Sharpe), MRS broth, Luria broth, Nutrient agar, Glucose Phosphate Broth were purchased from HiMedia, Mumbai, India.

Collection of samples

A total of 4 dairy products such as, curd (n=1), yoghurt (n=1), ice cream (n=1), buttermilk (n=1) were purchased from local dairy shops in Mysuru city followed by 3 pharmaceutical products such as bifilac, lactogut, pre and probiotic containing 1g of sample. The collected sample were brought to laboratory and stored at 4ºC until further use.

Isolation of bacteria

The bacteria were isolated from food samples derived from yogurts, buttermilk, curd, ice cream and pharmaceutical products. For isolation, 1g of respective sample was serially diluted with 0.85% of NaCl in aseptic condition. Later the samples were plated by pour plate and spread plate method[18]. For pour plate method, 1ml of the sample was poured into sterile Petri plate and later respective media were poured on to it. For spread plate method 0.1ml sample was poured on to Petri plate containing respective media. The plates incubated at 37Cº for 24 hrs in bacteriological incubator. After overnight incubation, the plates were observed for colony growth. Based on morphological characteristics the bacterial colonies were picked and streaked on fresh medium plates to obtain pure colony. Later the cultures were transferred into fresh sterile liquid broth.

Antibiotic susceptibility test

The antibiotic susceptibility of Bacillus and Lactic acid bacterial isolates was screened using the disc diffusion method. Antibiotic discs on the surface of MRS agar were used to help isolate LAB become more resistant to antibiotics, and the plates were incubated for 24 hours at 37Cº. The isolates' overnight-grown culture suspension (100µL) was spread out on MRS agar plates, and then the antibiotic disc containing ampicillin (10mcg), chloramphenicol (30mcg), erythromycin (15mcg), methicillin (5mcg), gentamicin (10mcg), kanamycin (30mcg), penicillin (100 units), streptomycin (10mcg), tetracycline (30mcg), Vancomycin (30mcg), ceftazidime (30mcg), ceftizoxime (30/10mcg), ofloxacin (5mcg), linezolid (30mcg), ciprofloxacin (5mcg), colistin (10mcg), nitrofurantoin (300mcg), nalidixic acid (5mcg), trimethoprim (5mcg), and amikacin (30mcg) were added. Following a 24-hour incubation period at 37°C, inhibition zone diameters were determined.

Similarly *Bacillus* isolates were also (S1, S3, S7, S8, S9, S15) tested for the above antibiotics by disc diffusion method and incubated at 37Cº for 24hrs.

Testing for Lactobacillus bacteria biochemically

The gram stain reactivity, cell shape, and cellular architecture of each isolate were investigated under a microscope. [19].

Catalase test

A sterile microscopic slide was coated with a drop of 3% hydrogen peroxide. Select cells from the middle of a well-isolated test culture colony using a nicrome wire loop, then move them into the hydrogen peroxide drop. Both were combined, and the formation of gas bubbles was monitored. While isolates that were gram positive and catalase positive were recognized as Bacillus sp., strains exhibiting gram-positive and catalase-negative isolates were classified as lactic acid bacteria.

Physiological and biochemical identification

Prior to usage, each isolate was activated in 5 ml of MRS broth for 24 hours at 30ºC. As a result, overnight cultures were employed for every identifying process. The techniques and standards of Sharpe and Fryer (1965) were followed in the physiological and biochemical identifications.

The following tests were used for the biochemical identification of lactic acid bacterial isolates

1. Gas generation from glucose;
2. Growth at various temperatures (5°C, 37°C, and 45°C);
3. Growth at 6.5% NaCl concentration; and
4. Voges-Proskauer (V-P) Test.

Gas production from Glucose

The CO_2 output from glucose test was used to better characterize homofermentative isolates. MRS broths and inverted Durham tubes were employed for this reason. Eight milliliters of test media were filled with fifty microliters of overnight cultures. Gas buildup in Durham tubes following five days of incubation at 30 ^{o}C was interpreted as proof that glucose was producing CO_2.

Voges-Proskauer (V-P) Test

Incubate the culture in the glucose phosphate broth (GPB) medium for 24 to 48 hours at 370 degrees Celsius. Add 0.6 ml of a-naphthol and 0.2 ml of KOH solution per milliliter of broth after incubation. Since a-naphthol only has a catalytic effect when added before KOH, the reagents should only be introduced in this order. After adding each reagent, give the tube a good shake and slant it to improve aeration. When a pinkred hue develops within 15 minutes or more, it indicates that the species is VP positive. Based on this, we may assume that the lactobacillus strain is either streptobacterium or thermobacterium, and the betabacterium is V-P negative.

Growth at different temperatures

The tubes containing 5 ml of temperature test media were filled with 50 µl of overnight cultures. Following inoculation, they were incubated at 5°C, 37°C, and 45°C for 24 hours. The cultures' color changed from purple to yellow, indicating the development of cells at any of these temperatures.

Growth at different NaCl concentrations

The tubes containing 5 ml of NaCl test media were filled with 50 µl of overnight cultures. Growth tests were performed on isolates at concentrations of 2%, 4%, or 6.5% NaCl. They were incubated at 30ºC for seven days. The purple to yellow hue shift is interpreted as proof of cell development.

Biochemical Characterization of *Bacillus* isolates

Gram-positive bacilli belong to the genus Bacillus. HiMedia, Mumbai, India's Hi-Bacillus Identification Kit (KB013) was used to characterize the isolates. The principles of pH change and substrate usage served as the foundation for the testing. A color shift in the media during incubation is a sign of metabolic changes in Bacillus species, which can be visually interpreted or, if necessary, confirmed by the addition of reagent.

RESULTS AND DISCUSSION

Isolation and Identification of Bacterial Isolates

A total of 8 different probiotic and dairy products were used to isolation of bacteria. A total of 20 different isolates among which 14 isolates belong to lactic acid bacterial species and 7 isolates belong to *Bacillus spp were isolated*. 20 different isolates exhibiting different morphological, characterization were selected for further studies. The isolates were designated as S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10}, S_{11}, S_{12}, S_{13}, S_{14}, S_{15}, S_{16}, S_{17}, S_{18}, S_{19}, S_{20}. Among them (S_1, S_2) were isolated from Curd, (S_3,S_4) bifilac, (S_5-S_8) lactogut, (S_9-S_{10}) Ice cream , (S_{11}-S_{14}) pre and probiotic, (S_{15}-S_{16}) buttermilk, (S_{17}-S_{18}) yogurt, (S_{19}-S_{20}) ice cream. All isolates were subjected to gram staining based on the morphology and the

isolates were gram positive bacteria among which S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_{11}, S_{18}, S_{17}, are Rod shaped and rest of the isolates were *Coccobaciili.*

Antibiotic susceptibility

A total of 20 isolates were isolated from 8 different probiotics of dairy and pharmaceutical products and were subjected to antimicrobial susceptibility testing using MRS agar by disc diffusion method. The bacterial isolates showed resistance to nalixdic acid (65%), ceftazidime (50%), ceftizoxime (50%), colistin (50%), amikacin (45%), methicillin (45%), erythromycin (35%), kanamycin (35%), streptomycin (35%), trimethoprim (35%), penicillin (30%), gentamycin (25%), ampicillin (25%), chloramphenicol (25%), ofloxacin (10%), linezolid (10%), nitrofurantrin(5%), ciprofloxacin (5%), Vancomycin (5%). The isolates of *Bacillus* and lactic acid bacteria were showing more resistant to nalixdic acid. This isolates were isolated from 2 dairy products followed by 3 pharmaceutical products.

CONCLUSION

In essence, many dried food supplements and to a lesser degree, dairy and pharmaceutical products are mislabeled with respect to the precise identification of the probiotic strains that are included. The problem of mislabeling has been brought to light in earlier reports, and the current data indicates that it has not substantially improved. The finding of resistance to nalidixic acid, ceftazidime, ceftizoxime, and colistin among the examined probiotic isolates raises concerns, even if some antibiotic resistance features in probiotic strains may be advantageous in particular situations. These results highlight the necessity of ongoing attention to detail when choosing probiotic strains devoid of genes linked to transferable antibiotic resistance. Customers must learn more about the connection between healthy eating and overall well-being. To guarantee that probiotic products used for health promotion consistently and responsibly provide the desired effects, they must be carefully assessed for safety and scientific validity.

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Conflict of Interest

The authors declare that there is no conflict of interest associated with this publication.

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Table 1: Different products of Probiotics of dairy and pharmaceutical products used for isolation.

Dairy products	Pharmaceuticals products
1. Acidophilus milk (Curd)	1. Lactogut
2. Yogurt	2. Pre and probiotic
3. Kefir	3. Bifilac
4. Cottage cheese	
5. Butter milk	
6. Cheese	
7. Fermented foods	
8. Milk powder	

Table 2: Gram staining of bacterial isolates

Isolates	Gram staining	Shape
S1	+	Rod
S2	+	Rod
S3	+	Rod
S4	+	Rod
S5	+	Rod
S6	+	Cocci
S7	+	Cocci
S8	+	Cocci
S9	+	Cocco bacilli
S10	+	Cocco bacilli
S11	+	Rod
S12	+	Cocco bacilli
S13	+	Cocco bacilli
S14	+	Cocco bacilli
S15	+	Cocco bacilli
S16	+	Cocco bacilli
S17	+	Rod

S18	+	Rod
S19	+	Rod
S20	+	Rod

Table 3: Biochemical characterization of *Bacillus*Isolates

SL.NO	Test	S1	S3	S4	S7	S9	S15
1	Malonate	-	-	-	-	-	-
2	Vogesproskauer's	-	-	-	-	+	-
3	Citrate	+	+	-	+	-	-
4	ONPG	-	-	-	-	-	-
5	Nitrate reduction	+	+	-	+	-	-
6	Catalase	+	+	+	+	+	-
7	Arginine	+	+	+	+	+	-
8	Sucrose	-	-	-	-	+	-
9	Mannitol	-	-	-	-	+	-
10	Glucose	-	-	-	-	+	-
11	Arabinose	-	-	-	-	+	-
12	Trehalose	-	-	-	-	+	-

Table 4: Antibiotic resistance pattern of bacterial isolates to 20 different classes of antibiotics(R- Resistance, S-Sensitive).

Sl.No	Antibiotics	Bacterial Isolates showing zone of inhibition in (millimeter)																			
		S1	S2	S3	S4	S5	S6	S7	S8	S9	S10	S11	S12	S13	S14	S15	S16	S17	S18	S19	S20
1	Ampicillin	5	43	31	38	R	R	48	32	S	18	25	R	R	R	S	S	R	30	S	S
2	chloroamphenicol	27	26	28	30	R	R	34	27	S	24	25	R	R	R	S	S	R	15	S	S
3	Erthyromycin	33	30	32	40	12	13	15	18	S	R	R	R	R	R	S	S	R	R	S	S
4	Methicillin	24	16	24	26	R	R	31	R	S	20	R	R	R	R	S	S	R	R	S	S
5	Gentamicin	20	18	16	20	13	15	18	20	S	17	R	R	R	R	S	S	R	13	S	S
6	Kanamycin	23	20	21	25	10	15	18	R	S	16	R	R	R	R	S	S	R	R	S	S
7	Pencillin G	23	40	27	38	R	R	33	25	S	15	23	R	R	R	S	S	R	R	S	S
8	Streptomycin	16	24	17	19	16	15	17	8	R	16	R	R	R	R	S	S	R	R	S	S
9	Tetracycline	21	28	18	23	28	28	39	28	S	33	30	28	28	28	S	S	R	27	S	S
10	Vancomycin	19	21	21	20	23	20	20	20	S	16	17	24	24	24	S	S	R	20	S	S
11	Ceftazidime	25	S	17	18	R	R	29	R	35	R	R	R	R	R	S	R	S	R	S	S
12	Ceftizoxime	35	S	30	30	R	R	37	R	50	R	R	R	R	R	S	R	S	R	S	S
13	Ofloxacin	38	S	37	19	R	15	38	15	47	17	18	17	18	18	S	R	S	18	S	S
14	Linezolid	36	S	40	31	20	23	38	30	40	R	30	30	32	32	S	R	S	35	S	S
15	Ciprofloxacin	40	S	37	25	17	25	33	20	36	20	21	22	16	23	S	R	S	22	S	S
16	Colistin	10	S	10	13	R	R	R	R	R	17	R	R	R	R	S	10	15	R	S	S
17	Nitrofurantoin	20	S	20	17	18	18	17	23	23	31	21	30	22	21	S	R	45	21	S	S
18	Nalidixic acid	17	S	17	R	R	R	R	R	15	R	R	R	R	R	S	R	R	R	S	S
19	Trimethaprime	30	S	35	R	R	R	20	20	32	R	R	16	22	26	S	R	R	25	S	S
20	Amikacin	20	S	22	21	R	R	18	R	23	13	R	R	R	R	S	R	15	R	S	S

Table 5: Biochemical characterization of Lactic acid bacteria.

Isolates	Gas Production from Glucose	Growth at Differ Concentration of NaCl (%)			Growth at Different Temperature (°C)			Voges-Proskauer Test	Catalase Test
		2	4	6.5	5	37	45		
S2	-	-	-	-	-	+	+	-	+
S5	+	+	+	-	-	+	+	+	_
S6	-	+	+	-	-	+	+	-	_
S8	-	+	+	-	-	+	+	-	_
S10	-	+	+	-	-	+	+	-	+
S11	-	+	-	-	-	+	-	-	_
S12	-	-	-	-	-	+	+	-	_
S13	-	+	+	-	-	+	+	-	_
S14	-	+	-	-	-	+	+	-	_
S16	-	+	-	-	-	+	+	-	+
S17	+	+	-	-	-	+	-	-	_
S18	-	+	+	-	-	+	+	-	_
S19	-	+	-	-	-	+	+	-	_
S20	-	+	-	-	-	-	-	-	_

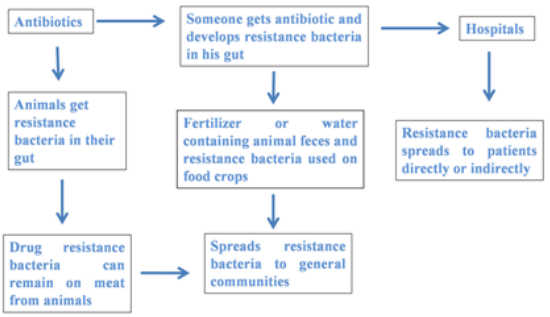


Fig.1: Flow chart showing development of antibiotic resistance in environment.



Fig2: Different pharmaceutical and dairy probiotic products.

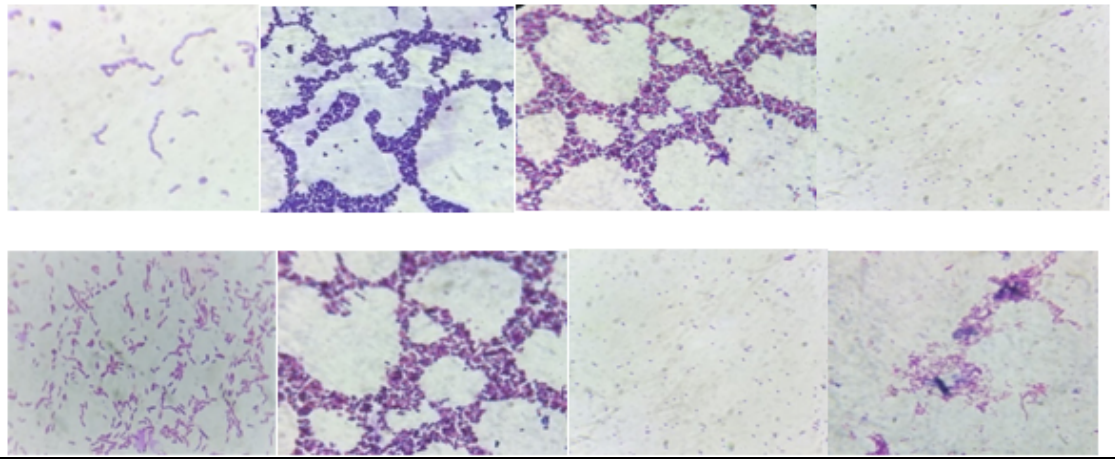


Fig 3. Microscopic images of some bacterial isolates observed under oil immersion (100X).

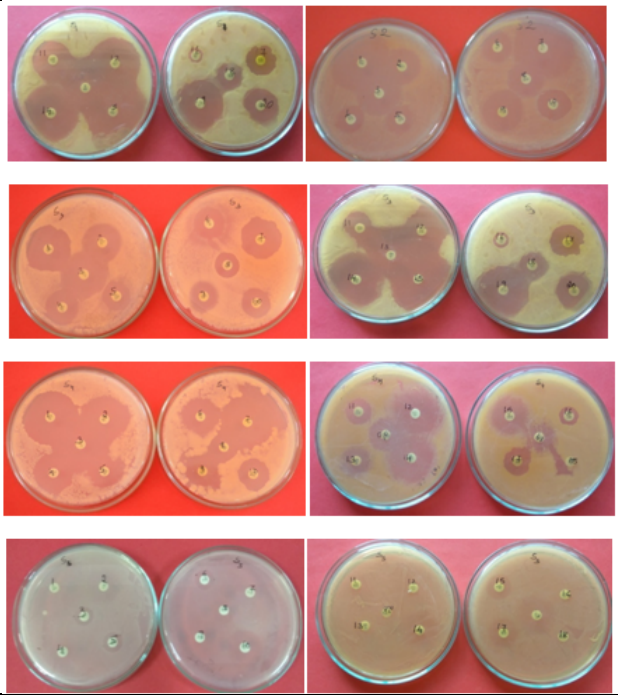


Fig 4: MRS agar plates showing resistance and zone of inhibition to 20 different antibiotics (S1 to S5)

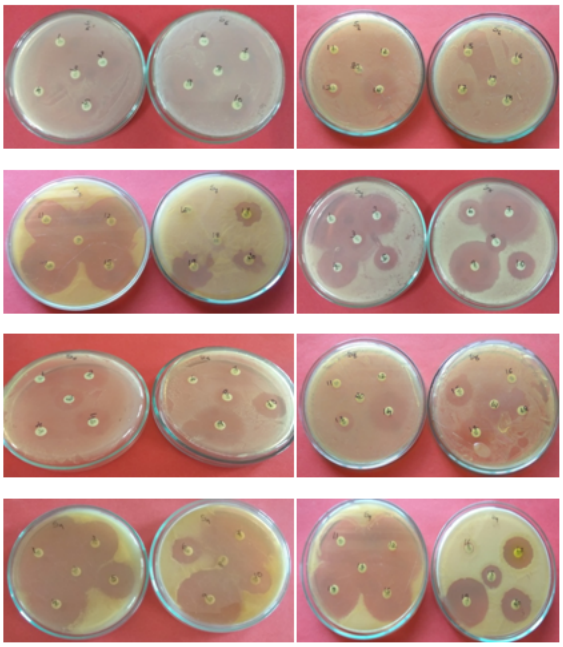


Fig 5: MRS agar plates showing resistance and zone of inhibition to 20 different antibiotics (S6 to S9)

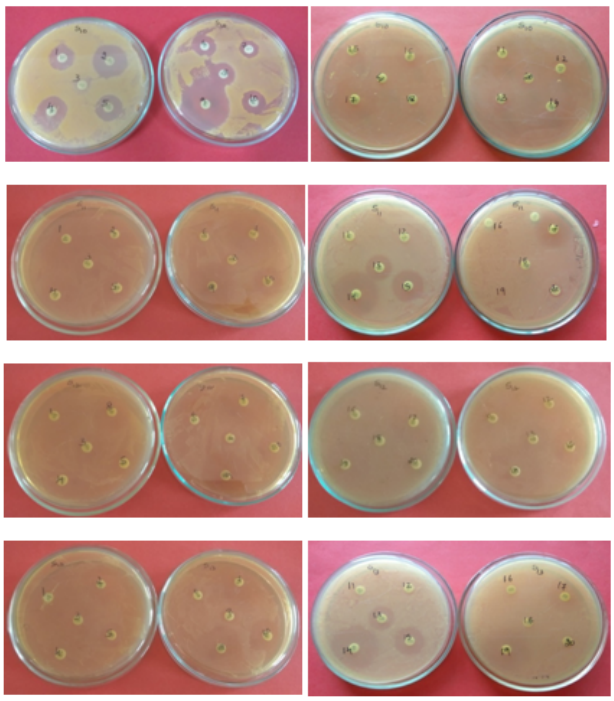


Fig. 6: MRS agar plates showing resistance and zone of inhibition to 20 different antibiotics (S10 to S13)

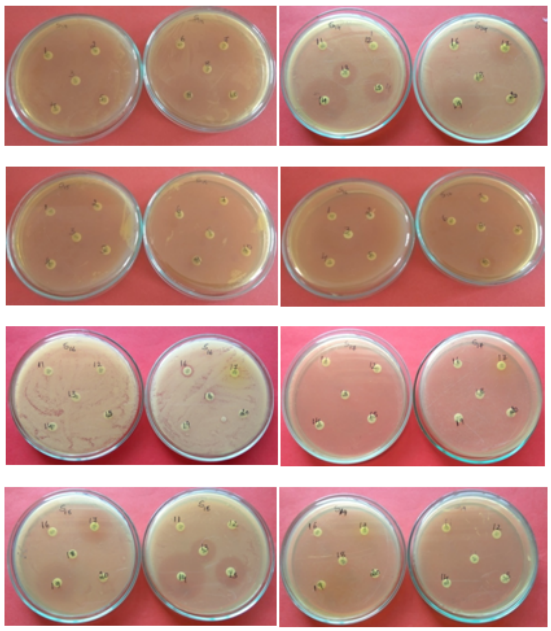


Fig. 7: MRS agar plates showing resistance and zone of inhibition to 20 different antibiotics (S10 to S13)



Fig 8: Biochemical test for Bacillus isolates.

RESEARCH ARTICLE

Study on Knowledge of Drug Dose Calculation among Pediatric Nurses in Gujarat

Rutu Devmurari[1], Renuka Gangoda[1], Khusbu Patel[1], Roshni Marvadi[1], Falguni Naika[1], Anil Sharma[2] and Sanket Patel[3]*

[1]Student, Department of Nursing, Manikaka Topawala Institute of Nursing, (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.
[2]Professor Cum Principal, Department of Nursing, Manikaka Topawala Institute of Nursing, (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.
[3]Tutor, Department of Nursing, Manikaka Topawala Institute of Nursing, (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.

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***Address for Correspondence**
Sanket Patel
Tutor, Department of Nursing,
Manikaka Topawala Institute of Nursing,
(Affiliated to Charotar University of Science and Technology),
Anand, Gujarat, India.
E.Mail: sanketpatel.nur@charusat.ac.in

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ABSTRACT

Accurate drug dose calculation is crucial in the pediatric unit due to the relatively weaker immunity of children, making them more susceptible to medications. The prescribed medication for children necessitates continuous monitoring from the prescription stage until the child achieves full recovery. Incorrect administration of medication can lead to adverse effects and pose risks to children's health. It is imperative for nurses to possess the proficiency to calculate drug doses accurately, as any lack of awareness in this regard may result in errors during drug administration. The study was carried out using quantitative research approach and non-experimental descriptive research design. The study was done on 110 sample using convenient sampling technique. Reliability was established by using Karl Pearson's formula. The collected data was analyzed using descriptive and inferential statistics. The result showed 12.7% samples had poor knowledge, average knowledge as 43.6% and good knowledge as 43.6%. There is no association found between gender, age in years, staff nurses working in pediatric unit, professional Qualification, total experience in clinical field, attended workshop/conference related to drug dose calculation and knowledge among staff nurses working in pediatric unit. The knowledge regarding pediatric drug dose calculation was found to be average hence it requires more training to be provided to the staff nurses.

Keywords: Knowledge, Pediatric Drug Dose Calculation, Pediatric Unit, Staff Nurse

INTRODUCTION

The drug dose calculation is a crucial part of every hospital. Drug dose calculation mistake happens very commonly in the paediatric patient unit[1]. Drug dose calculation is a fundamental mathematics required for awareness of normal equations, according to drug dose calculation, the drug should be administered to the children only if the nurse has adequate knowledge to calculate the drug dose[2]. The most important role and responsibility of nurse practitioner is to make certain protected medication dose computation during drug distribution. Drug problems are a frequent source of death rate and disease rates that can be incorrect during drug dose calculation[2]. The drug dose calculation is an essential part of the paediatric unit as the children's immunity is not so strong and children can become more prone to the medication[3]. If medication is administered wrongly, it can cause adverse effects and harm to children's health, if the nurse is not aware of drug dose calculation; it will create an error in administering the drug[3]. The doctor's written prescription is difficult to read so it causes dangerous problems for every patient[4]. Healthcare professionals are answerable for having enough knowledge about medication, medication preparation, and examination of medication[4]. Inadequate expertise in medication dose computation enlarges possibility of medication errors[5]. Nurses need to be careful with drug dosage calculations to prevent medication errors, which can lead to harm or even death[2]. Even a small mistake in calculating a drug dose can have serious consequences for the patient's life[6]. Medication inaccuracies are frequent and may threaten patient safety. As paediatric dose calculation is according to the children's body mass and age, the possibility of inaccuracy in dosage calculation rises[7]. Administration of paediatric drug differ from the typical adult medication dose. Children are dependent on their caretakers for their medicine. Also, they take more number of liquid medicines rather than capsules or tablets[8]. Nurses often do not realize the importance of pediatrics formulation, and it leads to inaccuracy in pediatric practice and ward treatment failure and even harm to the child.[8] According to NLM (National Library of Medicine), it is approximate 7.5 million avoidable drug inaccuracy occurs among paediatric patient in the United States[9]. The paediatric population are at remarkable risk for medication-related inaccuracy. Nurses operating with paediatric clients need adequate knowledge, and expertise about the complete attention during the drug delivering to children. Children are liable to drug mishaps. Role of the paediatric staff nurses is essential in administrating medication to children. The need for specific skills and knowledge is required by nurses working with paediatric patients, in order to ensure adequate care in administrating medication to children. Adequate preparation and medication administration for children is of great essence than that of adults[10].

METHODS AND MATERIALS

Study design: Descriptive research design
Study site: The study was carried out at selected hospital of central Gujarat
Sample size: Total 110 staff nurses include from different paediatric units
q= (1-p)
= (1- 0.0786)
= 0.9214
d= 5% = 0.05
Putting into the formula
$$n = \frac{(1.96)^2 \times (0.0786 * 0.9214)}{(0.05)^2}$$
n= 3.842x 0.0724 /0.0025
n= 110

Inclusion and Exclusion criteria
Nurses who are working in pediatric units in the last 6 months and qualified with GNM and B.Sc. Nursing course. Those who are not willing to participate, Nurse with ANM, M.Sc. nursing and Ph. D were excluded from the study.

Study tool

A: Demographic Tool consists of age, gender, professional qualification, clinical experience, pediatric experience and attended a workshop regarding drug calculation.

B: Self-structured Questionnaire To evaluate the knowledge regarding the drug dose calculation consisted of 22 questions.

Research Ethics

Ethical approval was taken from the Institutional ethical committee CHARUSAT, Changa, Gujarat.

Preparation and Investigation Phase

The researcher selected various pediatric hospitals from the Anand and Kheda districts. Then by using convenient sampling technique selected 110 staff nurses as a sample. Different demographic variables were used to select samples. By using a self-structured questionnaire, the knowledge regarding pediatric drug dose calculation was assessed.

Statistical Analysis

Data analysis is done by using Descriptive and inferential analysis. Demographic features of the sample were described using frequencies, percentages, mean and standard deviation**.** A self-structured knowledge questionnaire was applied to assess the knowledge of staff nurses by using frequency and percentage and mean, median. Then the chi-square test was employed to check association between knowledge of staff nurses about pediatric drug dose calculation with demographic variables.

RESULT

The Table 1 depicts the demographic characteristics, majority of the participants were females 90 (81.8%), whereas 20 (18.2%) were male. 94(85.5%), were between the ages of 20-30 whereas 31-40 years were 10(9.1%) and above 40 years were 6(5.5%). 89(80.9%)people have 0.5- 5years of experience, 13(11.8%) people have 6- 10 years of experience, and 8(7.3%) people have above 10 years' experience in clinical field. 91(82.7%) were qualified with GNM and 19(17.3%) were qualified with BSC nursing. 92 (83.6%) have a total pediatric experience of 0.5-3 years, 10(9.1%) have a total pediatric experience of 4-6years, 3(2.7%) have a total pediatric experience of 7-9years and 5(4.5%) has the total pediatric experience of above 10years. 38(34.5%) have attended conferences or workshops related to drug dose calculations while 72(65.5%) has not attended conferences or workshop related to drug dose calculation.

Table 2 shows the distribution of knowledge showing poor knowledge as 12.7%, average knowledge as 43.6% and good knowledge as 43.6%.

Table 3 From the above table Association between gender and knowledge among staff nurses working in pediatric unit is found to be non-significant 15.507>1.944 at 0.05 level. Association between age in years and knowledge among staff nurses working in pediatric unit is found to be non-significant 21.026>4.791 at 0.05 level. Association between Professional Qualification and knowledge among staff nurses working in pediatric unit is found to be non-significant 15.507> at 0.485 level. Association between Total Experience in clinical field and knowledge among staff nurses working in pediatric unit is found to be non-significant 21.026>5.501 at 0.05 level. Association between Total Experience in Paediatric field and knowledge among staff nurses working in pediatric unit is found to be non-significant 26.296>13.592 at 0.05 level.

DISCUSSION

The study was intended to assess the knowledge of staff nurses about drug dose calculation. By using convenient sampling technique 110 staff nurses were selected from different hospitals of Gujarat. In that majority81.1% samples

are female, and 18.2% samples are male. 94 samples are between the age group of 20 to 30 years whereas 5.5% people are above the age of 40 year. 80.9% samples have been experienced between 0.5 to 5 years whereas 7.3% samples have experienced more than 10 years. Majority 82.7% are qualified with the GNM and 17.3% are qualified with B.Sc. nursing. Majority 83.6% samples have total pediatric experience between the 0.50 to 3years whereas only 4.5% samples have pediatric experience above 10 years. Karen M. Stratton *et al*, 2004 conducted a descriptive study regarding the perception of the amount of medication errors reported and why medication errors occur in the pediatric departments. The study shows a sample of 57 pediatric nurses and 227 adult hospital nurses. The study shows that a higher proportion of error is seen in the pediatric unit (67%) than in the adult unit (56%). Also, in the pediatric unit, 14.80 per 1,000 patient days while in the adult unit, 5.56 per 1,000 patient days[11]. Jagjit Kaur *et al*, 2020 conducted a descriptive study to assess the knowledge and practices regarding drug administration among staff Nurses working in pediatric unit of DMC and Hospital, Ludhiana. The structured knowledge questionnaire and checklist were used to assess the knowledge and practice regarding drug administration. It was found that all of the staff Nurses had average practices regarding drug administration but the correlation between knowledge and practice was found to be weak. Its also shows that practice of staff nurses regarding drug administration was found to be average[12]. Babitha K Devu *et al*, May 2019 conducted an exploratory study to assess the level of knowledge and practice regarding the administration of drugs among the staff nurses working in pediatric hospitals in Bangalore. By using non-probability convenient sampling 50 staff nurses was selected and Self-structured questionnaires were used to collect data. The study revealed that 48 (96%) respondents have adequate knowledge of the administration of drugs and 2 (4%) have a moderate knowledge level[13].

CONCLUSION

In conclusion, this study highlights the importance of accurate drug dose calculation knowledge among pediatric nurses, as it directly impacts the quality of care for children. Despite the majority of participants demonstrating average or good knowledge, the findings underscore that maintaining merely average proficiency is inadequate for pediatric nursing. Furthermore, demographic variables such as gender, age, professional qualifications, and clinical experience showed no significant association with knowledge levels, emphasizing the need for continuous professional development across all groups. The low attendance at workshops or conferences related to drug dose calculation (34.5%) reveals a gap in ongoing education. This study reinforces the necessity for regular in-service training, workshops, and conferences to enhance and sustain nurses' competency in pediatric drug dose calculation, ultimately improving patient safety and care outcomes.

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Table 1: Frequency and Percentage Distribution of Demographic Variables

Variables	Frequency	Percentage
Gender		
Male	20	18.2
Female	90	81.2
Age in years		
20-30	94	8.5
31-40	10	9.1
>40	6	5.5
Professional Qualification		
GNM	91	82.7
B.Sc. Nursing	19	17.3
Total Experience in Clinical field		
0.5 – 5 Years	89	80.9
6 – 10 Years	13	11.8
>10Years	8	7.3
Total experience in paediatric field		
0 – 5 Years	92	83.6
4-6 Years	10	9.1
7-9 Years	3	2.7
>10 Years	5	4.5
Attended workshop/Conference related to Drug Dose calculation		
Yes	38	34.5
No	72	65.5

Table 2: Frequency and Percentage Distribution of Knowledge

Sr. No	Variables	Frequency	Percentage
1	Knowledge		
Poor	0-7	14	12.7
Good	8-14	48	43.6
Excellent	15-22	48	43.6

Table 3: Association between Knowledge with Demographic Variables

Variables	Score (<13.24)	Score (>13.24)	Total	X2	Df	Level of Significance
Gender						
Male	20	0	20	1.944	2	15.507>1.944 NS
Female	13	77	90			
Age in years						
20-30	11	83	94	4.791	4	21.026>1.944 NS
31-40	10	0	10			
>40	6	0	6			
Professional Qualification						
GNM	11	80	91	0.485	2	15.507>0.485 NS
B.Sc. Nursing	19	0	19			
Total Experience in Clinical field						
0.5 – 5 Years	9	80	89	5.501	4	21.026>5.501 NS
6 – 10 Years	13	0	13			
>10Years	8	0	8			
Total experience in paediatric field						
0 – 5 Years	8	84	92	13.592	6	26.296>13.592 NS
4-6 Years	10	0	10			
7-9 Years	30	0	3			
>10 Years	5	0	3			
Attended workshop/Conference related to Drug dose calculation						
Yes	13	25	38	11.941	2	11.941>11.941 NS
No	12	60	72			

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CASE STUDY ARTICLE

A Case Report on the Effectiveness of the Constitutional Approch its Managing Acid - Peptic Disorders : An Evaluation using the Final Perceived Stress Scale and Acid - Peptic Questionnaire

Komal Patel[1]* and Heena Rawal[2]

[1]Professor and Ph.D Scholar, Department of Organon of Medicine, Ahmedabad Homoeopathic Medical College, Ahmedabad, Parul University, Vadodara, Gujarat, India.
[2]Professor and Ph.D Guide, Department of Organon of Medicine, Ahmedabad Homoeopathic Medical College, Ahmedabad, Parul University, Vadodara, Gujarat, India.

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***Address for Correspondence**
Komal Patel
Professor and Ph.D Scholar,
Department of Organon of Medicine,
Ahmedabad Homoeopathic Medical College, Ahmedabad,
Parul University, Vadodara, Gujarat, India.
E.Mail: komalbrijesh18@gmail.com

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ABSTRACT

Acid peptic disorders are one of the commonest disorders encountered in clinical practice, although not life threatening as other major illness. The symptoms can be distressing and cause discomfort to normal life. Recent studies have found that people who face general stresses pessimistically are apt to experience psychosomatic disorders; in these very common is Acid- peptic disorders. According to Homoeopathy, it is the man who is sick and not his body and as a matter of fact he needs to be treated. It is here the concept of individualization comes into practice, where the physical as well as the mental characteristics of the individual is taken. This study has documented the prevalence, associated risk factors and the impact of this health issue along with homoeopathic constitutional medicine given to patient. An interventional study in the homoeopathic hospital to ascertain the role of constitutional medicine Arsenic album in case of acid peptic disorder was assessed by using of questionnaire, i.e., Acid peptic disorders and perceiving stress scale. Mr. BP with peptic ulcer disease aged 32 years working as bank manager is considered. After case taking and filling theFinal Perceiving stress scale questionnaire the stress level score of patient was 25 and Acid peptic questionnaire score was 26 before treatment. After taking proper analysis and evaluation of the case according to homoeopathic principles, his constitutional medicine Arsenic album in 200was prescribed. Arsenic Album 1M in the follow-up, according to the change in the susceptibility was administered. After 6 months treatment patient showed improvement. At the end of June 2024 further final perceiving stress scale and acid peptic questionnaire were filled by patient and the score was 5 and 6. It also reflected significant improvement in patient's psychological and physical

ailments. Acid peptic disorders are generally observed in patients with high stress as a common health problem due to its prevalence, manageable nature and associated social psychological implications. The acid peptic disorder in a patient is also influenced by the various stress factors and homoeopathy as a holistic system of medicine is helpful in relieving the disease, not only removing the physical symptoms but also helps the patient to increasing the coping mechanism of stress.

Keywords: Acid-peptic disorders,Psychosomatic, Stress, constitution, Psychosomatic, Peptic ulcer, GERD, Gastritis, vagal hyper activity, food habits, neuro-endocrine, immunological, Individualisation

INTRODUCTION

The concept of psychosomatic medicine is subsumed in the diagnostic entity of "Psychological factors Affection other medical condition." This category covers physical disorders caused by or adversely affected by emotional or psychological factors. A medical condition must always be present for the diagnosis to be made.[1] Fourth edition of DSM-IV describes psychological factors affecting medical condition as one or more psychological or behavioural problems that adversely affect the course or outcome of a general medical condition or that significantly increases the person's risk of an adverse outcome.[1] Disease represents to us the reaction of the patient to the unfavorable environmental factors and this reaction manifests as signs and symptoms. The pattern of reaction is not only determinedby factors that caused illness, but also by the constitution of the affectedperson.[11] The gastro intestinal tract is one of the most common sites of psychogenetically triggered somatic symptoms and psychosomatic disorders. Problems associated with appetite and eating or elimination are part of the symptom profile of many psychiatric diseases.Studies have found convincing evidence that the onset, perpetuations and recurrence of peptic ulcers are associated with stressful life events. Acid peptic disease by definition is a disorder of gastric and duodenal mucosal barrier due to hyper or hypo secretion of acid and pepsin into the gastric juice which result in destruction of mucosal and muscular layers of stomach and duodenum. Diet habits including irregularity in food habits and rich foods, smoking and alcohol also provides a major role in the causation of the disease in addition to psychological factors. This constitution is the resultant of the early environmental factors, right from the time of conception, acting on the hereditary factors which determined by genes.[12]This constitution is the resultant of the early environmental factors, right from the time of conception, acting on the hereditary factors which determined by genes.[24]

MATERIALS AND METHODS

Clinical study: The study will be carried out with detailed case study and follow up at Sainath Hospital attached to Ahmedabad Homoeopathic Medical College, Parul University, Ahmedabad.
***Case definition*:** Cases presenting with complaints of Acid peptic diseases, case of age 32 was taken in study.

Inclusive criteria
The diagnostic criteria are mainly based on clinical history, presentation and examination findings.

Exclusion criteria
1. Ulcers secondary to burns, uraemia and drugs.
2. Cases complicated with gastric ulcer, haemorrhage, perforation, and gastric outlet obstruction.
3. Malignant conditions.
4. The patients who were unwilling to give consent to participate in the study

Data Collection It done by individual history taking according to Homoeopathic principles. A self-designed self-assessed questionnaire consisting of socio demographic characteristics, travel history, co morbidity patterns, health seeking behaviors, and outcome of the treatment had been prepared in English and local language.

Case Report
Preliminaries
Date: 09/01/2024
OPD no.: 23006434
Name: Mr. BP
Sex: Male
Age: 32
Education: MBA
Occupation: Bank Manager
Religion: Hindu
Address: Ahmedabad
Chief complains: Burning pain in epigastrium and left hypochondrium, with vomiting and retching for 1-year months, Flatulence, Nausea, generalized weakness.
<by touching, after eating and warm liquids
>After eructation, vomiting

History of Present Complain with ODP
Burning pain in epigastrium and left hypochondrium, with vomiting and retching for 1 year, generalized weakness. Patient taken allopathic treatment for 6 months but it's given no relief.
Past history: Dengue at the age of 16
Family history: Mother: DM-II, Father: HTN

Personal History
Physical Generals
- Appetite: Decreased
- Thirst: Increased
- Craving: not specific
- Aversion: food, milk
- Aggravation: warm liquids
- Sleep: Disturbed
- Perspiration: profuse on whole body
- Stool: once /day, not satisfactory
- Urine: 7-8 times /day
- Thermals: Chilly patient

Mentals
Financial stress due to loss in business, anxious, Irritable, anxiety about disease fearful dreams

Physical examination
General Examination
- Conscious
- Intelligence and education level: Intelligent
- General built and nutrition: week looking
- Height: 6' 1-inch Weight: 70 kg BMI:
- Anaemia: absent, Jaundice: not present, Cyanosis: not present, Oedema: not present
- Skin (Pigmentation, Hair distribution, warts, etc.): no any
- Nails: pinkish, Gait: normal
- Lymphadenopathy (Cervical, axillary, inguinal, etc.): no any
- Blood pressure:126/76 mm of hgPulse:80 /minutes Temperature: 98.8 F
- Respiration Rate: 18 b /min

Systemic Examination(Table 1)
Laboratory investigations and findings: Endoscopy of upper GI tract done on 9/3/2023
Provisional diagnosis: Gastritis
Final diagnosis: Body- antral Gastritis (Fig:1,2)

Miasm: Dominant miasm: Psora, Fundamental miasm: Sycosis

Analysis and evaluation
Mentals: Financial stress, Irritable, anxiety about disease

General symptoms
- Increased Thirst
- Aversion to food, milk
- Aggravation from warm liquids
- Sleep is disturbed
- Profuse on whole body

Particular symptoms
- Burning pain in epigastrium and left hypochondrium
- <by touching, after eating, >After eructation, vomiting
- Flatulence in epigastrium region
- Nausea and vomiting occasionally

Common symptoms
Weakness

Totality of symptoms
- Financial stress
- Irritable, anxiety about disease
- Increased Thirst
- Aversion to food, milk
- Aggravation from warm liquids
- Sleep is disturbed
- Profuse on whole body
- Burning pain in epigastrium and left hypochondrium <by touching, after eating
- >After eructation, vomiting
- Flatulence

Susceptibility: High
Selection of remedy: Arsenic album
Prescriptions: Ars Alb 200 single dose stat, SL 4 pills BD for 7 days
General management and auxiliary measures: Take light nutritional food

Follow ups
19/01/2024: No vomiting, pain decreased, weakness present, sleep sound
Prescription: Ars Alb 200 single dose stat, SL 4 pills BD for 15 days
04/02/2024: better in pain in abdomen, can eat food as much as he likes, weakness decreased
Prescription: SL 4 pills BD for 15 days
19/02/2024: Better in weakness, sometime burning in epigastric region, sleep disturbed
Prescription: Arsenic Alb 1m one dose, SL 4 pills BD for 1month
20/03/2024: Better in all complain, Weigh as it is

Prescription: SL 4 pills BD for 1 month
22/04/2024: Burning in epigastrium region since 5 days, no nausea, vomiting
Prescription: Arsenic Alb 1m one dose, SL 4 pills BD for 1month
27/06/2024: Better in all complain

RESULT

Final Perceiving stress scale (Fig:3,4)
Questionaries for acid-peptic disorders(Fig:5,6)
Marked improvement

DISCUSSION

Considering the psychosomatic relationship in acid peptic diseases, a detailed history taking is necessary to obtain a psychological profile and constitutional prescription. Mr. BP with peptic ulcer disease aged 32 years working as bank manager is considered. After case taking and filling the Final Perceiving stress scale questionnaire the stress level score of patient was 25 and Acid peptic questionnaire score was 26 before treatment. After taking proper analysis and evaluation of the case according to homoeopathic principles, his constitutional medicine Arsenic album in 200 was prescribed. Arsenic Album 1M in the follow-up, according to the change in the susceptibility was administered. After 6 months treatment patient showed improvement. At the end of June 2024 further final perceiving stress scale and acid peptic questionnaire were filled by patient and the score was 5 and 6. It also reflected significant improvement in patient's psychological and physical ailments. Ethical issue: Study protocol was approved through Institutional Ethical Committee. Written informed consent was taken from the participant and full confidentiality for the information provided was ensured.

CONCLUSION

Acid peptic disorders were observed in a person with high stress as a common health issue due to its prevalence, curable nature and associated social psychological problems. During this study it was found that people working at professional level and intellectually sharp has observed more acid peptic disorders. After constitutional similimum medicine Arsenic album was given patient show marked improvement. So that Homoeopathic constitutional similimum medicine has marked effect in APD.

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Table: 1 Systemic Examination

System	Findings
Respiratory System	Air entry clear
Cardiovascular System	S1, S2 heard
Nervous System	All reflexes normal
Gastro-intestinal System	Soft, increases peristaltic movement
Genitourinary System	NAD
Locomotor System	NAD



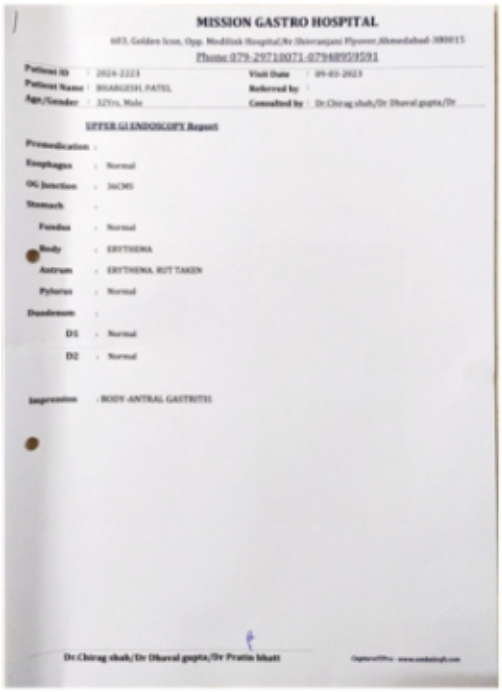


Fig.1 . Systemic Examination

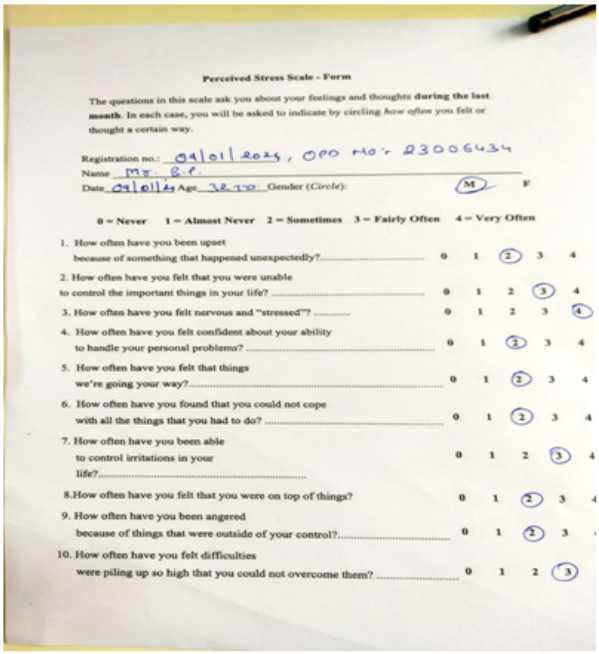


Fig.3: Final Perceiving stress scale Before: 25

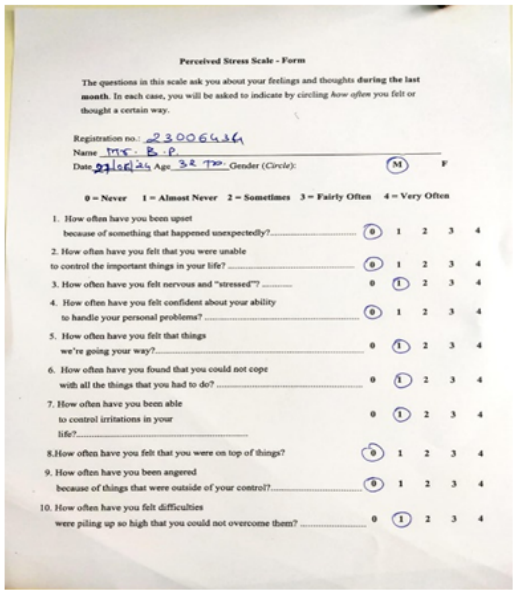
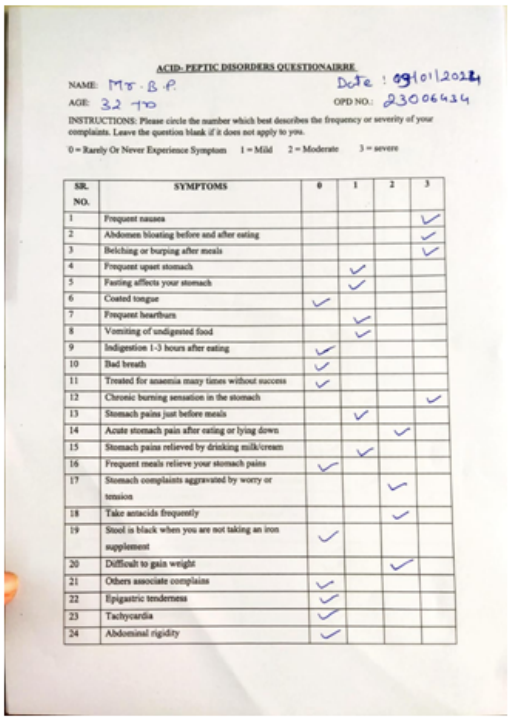


Fig.4: Final Perceiving stress scale After: 5



ACID- PEPTIC DISORDERS QUESTIONAIRRE

NAME: Mr. B.P. Date : 09/01/2024

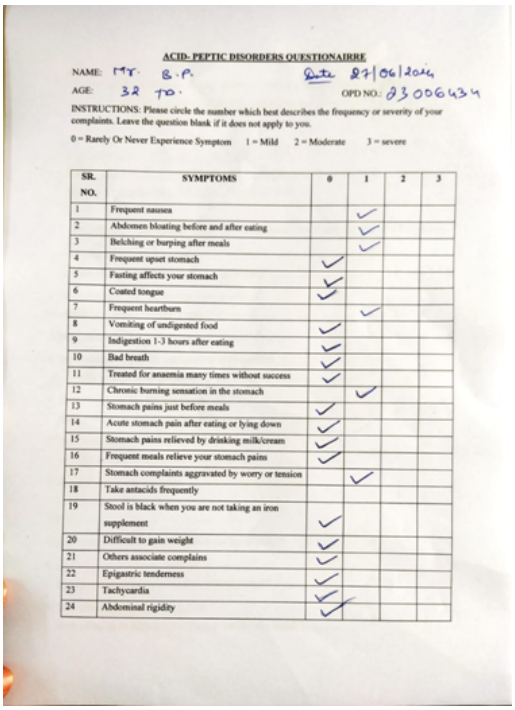
AGE: 32 YO OPD NO.: 23006434

INSTRUCTIONS: Please circle the number which best describes the frequency or severity of your complaints. Leave the question blank if it does not apply to you.

0 = Rarely Or Never Experience Symptom 1 = Mild 2 = Moderate 3 = severe

SR. NO.	SYMPTOMS	0	1	2	3
1	Frequent nausea				✓
2	Abdomen bloating before and after eating				✓
3	Belching or burping after meals				✓
4	Frequent upset stomach		✓		
5	Fasting affects your stomach		✓		
6	Coated tongue	✓			
7	Frequent heartburn		✓		
8	Vomiting of undigested food		✓		
9	Indigestion 1-3 hours after eating	✓			
10	Bad breath	✓			
11	Treated for anaemia many times without success	✓			
12	Chronic burning sensation in the stomach				✓
13	Stomach pains just before meals		✓		
14	Acute stomach pain after eating or lying down			✓	
15	Stomach pains relieved by drinking milk/cream		✓		
16	Frequent meals relieve your stomach pains	✓			
17	Stomach complaints aggravated by worry or tension			✓	
18	Take antacids frequently			✓	
19	Stool is black when you are not taking an iron supplement	✓			
20	Difficult to gain weight			✓	
21	Others associate complains	✓			
22	Epigastric tenderness	✓			
23	Tachycardia	✓			
24	Abdominal rigidity	✓			

Fig.5: Questionaries for acid-peptic disorders: Before: 26



ACID- PEPTIC DISORDERS QUESTIONAIRRE

NAME: Mr. B.P. Date 27/06/2024

AGE: 32 YO. OPD NO.: 23006434

INSTRUCTIONS: Please circle the number which best describes the frequency or severity of your complaints. Leave the question blank if it does not apply to you.

0 = Rarely Or Never Experience Symptom 1 = Mild 2 = Moderate 3 = severe

SR. NO.	SYMPTOMS	0	1	2	3
1	Frequent nausea		✓		
2	Abdomen bloating before and after eating		✓		
3	Belching or burping after meals		✓		
4	Frequent upset stomach	✓			
5	Fasting affects your stomach	✓			
6	Coated tongue	✓			
7	Frequent heartburn		✓		
8	Vomiting of undigested food	✓			
9	Indigestion 1-3 hours after eating	✓			
10	Bad breath	✓			
11	Treated for anaemia many times without success	✓			
12	Chronic burning sensation in the stomach		✓		
13	Stomach pains just before meals	✓			
14	Acute stomach pain after eating or lying down	✓			
15	Stomach pains relieved by drinking milk/cream	✓			
16	Frequent meals relieve your stomach pains	✓			
17	Stomach complaints aggravated by worry or tension		✓		
18	Take antacids frequently				
19	Stool is black when you are not taking an iron supplement	✓			
20	Difficult to gain weight	✓			
21	Others associate complains	✓			
22	Epigastric tenderness	✓			
23	Tachycardia	✓			
24	Abdominal rigidity	✓			

Fig.6: Questionaries for acid-peptic disorders: After: 6

RESEARCH ARTICLE

Molecular Docking Studies on Phytoconstituents of Siddha Formulation Eladi Chooranam against RAS/RAF/MEK/ERK Pathway with ADMET Predictions

Jayaraj .S[1]*, Gokul .G[1], Viknesh .M[1] and R.Vimalavathini[2]

[1]B. Pharm Student, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, (Affiliated to Pondicherry University), Puducherry, India.
[2]Associate Professor, Department of Pharmacology, College of Pharmacy, Mother Theresa Post Graduate and Research Institute of Health Sciences, (Affiliated to Pondicherry University), Puducherry, India.

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***Address for Correspondence**
Jayaraj .S
B. Pharm Student, College of Pharmacy,
Mother Theresa Post Graduate and
Research Institute of Health Sciences,
(Affiliated to Pondicherry University),
Puducherry, India.
E.Mail: jayaraj2453@gmail.com

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ABSTRACT

To investigate the *in silico* RAS-RAF-MEK-ERK modulatory potential of 20 Phyto-Chemicals present in the poly herbal Siddha formulation Eladi Chooranam (α-Cubebene, α-Copaene, β-Humulene, Eugenol, α- Thujene, β-Bisabolene, δ-Cadinol, Nerolidol, β-Sitosterol, β-Amyrin, α-Conidendrin, Hydroxy matairesinol, Texiresinol, Ferulic acid, Dresgenin. , α -Farnesene, Glycidol, α-Terpinyl Acetate, Linalool, Sabinene.) against four protein targets (K-Ras G12C: Kirsten Rat Sarcoma viral oncogene homolog G12C mutant, c-Raf1 kinase: Rapidly Accelerated Fibrosarcoma 1 kinase, MEK-1 kinase: Mitogen-Activated Protein Kinase Kinase 1, ERK2: Extracellular Signal-Regulated Kinase 2) using PyRx 0.8 in addition to study ADME properties and Toxicity Predictions using SwissADME and OSIRIS Property Explorer respectively. Based on the literature review it is evident that the foretold ligands exhibiting anti-inflammatory, antioxidant, anticancer, immunomodulatory, free radical scavenging, and anti-angiogenic properties. The Modulation of RAS-RAF-MEK-ERK pathway has its significant role in mediations of forementioned properties. Hence, the aim of our study is to investigate the phytochemicals present in poly herbal Siddha formulation Eladi Chooranam for its potential RAS-RAF-MEK-ERK modulation. All the 20 Phyto Chemicals were obtained from PubChem database. All the proteins were downloaded from RCSB PDB. Twenty Ligands were docked with 4 target proteins using PyRx and the binding interactions were studied. Apart from Glycidol other 19 ligands elicits better Binding Affinities greater than 5 Kcal/Mol. In addition, the ADMET Properties of many ligands are much favorable or comparable to that

of Standard Inhibitors of respective proteins. Thus, the *in silico* investigation of the Phyto chemicals present in Eladi Chooranam displays potential RAS-RAF-MEK-ERK modulation with favorable ADMET Properties and their activities were comparable to that of their standard inhibitors namely Sotorasib, Vemurafenib, Trametinib, Ulixertinib.

Keywords: Siddha Formulation, Molecular Docking, Eladi Chooranam, ADMET predictions, Phyto chemicals.

INTRODUCTION

RAS-RAF-MEK-ERK Pathway

The RAS-RAF-MEK-ERK Pathway is a well-studied Signal transduction pathway in cellular Biology. The elementary function of the pathway is to cascade signals from extra cellular space to the specific genes which upon activation causes functions like Cell growth, division and differentiation [1]. Wound healing, tissue repair, cell cycle regulation and its migration are the functions which occurs upon cascade of the pathway. Tumorigenesis and Oncogenesis occurs upon the mutations of the proteins in the pathway [2]. The Mutant proteins cause many types of cancers such as neurofibromas, colorectal, lung, pancreatic carcinomas, along with metastasis [3]. Various inhibitors are administered to these mutant proteins for treating these melanomas drugs like Sotorasib (Lumakras™), Vemurafenib (Zelboraf®), Encorafenib (Braftovi®), Trametinib (Mekinist®), Selumetinib (Koselugo®) [4] [5] [6] [7] [8].

Eladi Chooranam

Siddha medicinal system is one of the prominent traditional medicinal systems in India (especially in Tamil Nadu). Eladi Chooranam is a poly herbal powder formulation. Etymologically the word Eladi was derived from its one of the key ingredient 'Elam' (cardamom) [9]. The 7 ingredients of the Eladi Chooranam are *Eletaria cardamomum* (Elam), *Syzygium aromaticum* (Kirambu), *Piper nigrum* (Milagu), *Taxus baccata* (Thalisapathri), *Zingiber officinale* (Chukku), *Curcuma angustifolia* (koogaineeru), Nagapoo (*Mesua ferrea*). The above ingredients are pulverized to fine homogenous powder [10]. This formulation is indicated for the humoral imbalances (Piththam, Vaatham), flatulence (Vaayu), skin eruptions (Kiranthi, Arikiranthi, Sorisirangu, Kuttam), insect bites, urogenital disorders (Megavooral), menorrhagia (Perumpaadu), Peptic Ulcer (Kunmam) and wasting diseases (Kurainoy, Elumburukki) [9] [10].

***In silico* analysis; As a Primordial in Drug Discovery**

Old school methodology of Lead identification involves a very extensive experimental screening of chemical libraries and requires too much time and resources with little to no fruitful results [11]. In order to overcome these hurdles, computational methods were emerged as the effective tools to screen the lead candidates with selectivity and precisely [12]. The major Virtual Screening methodologies are divided into two: Ligand based screening (LBVS) and Structure based screening (SBVS) [11] [12]. The LBVS involves utilization of Physio Chemical properties of known ligand data set to predict potential new lead molecule's ADMET properties (Absorption, Distribution, Metabolism, Excretion and Toxicity) [13]. In this present study we used SwissADME and Osiris Property Explorer for the predictions. Secondly, the Structure-based virtual screening deals with Molecular Docking Studies between Biological Targets (i.e., Proteins) and a Ligand to predict the binding affinities, interaction and orientation [14]. Current study utilized PyRx for Multiple Ligand Docking.

Rationale behind Eladi Chooranam's Pathway Modulation

By reviewing over 80 Phyto constituent's pharmacological profile of the Eladi Chooranam. 20 Phyto chemicals were selected exhibiting Anti-inflammatory, anti-oxidant, anti-cancer, free radicle scavenging, anti-angiogenic properties. As it is evident that the inflammatory and oncogenic state was modulated by RAS-RAF-MEK-ERK Pathway, so with this background this paper aims to study the molecular interaction between the constituents and protein targets using in silico docking analysis, additionally ADMET predictions are also performed.

MATERIALS AND METHODS

Protein Preparation

The 3D crystallographic structures of four target proteins of RAS-RAF-MEK-ERK signaling pathway i.e., K-Ras G12C (PDB ID: 4L8G), c-Raf1 kinase (PDB ID: 1C1Y), MEK-1 kinase (PDB ID: 3SLS), and ERK2 (PDB ID: 6SLG) were downloaded from Research Collaboratory for Structural Bioinformatics Protein Data Bank (RCSB PDB). From all the Structures, co-factors, hetero atoms and water molecules were removed [15] computationally using BioVia Discovery Studio and saved in Protein Data Bank (PDB) format.

Ligand Preparation

The 20 selected Phyto constituents of Eladi Chooranam namely α-Terpinyl Acetate, Sabinene, Linalool, Eugenol, β-Humulene, α-Cubebene, α-Copaene, α-Thujene, β-Bisabolene, Nerolidol, ð-Cadinol, β-Amyrin, β-Sitosterol, Matairesinol, Hydroxy matairesinol, α-Conidendrin, Glycidol, α-Farnesene, Dresgenin, Ferulic acid were downloaded from the NCBI PubChem in 2D *SDF format alongside the 4 Standard drugs Sotorasib, Vemurafenib, Trametinib, Ulixertinib.

Molecular Docking Using PyRx

The 4 Proteins and 20 Ligands and their respective inhibitor were loaded into the PyRx 0.8 in order to perform the multiple ligands Docking. The grid box dimensions and center co-ordinates were maximized to envelope the entire protein. Docking simulations employed an exhaustiveness parameter of 8, then 'Forward' button is clicked to start Docking [16]. Then the binding affinities of the docked molecules were tabulated and analyzed. The Inhibition constant was calculated by the following formula [17] [18].

$Ki = \exp(\Delta G/(R*T))$

ΔG: Binding affinity (in kcal/mol).

R: Gas constant = 1.987×10^{-3} kcal/(mol·K).

T: Temperature in Kelvin (typically 298 K)

Subsequently the amino acid interactions are visualized using BioVia Discovery Studio.

Pharmacokinetic and Toxicity Predictions

The *in silico* pharmacokinetic properties (Absorption, Distribution, Metabolism, Excretion) were predicted using open source online server SwissADME by providing the SMILES notations of the compounds, from the server the Physio-Chemical properties (such as Molecular weight, Lipophilicity (M Log P), Lipinski violations and Bioavailability score) and Pharmacokinetic properties (such as GI absorption, BBB Permeant, P-gp substrate, Enzyme inhibited, Skin permeation) were assessed and tabulated. The Potential Toxic effects (mutagenic, tumorigenic, irritant, reproductive effects) of all the compounds were assessed by OSIRIS Property Explorer and the results were tabulated.

RESULTS

In this current *in silico* study, molecular docking analyzed the inhibitory activity of 20 Eladi Chooranam phytoconstituents against the RAS-RAF-MEK-ERK pathway kinases (K-RAS: 4L8G, C-RAF: C1Y1, MEK: 3SLS, ERK: 6SLG), in comparison to corresponding inhibitors (Sotorasib, Vemurafenib, Trametinib, Ulixertinib). As shown in Table 1, against K-RAS, Dresgenin showed the greatest binding affinity (-9.3 Kcal/Mol; Ki=0.15 μM), followed by Sotorasib (-9.1 Kcal/Mol; Ki=0.21 μM), then α-Conidendrin (-9.0 Kcal/Mol), Hydroxy matairesinol (-8.5 Kcal/Mol), β-Amyrin (-8.3 Kcal/Mol), and β-Sitosterol (-8.2 Kcal/Mol). Against C-RAF, though Vemurafenib bound well (-10.2 Kcal/Mol), β-Amyrin (-8.0 Kcal/Mol), β-Sitosterol (-7.8 Kcal/Mol), and α-Conidendrin (-7.2 Kcal/Mol) had the best affinities of the phytochemicals. Docking with MEK unveiled β-Amyrin (-8.8 Kcal/Mol; Ki=0.35 μM) as the best phytochemical binder, coming close to that of Trametinib (-9.2 Kcal/Mol; Ki=0.18 μM), with Dresgenin (-8.6 Kcal/Mol) and α-Conidendrin (-8.0 Kcal/Mol) also being highly promising. Against ERK, Dresgenin once again exhibited better binding (-8.9 Kcal/Mol; Ki=0.30 μM) in comparison to Ulixertinib (-8.4 Kcal/Mol; Ki=0.69 μM), with α-Conidendrin (-

8.7 Kcal/Mol) and β-Amyrin (-8.6 Kcal/Mol) also demonstrating strong binding affinities. SwissADME predictions (Table 2 and Table 3) predicted good drug-likeness for all constituents with moderate oral bioavailability (score 0.55) and variable GI absorption and BBB permeability; potential CYP enzyme interactions were observed. OSIRIS toxicity predictions shown in table 4, flagged some compounds (including β-Amyrin, Dresgenin, *α*-Conidendrin, β-Sitosterol, Hydroxy matairesinol) as non-toxic in all mutagenicity, tumorigenicity, and reproductive effect endpoints and indicated potentially safer profiles than comparable drugs such as Sotorasib and Vemurafenib, which were predicted toxicities. Overall, five phytochemicals, β-Amyrin, Dresgenin, *α*-Conidendrin, β-Sitosterol, and Hydroxy matairesinol, have desirable ADMET attributes and exhibit high binding affinity (> -8.0 kcal/mol) against the RAS-RAF-MEK-ERK pathway, indicating their possible modulatory role in this pathway, in line with reported bioactivities [19 - 23].

DISCUSSION

The current *in silico* study aimed to investigate the molecular interactions of phytoconstituents of the traditional Siddha drug, Eladi Chooranam, and major protein kinases of the oncogenic RAS-RAF-MEK-ERK pathway. This computational approach, encompassing molecular docking alongside ADMET predictions, provides valuable preliminary insights into the potential mechanisms underlying the formulation's documented therapeutic effects, particularly those related to inflammatory and proliferative conditions where this pathway is implicated [1, 2, 9, 10].
Our findings reveal that several phytochemicals derived from Eladi Chooranam exhibit promising binding affinities towards K-RAS, C-RAF, MEK, and ERK kinases. Most noteworthy were Dresgenin's high inhibitory capability, notably against K-RAS and ERK, whose binding energies (-9.3 Kcal/Mol and -8.9 Kcal/Mol, respectively) were better than or equivalent to respective standard drugs Sotorasib and Ulixertinib. Likewise, β-Amyrin was a strong binder of MEK (-8.8 Kcal/Mol), second only to Trametinib, and was the highest phytochemical binder versus C-RAF (-8.0 Kcal/Mol). The complex *α*-Conidendrin consistently exhibited high binding over K-RAS, MEK, and ERK, proposing a multi-targeted inhibitory profile. The computationally predicted interactions, usually involving central residues within or at the proximity of the binding sites targeted by current inhibitors, gives merit to the likelihood that such phytochemicals could functionally regulate kinase activity. This observation is consistent with the rationale of the study, which selected constituents on the basis of previously documented anti-inflammatory and anti-cancer activities, activities commonly exerted via mechanisms such as RAS-RAF-MEK-ERK. In addition, the combined ADMET analysis increases the value of these docking outcomes. The prediction that these important compounds such as β-Amyrin, Dresgenin, *α*-Conidendrin, β-Sitosterol, and Hydroxy matairesinol have good drug-like properties based on Lipinski's rule, along with moderate predicted oral bioavailability and generally good non-toxic profiles (non-mutagenic, non-tumorigenic, no reproductive effects through OSIRIS), is especially promising. This is contrasted positively with the anticipated toxicities of certain conventional inhibitors such as Sotorasib and Vemurafenib, highlighting the value of these natural compounds as leads with potentially wider safety margins. Although in silico predictions present a powerful and effective tool for lead identification [11, 12], there is a need to recognize their limitations. Such computational results are hypotheses that require stringent experimental proof. Thus, future research should focus on in vitro enzyme inhibition assays and cell-based studies focused on the identified lead compounds (Dresgenin, β-Amyrin, *α*-Conidendrin, Hydroxy matairesinol, β-Sitosterol) to validate their inhibitory activity against the target kinases and their effect on the overall RAS-RAF-MEK-ERK pathway signaling. Follow-up in vivo studies would be crucial to further assess efficacy and validate the predicted pharmacokinetic and safety profiles. Thus, the current *in silico* study identifies the therapeutic potential of specific phytoconstituents of the poly herbal Siddha formulation Eladi Chooranam, demonstrating their ability to modulate the RAS-RAF-MEK-ERK pathway by means of substantial interactions with important kinase targets (K-RAS, C-RAF, MEK, ERK) and showing good ADMET properties indicative of desirable drug-like behavior.

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Nil

Conflict of Interest
Nil

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Table 1: Docking results of 20 phytochemicals of Eladi Chooranam and with 4 standard drugs with target receptor proteins

S.No	Protien Target	Constituents	Binding Affinity (Kcal/Mol)	Inhibition Constant (μM)	No. of H bonds
1	RAS (PDB:4L8G)	Sotorasib (K-RAS INHIBITOR)	-9.1	0.211646	4
2		-Terpinyl Acetate	-6.6	14.42892	3
3		Linalool	-5.2	153.48396	1
4		Sabinene	-5.1	181.7221	----
5		α-Cubebene	-7.1	6.201675	----
6		α-Copaene	-6.5	17.08357	----
7		β-Humulene	-6.9	8.693584	----
8		β-Bisabolene	-7	7.342669	----
9		δ-cadinol	-6.6	14.42892	1
10		-Thujene	-5.9	47.05961	----
11		Eugenol	-6	39.74691	3
12		Nerolidol	-6.3	23.94796	2
13		α-Conidendrin	-9	0.250585	4
14		Hydroxy matairesinol	-8.5	0.5830151	6
15		Texiresinol	-7.8	1.901491	4
16		α –Farnesene	-6.5	17.08357	----
17		Glycidol	-3.6	2288.664	5
18		Ferulic acid	-6.3	23.94796	3
19		Dresgenin	-9.3	0.15098036	5
20		β-Sitosterol	-8.2	0.9676418	----
21		β-Amyrin	-8.3	0.492419	1
22	RAF (PDB: C1Y1)	Vemurafenib (C-RAF INHIBITOR)	-10.2	0.03302294	5
23		-Terpinyl Acetate	-5.6	78.10578	1
24		Linalool	-5.2	153.4839	2
25		Sabinene	-4.6	422.7977	----
26		α-Cubebene	-6.9	8.693584	----
27		α-Copaene	-6.2	28.35394	----
28		β-Humulene	-6.9	8.693584	----
29		β-Bisabolene	-7.1	6.201675	----
30		δ-cadinol	-6.7	12.18677	----
31		-Thujene	-5.6	78.10578	----
32		Eugenol	-5.8	55.71771	4
33		Nerolidol	-6.5	17.08357	1
34		α-Conidendrin	-7.2	5.237983	----
35		Hydroxy matairesinol	-6.8	10.29304	5
36		Texiresinol	-6.3	23.94796	1

37		α –Farnesene	-6.7	12.18677	----
38		Glycidol	-3.8	1632.646	4
39		Ferulic acid	-6.7	12.18677	5
40		Dresgenin	-6.8	10.29304	2
41		β-Sitosterol	-7.8	1.901491	1
42		β-Amyrin	-8.0	1.350	1
43	MEK (PDB: 3SLS)	Trametinib (MRK INHIBITOR)	-9.2	0.1787579	3
44		-Terpinyl Acetate	-6.4	20.22663	1
45		Linalool	-5	215.1557	1
46		Sabinene	-5.9	47.05961	----
47		α-Cubebene	-6.5	17.08357	----
48		α-Copaene	-6.8	10.29304	----
49		β-Humulene	-6.5	17.08357	----
50		β-Bisabolene	-6.1	33.57055	----
51		δ-cadinol	-6.7	12.18677	----
52		-Thujene	-5.9	47.05961	----
53		Eugenol	-5.8	55.71771	1
54		Nerolidol	-6.2	28.35394	----
55		α-Conidendrin	-8	1.356452	5
56		Hydroxy matairesinol	-7	7.342669	2
57		Texiresinol	-8	1.356452	3
58		α –Farnesene	-5	215.1557	----
59		Glycidol	-3.6	2288.664	3
60		Ferulic acid	-6.2	28.35394	4
61		Dresgenin	-8.6	0.492419	6
62		β-Sitosterol	-7	7.342669	----
63		β-Amyrin	-8.8	0.3512731	1
64	ERK (PDB: 6SLG)	Ulixertinib (ERK INHIBITOR)	-8.4	0.6902791	1
65		-Terpinyl Acetate	-5.8	55.71771	2
66		Linalool	-4.4	592.683	----
67		Sabinene	-5.2	153.4839	----
68		α-Cubebene	-7	7.342669	----
69		α-Copaene	-6.3	23.94796	----
70		β-Humulene	-7.3	4.424041	----
71		β-Bisabolene	-5.9	47.05961	----
72		δ-cadinol	-7.2	5.237983	----
73		-Thujene	-5.2	153.4839	----
74		Eugenol	-5.3	129.6337	2
75		Nerolidol	-5.2	153.4839	2
76		α-Conidendrin	-8.7	0.4159009	4
77		Hydroxy matairesinol	-7.7	2.251331	4
78		Texiresinol	-7.6	2.665534	1
79		α –Farnesene	-5.2	153.4839	----
80		Glycidol	-3	6304.515	3
81		Ferulic acid	-6.3	23.94796	2
82		Dresgenin	-8.9	0.296688	3

83		β-Sitosterol	-8.3	0.492419	----
84		β-Amyrin	-8.6	0.8172777	----

Table 2: Physio-Chemical Predictions of the Phyto-Chemical of Eladi Chooranam and Standard drugs from Swiss ADME

S.No	Name of constituent	Molecular Formula	Molecular Weight (g/mol)	Lipophilicity (M Log P)	Lipinski Violations	Bioavailability score
1	α-Terpinyl Acetate	C12H20O2	196.29	3.67	0	0.55
2	Linalool	C10H18O	154.25	2.59	0	0.55
3	Sabinene	C10H16	136.23	4.29	1	0.55
4	α-Cubebene	C15H24	204.35	5.65	1	0.55
5	α-Copaene	C15H24	204.35	5.65	1	0.55
6	β-Humulene	C15H24	204.35	2.01	0	0.55
7	β-Bisabolene	C15H24	204.35	4.53	1	0.55
8	δ-cadinol	C15H26O	222.37	3.67	0	0.55
9	α-Thujene	C10H16	136.23	4.29	1	0.55
10	Eugenol	C10H12O2	164.2	2.01	0	0.55
11	Nerolidol	C15H26O	222.37	3.86	0	0.55
12	α-Conidendrin	C20H20O6	356.37	1.9	0	0.55
13	Hydroxy matairesinol	C20H22O7	374.38	1.1	0	0.55
14	Texiresinol	C20H22O6	358.39	1.9	0	0.55
15	α -Farnesene	C15H24	204.35	4.84	1	0.55
16	Glycidol	C3H6O2	74.08	-1.03	0	0.55
17	Ferulic acid	C10H10O4	194.18	1	0	0.85
18	Dresgenin	C28H40O7	488.61	2.22	0	0.55
19	β-Sitosterol	C29H50O	414.71	6.73	1	0.55
20	β-Amyrin	C30H50O	426.72	6.92	1	0.55
21	Sotorasib	C30H30F2N6O3	560.59	3.67	1	0.55
22.	Vemurafenib	C23H18ClF2N3O3S	489.92	3.14	0	0.55
23	Trametinib	C26H23FIN5O4	615.39	4.21	2	0.17
24	Ulixertinib	C21H22Cl2N4O2	433.33	2.36	0	0.55

Table 3: Pharmacokinetic Predictions of the Phyto-Chemical of Eladi Chooranam and Standard drugs from Swiss ADME

S.no	Name of constituent	GI absorption	BBB permeant	P-gp substrate	Enzymes Inhibited	Log Kp (skin permeation-cm/s)
1.	α-Terpinyl Acetate	LOW	NO	NO	CYP1A2, CYP2C9	-3.2
2.	Linalool	HIGH	YES	NO	----	-5.13
3.	Sabinene	LOW	YES	NO	----	-4.94
4.	α-Cubebene	LOW	YES	NO	CYP1A2, CYP2C19, CYP2C9	-4.37
5.	α-Copaene	LOW	YES	NO	CYP1A2, CYP2C19, CYP2C9	-4.37
6.	β-Humulene	HIGH	YES	NO	CYP1A2	-5.69

7.	β-Bisabolene	LOW	NO	NO	CYP2C9	-2.98
8.	δ-cadinol	HIGH	YES	NO	CYP2C19	-5.29
9.	α-Thujene	LOW	YES	NO	----	-5.11
10.	Eugenol	HIGH	YES	NO	CYP1A2	-5.69
11.	Nerolidol	HIGH	YES	NO	CYP1A2, CYP2C9	-4.23
12.	α-Conidendrin	HIGH	NO	YES	CYP2D6	-6.49
13.	Hydroxy matairesinol	HIGH	NO	YES	----	-7.06
14.	Texiresinol	HIGH	NO	NO	CYP2D6, CYP3A4	-6.17
15.	α -Farnesene	LOW	NO	NO	CYP1A2, CYP2C9	-3.2
16.	Glycidol	HIGH	NO	NO	----	-7.43
17.	Ferulic acid	HIGH	YES	NO	----	-6.41
18.	Dresgenin	HIGH	NO	YES	----	-7.62
19.	β-Sitosterol	LOW	NO	NO	----	-2.2
20.	β-Amyrin	LOW	NO	NO	----	-2.41
21.	Sotorasib	HIGH	NO	YES	CYP2C9	-6.9
22.	Vemurafenib	LOW	NO	NO	CYP2C19, CYP2C9, CYP3A4	-5.76
23.	Trametinib	HIGH	NO	NO	CYP2C19, CYP2C9, CYP3A4	-7.62
24.	Ulixertinib	HIGH	NO	NO	CYP1A2, CYP2C19, CYP2C9, CYP2D6, CYP3A4	-6.03

Table 4: Toxicity Predictions of the Phyto-Chemical of Eladi Chooranam and Standard drugs from OSIRIS Property Explorer

S.No	Name of constituent	Mutagenic	Tumerogenic	Irritant	Reproductive Effect
1.	α-Terpinyl Acetate	NON-TOXIC	NON-TOXIC	TOXIC	NON-TOXIC
2.	Linalool	TOXIC	NON-TOXIC	TOXIC	NONTOXIC
3.	Sabinene	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
4.	α-Cubebene	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
5.	α-Copaene	NON-TOXIC	NON-TOXIC	TOXIC	NON-TOXIC
6.	β-Humulene	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
7.	β-Bisabolene	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
8.	δ-cadinol	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
9.	α-Thujene	NON-TOXIC	NON-TOXIC	MODERATELY TOXIC	NON-TOXIC
10.	Eugenol	TOXIC	TOXIC	TOXIC	NON-TOXIC
11.	Nerolidol	NON-TOXIC	NON-TOXIC	TOXIC	NON-TOXIC
12.	α-Conidendrin	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
13.	Hydroxy matairesinol	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
14.	Texiresinol	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
15.	α -Farnesene	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
16.	Glycidol	TOXIC	TOXIC	TOXIC	TOXIC
17.	Ferulic acid	TOXIC	TOXIC	NON-TOXIC	TOXIC
18.	Dresgenin	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC

19.	β-Sitosterol	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
20.	β-Amyrin	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
21.	Sotorasib (RAS Inhibitor)	TOXIC	MODERATELY-TOXIC	MODERATELY-TOXIC	MODERATELY-TOXIC
22.	Vemurafenib (RAF Inhibitor)	TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
23.	Trametinib (MEK Inhibitor)	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC
24.	Ulixertinib (ERK Inhibitor)	NON-TOXIC	NON-TOXIC	NON-TOXIC	NON-TOXIC

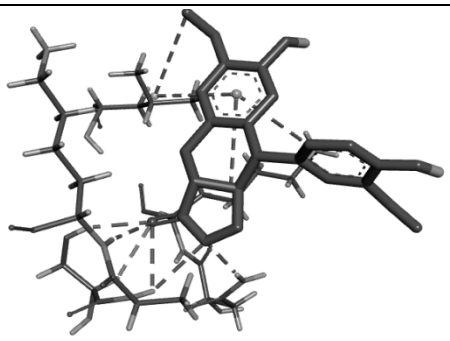


Figure 1: α-Conidendrin docked with K-RAS

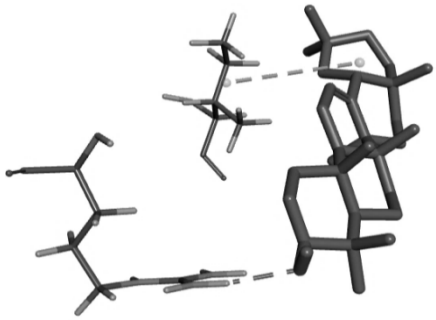


Figure 2: β-Amyrin docked with C-RAF

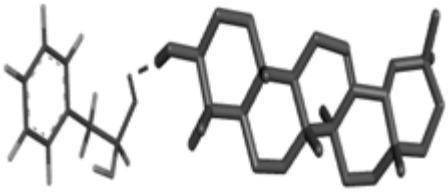


Figure 3: β-Amyrin docked with MEK

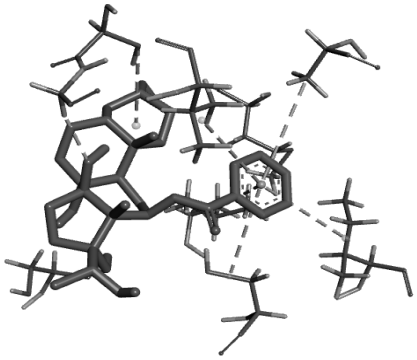


Figure 4: Dresgenin docked with ERK

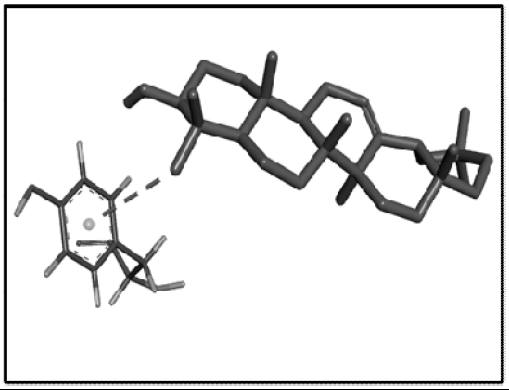


Figure 5: β-Amyrin docked with ERK

RESEARCH ARTICLE

4-Total Geometric Mean Cordial Labeling on Duplication of some Graphs

L. Vennila[1]* and P. Vidhyarani[2]

[1]Research Scholar (Reg. No: 19211202092025), Department of Mathematics, Sri Parasakthi College for Women, Courtallam, (Affiliated to Manonmaniam Sundaranar University, Abisekapatti), Tirunelveli, Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, Sri Parasakthi College for Women, Courtallam, (Affiliated to Manonmaniam Sundaranar University, Abisekapatti), Tirunelveli, Tamil Nadu, India.

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***Address for Correspondence**
L. Vennila
Research Scholar (Reg. No: 19211202092025),
Department of Mathematics,
Sri Parasakthi College for Women, Courtallam,
(Affiliated to Manonmaniam Sundaranar University, Abisekapatti),
Tirunelveli, Tamil Nadu, India.
E.Mail: vennila319@gmail.com

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ABSTRACT

In this paper we explore the 4-total geometric mean cordial (4-TGMC) labeling behavior of duplication on some graphs.

Keywords: Path, Cycle, Duplication, 4- total geometric mean cordial labeling, 4- total geometric mean cordial graphs.

INTRODUCTION

The concept of k-total geometric mean cordial labeling of graphs was introduced in [7]. In this paper we investigate 4-total geometric mean cordial (4-TGMC) labeling behavior of duplication on some graphs like Path, and Cycle graphs.

AMS Mathematics Subject Classification 2010 : 05C78.

MAIN RESULTS

Theorem 2.1.
Duplicating all the points of the graph P_t is a 4- total geometric mean cordial graph for all $t \geq 2$.

Proof

Let H = P_t be the path graph with points $g_1, g_2,\ldots,g_t$. Let H' be the graph obtained by duplicating all the points $g_1, g_2,\ldots,g_t$ by a new lines $g_i'\ g_i''$ $(1\le i\le t)$.

Then $|V(H')| + |E(H')| = 7t - 1$. The function f : V($H'$) → { 1, 2, 3, 4} is defined by

Case 1: When t ≡ 1 (mod 4)

The labeling pattern of g_i, g_i' *and* g_i'' are as follows,

$$f(g_i) = \begin{cases} 3, & 1 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le t \end{cases}$$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 2, & \frac{t+3}{2} \le i \le \frac{3t+5}{4} \\ 1, & \frac{3t+9}{4} \le i \le t \end{cases}$$

$$f(g_i'') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le \frac{3t+5}{4} \\ 3, & \frac{3t+9}{4} \le i \le t \end{cases}$$

Case 2: when t ≡ 2 (mod 4)

It is easy to verify for t =2, when t ≥ 6,

$$f(g_i) = \begin{cases} 3, & 1 \le i \le \frac{t}{2} \\ 4, & \frac{t+2}{2} \le i \le t \end{cases}$$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t-2}{4} \\ 2, & \frac{3t+2}{4} \le i \le t \end{cases}$$

$$f(g_i'') = \begin{cases} 1, & 1 \le i \le \frac{t}{2} \\ 3, & \frac{t+2}{2} \le i \le \frac{3t-2}{4} \\ 4, & \frac{3t+2}{4} \le i \le t \end{cases}$$

Case 3: when t ≡ 3 (mod 4)

It is easy to verify for t =3, when t ≥ 7, the label of the points are

f (g_1) = 2,

$$f(g_i) = \begin{cases} 3, & 2 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le t \end{cases}$$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 2, & \frac{t+3}{2} \le i \le \frac{3t+7}{4} \\ 1, & \frac{3t+11}{4} \le i \le t \end{cases}$$

$$f(g_i'') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le \frac{3t+7}{4} \\ 3, & \frac{3t+11}{4} \le i \le t \end{cases}$$

Case 4: when $t \equiv 4 \pmod 4$

$$f(g_i) = \begin{cases} 3, & 1 \le i \le \frac{3t}{4} \\ 4, & \frac{3t+4}{4} \le i \le t \end{cases}$$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t}{4} \\ 4, & \frac{3t+4}{4} \le i \le t \end{cases}$$

$$f(g_i'') = \begin{cases} 1, & 1 \le i \le \frac{t}{2} \\ 2, & \frac{t+2}{2} \le i \le \frac{3t}{4} \\ 4, & \frac{3t+4}{4} \le i \le t \end{cases}$$

From the above four cases,(Table: 1)

The function f satisfies the condition $|t_{gmf}(i) - t_{gmf}(j)| \le 1$, for all i, j∈{1, 2, 3, 4}. Hence $H'$is 4- total geometric mean cordial graph.

Theorem 2.2.

Duplicating all the lines of the graph C_t, $t \ge 3$, admits 4- TGMC labeling.

Proof

Let $H = C_t$ be the cycle graph with points $g_1, g_2, g_3, \ldots, g_t$ and H' be the graph obtained from the cycle C_t by duplicating all the lines $g_1g_2, g_2g_3, \ldots, g_ng_1$ by a new points $h_1, h_2, h_3, \ldots, h_t$ respectively. Then $|V(H')| + |E(H')| = 5t$. The function $f : V(H') \to \{1, 2, 3, 4\}$ is defined by

Case 1: when $t \equiv 1 \pmod 4$

The labeling pattern of g_i and h_i are

$$f(g_i) = \begin{cases} 1, & 1 \le i \le \frac{t+3}{4} \\ 2, & \frac{t+7}{4} \le i \le \frac{t+1}{2} \\ 3, & \frac{t+3}{2} \le i \le \frac{3t+1}{4} \\ 4, & \frac{3t+5}{4} \le i \le t \end{cases}$$

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t-1}{4} \\ 2, & \frac{t+3}{4} \le i \le \frac{t-1}{2} \\ 3, & \frac{t+1}{2} \le i \le \frac{3t-3}{4} \\ 4, & \frac{3t+1}{4} \le i \le t-1 \end{cases}$$

$f(h_t) = 3.$

Case 2: when $t \equiv 2 \pmod 4$

The labeling pattern of g_i and h_i are

$$f(g_i) = \begin{cases} 1, & 1 \le i \le \frac{t+2}{4} \\ 2, & \frac{t+6}{4} \le i \le \frac{t}{2} \\ 3, & \frac{t+2}{2} \le i \le \frac{3t+2}{4} \\ 4, & \frac{3t+6}{4} \le i \le t \end{cases}$$

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t+2}{4} \\ 2, & \frac{t+6}{4} \le i \le \frac{t}{2} \\ 3, & \frac{t+2}{2} \le i \le \frac{3t-2}{4} \\ 4, & \frac{3t+2}{4} \le i \le t \end{cases}$$

Case 3: when $t \equiv 3 \pmod 4$

It is easy to verify for $t = 3$, when $t \ge 7$, The label of the points g_i are

$$f(g_i) = \begin{cases} 1, & 1 \le i \le \frac{t+1}{4} \\ 2, & \frac{t+5}{4} \le i \le \frac{t+1}{2} \\ 3, & \frac{t+3}{2} \le i \le \frac{3t+3}{4} \\ 4, & \frac{3t+7}{4} \le i \le t \end{cases}$$

The labeling pattern of h_i are the following two subcases,

Subcase (i): when $t = 7$

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t-1}{2} \\ 3, & i = \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le t-1 \end{cases}$$

$f(h_t) = 3$.

Subcase (ii): when $t > 7$

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t+5}{4} \\ 2, & \frac{t+9}{4} \le i \le \frac{t-1}{2} \\ 3, & \frac{t+1}{2} \le i \le \frac{3t-5}{4} \\ 4, & \frac{3t-1}{4} \le i \le t-1 \end{cases}$$

$f(h_t) = 3$.

Case 4: when $t \equiv 4 \pmod 4$

The label of the points g_i are

$$f(g_i) = \begin{cases} 1, & 1 \le i \le \frac{t}{4} \\ 2, & \frac{t+4}{4} \le i \le \frac{t}{2} \\ 3, & \frac{t+2}{2} \le i \le \frac{3t}{4} \\ 4, & \frac{3t+4}{4} \le i \le t \end{cases}$$

The labeling pattern of h_i are as follows

Subcase (i): when t = 4

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t}{4} \\ 3, & \frac{t+4}{4} \le i \le \frac{t}{2} \\ 4, & \frac{t+2}{2} \le i \le t-1 \end{cases}$$

$f(h_t) = 1.$

Subcase (ii): when t > 4

$$f(h_i) = \begin{cases} 1, & 1 \le i \le \frac{t}{4} \\ 2, & \frac{t+4}{4} \le i \le \frac{t-2}{2} \\ 3, & \frac{t}{2} \le i \le \frac{3t-4}{4} \\ 4, & \frac{3t}{4} \le i \le t-1 \end{cases}$$

$f(h_t) = 1.$

From the above four cases,(Table: 2)

Hence $H'$is a 4- total geometric mean cordial graph.

Theorem 2.3.

Duplicating all the points of the graph C_t, $t \ge 3$, admits 4- TGMC labeling.

Proof

Let $g_1, g_2, g_3, \ldots, g_t$ be the points of the cycle C_t and C_t' be the graph obtained by duplicating all the points $g_1, g_2, g_3, \ldots, g_t$ by a new lines $g_i'\ h_i'$ $(1 \le i \le t)$. Then $|V(C_t')| + |E(C_t')| = 7t$. The function f : $V(C_t') \to \{1, 2, 3, 4\}$ is defined by

Case 1: when t is odd

The label of the points g_i are

$$f(g_i) = \begin{cases} 3, & 1 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le t \end{cases}$$

The labeling pattern of g_i'*and* h_i' are the following two subcases,

subcase (i): when $t \equiv 1 \pmod 4$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t-3}{4} \\ 2, & \frac{3t+1}{4} \le i \le t \end{cases}$$

$$f(h_i') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 4, & \frac{t+3}{2} \le i \le \frac{3t+5}{4} \\ 3, & \frac{3t+9}{4} \le i \le t \end{cases}$$

Subcase (ii): when $t \equiv 3 \pmod 4$

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t-1}{4} \\ 2, & \frac{3t+3}{4} \le i \le t \end{cases}$$

$$f(h_i') = \begin{cases} 1, & 1 \le i \le \frac{t+1}{2} \\ 3, & \frac{t+3}{2} \le i \le \frac{3t-1}{4} \\ 4, & \frac{3t+3}{4} \le i \le t \end{cases}$$

Case 2: when t is even

The label of the points g_i are

$$f(g_i) = \begin{cases} 3, & 1 \le i \le \frac{t}{2} \\ 4, & \frac{t+2}{2} \le i \le t \end{cases}$$

The labeling pattern of g_i' *and* h_i' are as follows

Subcase (i): when t ≡ 2 (mod 4)

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t-2}{4} \\ 2, & \frac{3t+2}{4} \le i \le t \end{cases}$$

$$f(h_i') = \begin{cases} 1, & 1 \le i \le \frac{t}{2} \\ 4, & \frac{t+2}{2} \le i \le \frac{3t-2}{4} \\ 3, & \frac{3t+2}{4} \le i \le t \end{cases}$$

Subcase (ii): when t ≡ 4 (mod 4)

$$f(g_i') = \begin{cases} 1, & 1 \le i \le \frac{3t}{4} \\ 2, & \frac{3t+4}{4} \le i \le t \end{cases}$$

$$f(h_i') = \begin{cases} 1, & 1 \le i \le \frac{t}{2} \\ 3, & \frac{t+2}{2} \le i \le \frac{3t+4}{4} \\ 4, & \frac{3t+8}{4} \le i \le t \end{cases}$$

From the above two cases,(Table: 3)

Hence s$C_t'$is a 4- TGMC graph.

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Table: 1

Nature of t	$t_{gmf}(1)$	$t_{gmf}(2)$	$t_{gmf}(3)$	$t_{gmf}(4)$
t≡ 1 mod 4	$\frac{7t+1}{4}$	$\frac{7t-3}{4}$	$\frac{7t+1}{4}$	$\frac{7t-3}{4}$
t≡ 2 mod 4	$\frac{7t-2}{4}$	$\frac{7t-2}{4}$	$\frac{7t-2}{4}$	$\frac{7t+2}{4}$
t≡ 3 mod 4	$\frac{7t-1}{4}$	$\frac{7t-1}{4}$	$\frac{7t-1}{4}$	$\frac{7t-1}{4}$
t≡ 4 mod 4	$\frac{7t}{4}$	$\frac{7t}{4}$	$\frac{7t-4}{4}$	$\frac{7t}{4}$

Table: 2

Nature of t	$t_{gmf}(1)$	$t_{gmf}(2)$	$t_{gmf}(3)$	$t_{gmf}(4)$
t≡ 1 mod 4	$\frac{5t-1}{4}$	$\frac{5t+3}{4}$	$\frac{5t-1}{4}$	$\frac{5t-1}{4}$
t≡ 2 mod 4	$\frac{5t+2}{4}$	$\frac{5t-2}{4}$	$\frac{5t+2}{4}$	$\frac{5t-2}{4}$
t≡ 3 mod 4	$\frac{5t+1}{4}$	$\frac{5t+1}{4}$	$\frac{5t-3}{4}$	$\frac{5t+1}{4}$
t≡ 4 mod 4	$\frac{5t}{4}$	$\frac{5t}{4}$	$\frac{5t}{4}$	$\frac{5t}{4}$

Table: 3

Nature of t	$t_{gmf}(1)$	$t_{gmf}(2)$	$t_{gmf}(3)$	$t_{gmf}(4)$
t≡ 1 mod 4	$\frac{7t+1}{4}$	$\frac{7t-3}{4}$	$\frac{7t+1}{4}$	$\frac{7t+1}{4}$
t≡ 2 mod 4	$\frac{7t-2}{4}$	$\frac{7t-2}{4}$	$\frac{7t+2}{4}$	$\frac{7t+2}{4}$
t≡ 3 mod 4	$\frac{7t+3}{4}$	$\frac{7t-1}{4}$	$\frac{7t-1}{4}$	$\frac{7t-1}{4}$
t≡ 4 mod 4	$\frac{7t}{4}$	$\frac{7t}{4}$	$\frac{7t}{4}$	$\frac{7t}{4}$

CASE STUDY ARTICLE

Case Report: Surgical Management of a Periauricular Tag

Adithya J.V[1]*, Punith.C[2], Siddayya Aradhyamath[3] and Ganavi.D[4]

[1]Assistant Professor, Department of Shalya Tantra, JSS Ayurveda Medical College, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru), Mysuru, Karnataka, India.
[2]Third Year PG Scholar, Department of Shalya Tantra, JSS Ayurveda Medical College, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru), Mysuru, Karnataka, India.
[3]Head and Professor, Department of Shalya Tantra, JSS Ayurveda Medical College, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru), Mysuru, Karnataka, India.
[4]Second Year PG Scholar, Department of Shalya Tantra, JSS Ayurveda Medical College, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru), Mysuru, Karnataka, India.

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***Address for Correspondence**
Adithya J.V
Assistant Professor,
Department of Shalya Tantra,
JSS Ayurveda Medical College,
(Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru),
Mysuru, Karnataka, India.
E.Mail: ganavidevaraj8@gmail.com

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ABSTRACT

Periauricular tags, benign congenital anomalies located near the ear, are often asymptomatic but may present cosmetic concerns. This case report details the surgical management of a significant periauricular tag in a 7-year-old patient. The report highlights the diagnostic approach, surgical technique, and outcomes, emphasizing the effectiveness of surgical intervention in improving both cosmetic appearance and patient satisfaction.

Keywords: Periauricular Tag, Surgical Management, Cosmetic Surgery, Congenital Anomalies.

INTRODUCTION

Periauricular tags are congenital skin growths located near the anterior auricle, frequently identified in childhood. Though typically benign and asymptomatic, these tags can cause cosmetic concerns or discomfort. Surgical removal is a common management strategy for tags that impact appearance or function. This case report discusses the surgical approach for a periauricular tag, providing insights into effective management strategies.

CASE REPORT
Age:14 years
Gender: Male
Medical History: No significant past medical or surgical history. Developmental milestones were appropriate for age. No associated symptoms or syndromes.

Chief Complaint
The patient presented with a noticeable nodular swelling on the left side of the ear, which had been present since birth. The tag was approximately 2.0x2.0cm in size, raised, elliptical and skin-colored. The primary concern was cosmetic, as the tag affected the patient's self-esteem and social interactions.

Physical Examination
On examination, the periauricular tag was located anterior to the left ear, above the ear canal. The lesion was smooth, non-tender, mobile and pedunculated. There were no signs of inflammation, infection, or associated ear malformations. The rest of the ear examination was unremarkable, and no other congenital anomalies were observed.

Diagnostic Work-Up
Given the benign appearance of the tag, further diagnostic imaging was not deemed necessary. The diagnosis was based on clinical evaluation, with no signs of associated congenital syndromes or additional anomalies. Parental concerns about cosmetic appearance and potential psychological impact motivated the decision for surgical intervention.

Surgical Procedure
Pre operative measures
Informed Consent: Detailed discussion with parents about the procedure, risks, and benefits. Written consent obtained.
Anaesthesia: Local anaesthesia was administered using lidocaine to minimize bleeding and provide adequate pain control.
Injection tetanus was given IM stat
Positioning: The patient was positioned comfortably on the operating table, with the head slightly tilted to expose the left ear.
The area was painted with the antiseptic lotion
Operative measures
Incision: Aelliptical incision was made around the periauricular tag using a #15 blade, ensuring clear margins. At the base of the swelling.
Dissection: The tag was dissected from the surrounding tissue with a combination of scissors and blunt dissection. Care was taken to avoid damaging the underlying cartilage and ear structures.
Haemostasis: Bleeding was controlled with electric cautery.
Closure: The wound was closed in layers using 3.0 catgut sutures. A small, sterile dressing was applied.

Postoperative Care
Instructions: Parents were advised to keep the area clean and dry, and to apply an antibiotic ointment as directed.
Follow-Up: The patient was scheduled for follow-up in 1 week to assess wound healing and suture removal.

RESULTS

The patient recovered without complications. At the 1-week follow-up, the surgical site showed good healing with no signs of infection or wound dehiscence. Sutures were removed, and the cosmetic result was satisfactory to both the patient and her parents. The patient reported an improvement in self-esteem and social interactions.

DISCUSSION

Periauricular tags are common congenital anomalies that are generally benign. Surgical removal is indicated primarily for cosmetic reasons or if the tag causes discomfort. In this case, the decision to proceed with surgery was guided by the significant cosmetic impact on the patient and her reported psychological distress. The surgical approach used was effective in removing the tag with minimal complications. Local anaesthesia proved adequate for pain management, and postoperative care was straightforward. The positive outcome highlights the benefits of surgical intervention in improving patient quality of life and satisfaction.

CONCLUSION

Surgical management of periauricular tags is a safe and effective option for addressing cosmetic concerns. This case report demonstrates that with proper surgical technique and follow-up care, patients can achieve excellent cosmetic outcomes and improved self-esteem. Ongoing evaluation and individualized care plans are essential to ensuring optimal results and patient satisfaction.

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Fig. 1: Physical Examination

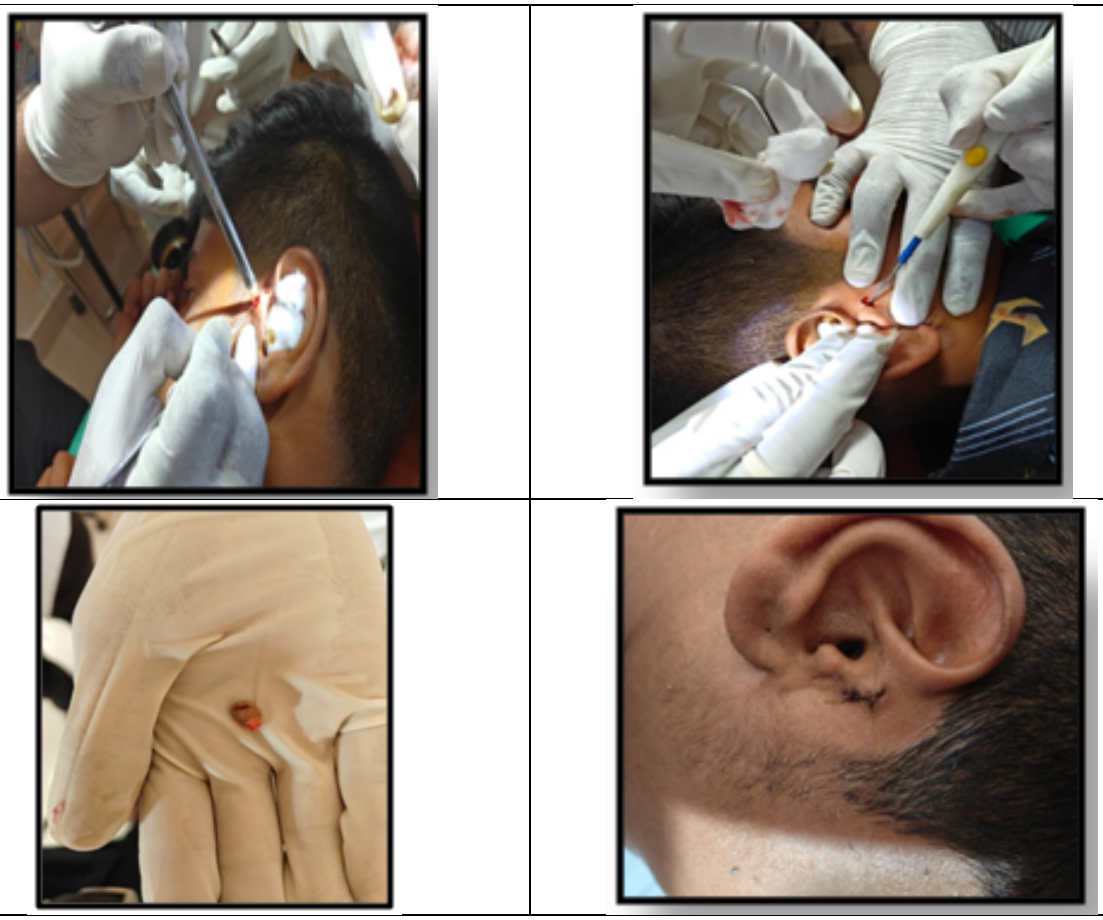


Fig. 2: Postoperative Care



Fig. 3: Result

RESEARCH ARTICLE

Lexi–AI

C. Daniel Nesa Kumar[1]*, S.S.Saravana Kumar[1], S.K.Komagal Yallini[2], R. Aruna[3], and P. Jayasree[4]

[1]Assistant Professor, Department of Computer Applications, Sri Ramakrishna College of Arts and Science, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Computer Science with Artificial Intelligence and Data Science, Sri Ramakrishna College of Arts and Science, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[3]Assistant Professor, Department of Artificial Intelligence and Machine Learning, KGISL Institute of Technology, Saravanampatti, Coimbatore, (Affiliated to Anna University, Chennai) Tamil Nadu, India.
[4]Assistant Professor, Department of Department of Computer Applications, Hindusthan College of Arts and Science, Udayampalayam, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
C. Daniel Nesa Kumar
Assistant Professor,
Department of Computer Applications,
Sri Ramakrishna College of Arts and Science,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: daniel@srcas.ac.in

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ABSTRACT

Lexi AI is a cutting-edge project designed to provide users with an efficient and secure platform for interacting with a locally hosted Large Language Model (LLM). The project leverages Gradio, a Python-based library, to create an intuitive and interactive user interface, enhancing usability while maintaining simplicity. By running entirely on local systems, Lexi AI ensures data privacy, eliminating the need for external servers or cloud-based processing. This project demonstrates the potential of integrating AI capabilities into standalone systems, offering a versatile tool for various applications such as learning support, content generation, and interactive problem-solving. The reliance on Gradio for the frontend ensures ease of use and accessibility, while the absence of a complex backend simplifies deployment and reduces overhead. Lexi AI is further enhanced by integrating Ollama deployment, which provides a seamless and efficient method for managing and running the locally hosted LLM. This framework ensures smooth performance and accessibility, making it easier for users to interact with the AI model without technical barriers. With its focus on privacy, efficiency, and accessibility, Lexi AI empowers users by bringing advanced natural language processing capabilities directly to their systems. Whether for

education, research, or creative writing, this solution represents a step forward in AI-powered solutions for individuals and businesses alike.

Keywords: Large Language Model (LLM), Gradio Interface, Ollama Deployment, Data Privacy, AI-Powered Solutions

INTRODUCTION

Artificial Intelligence (AI) has significantly transformed human-computer interactions, making systems more intelligent and responsive. With the increasing demand for AI-driven applications, local deployment of language models has gained prominence due to its ability to function independently without relying on cloud-based services. Lexi AI is an AI-based chatbot designed to answer user queries efficiently while ensuring data privacy and fast response times. Built using Python and Gradio, this system leverages an LLM (Large Language Model) deployed locally through Ollama, ensuring seamless query processing without dependence on external APIs. Unlike traditional AI chatbots that require internet connectivity, Lexi AI runs entirely on local hardware, making it ideal for restricted or offline environments.

Key Features of LEXI AI
Local Deployment: Functions without internet access, ensuring independence from cloud services.
Data Confidentiality: User queries and responses remain stored locally, preventing unauthorized access.
Fast and Efficient Processing: Optimized for quick, contextually relevant answers with minimal computational overhead.
User-Friendly Interface: Powered by Gradio for an intuitive and interactive chatbot experience.

Development Process
System Specification and Design: Selection of appropriate hardware and software configurations.
Development and Implementation: Building the chatbot with Python and integrating Gradio for user interaction.
Testing and Deployment: Ensuring optimal performance through robust testing and evaluation strategies.

Key Focus Areas
Privacy and Security: Prevents external data breaches by storing all interactions locally.
Optimized Performance: Ensures low-latency response times and smooth operation.
Accessibility: Designed for both technical and non-technical users.
Lexi AI stands out as a reliable AI chatbot, offering secure, efficient, and intelligent AI-driven interactions in offline and privacy-sensitive applications.

LITERATURE REVIEW

Cloud - Based AI Chatbots

- Many AI chatbots, such as Open AI's ChatGPT (https://openai.com/research/gpt-4) and Google's Bard (https://ai.google/discover/bard), rely on cloud-based processing, offering powerful computational resources and large-scale language modeling.
- These systems utilize transformer-based architectures to generate human-like responses, making them effective for various applications (https://papers.nips.cc/ paper_files/paper/ 2017/file/3f5ee243547dee91fbd053c1c4a845aa-Paper.pdf).
- However, cloud dependency introduces challenges such as data privacy risks, latency issues, and the need for an active internet connection (https://ieeexplore.ieee.org/document/9349192).

- Additionally, concerns about external data processing and storage limit the adoption of cloud-based models in sensitive domains (https://link.springer.com/article/10.1007/s43681-022-00123-4).

Locally Hosted AI Systems

- Locally hosted AI chatbots have emerged as a privacy-preserving alternative to cloud-based models.
- Solutions like Meta's LLaMA (https://ai.facebook.com/blog/large-language- model-llama-meta-ai/) and Stanford's Alpaca (https://crfm.stanford.edu/2023/03/13/ alpaca.html) allow users to deploy large language models on personal hardware, eliminating reliance on external servers.
- These models leverage fine-tuned transformer architectures optimized for on-device execution (https://www.springer.com/ journal/12345).
- However, despite improved privacy and offline functionality, locally hosted systems require substantial computational resources, making them less accessible to users with limited hardware capabilities (https://dl.acm.org/doi/10.1145/3468626).

Hybrid AI Deployment

- Hybrid AI frameworks integrate both local and cloud-based processing to balance performance and accessibility.
- Platforms like Rasa (https://rasa.com/docs/rasa/) and DeepPavlov (http://docs.deeppavlov.ai/en/master/) offer hybrid deployment models that allow chatbots to operate offline while synchronizing data with online services when needed.
- This approach enhances flexibility but also introduces challenges in maintaining consistency between local and cloud models, as real-time synchronization can affect system responsiveness (https://dl.acm.org/doi/10.1145/3490485)
- Additionally, security risks persist when transitioning between local and online environments, requiring robust encryption and access control measures.

User Interface and Interaction

- The usability of AI chatbots is influenced by their user interfaces.
- Frameworks like Gradio (https://www.gradio.app/) and Streamlit (https://docs.streamlit.io/) have simplified chatbot interactions through intuitive, web-based UI solutions, making AI models more accessible.
- Studies indicate that a well-structured chatbot interface enhances user engagement and response interpretability (https://www.tandfonline.com/doi/full/10.1080/).
- However, many existing UI frameworks prioritize cloud-based deployment, leading to challenges in optimizing them for low-latency, locally hosted AI applications (https://papers.nips.cc/paper_files/paper/2019/file/b1f62f3d0d4d8afc3e5b3d31394f727d- Paper.pdf).

Existing System Challenges

Traditional chatbot systems or AI assistants often rely on cloud-based models and third-party APIs for query resolution. These systems typically involve

Internet dependency: Most AI-based systems require a stable internet connection, making them inaccessible in offline environments.

Data privacy concerns: User queries and responses are sent to external servers, raising concerns over data security and confidentiality.

Latency issues: Since queries must be processed over the internet, users may experience delays, especially during high traffic periods.

Limited customization: Many existing systems are pre-trained with fixed models and offer limited flexibility for local fine-tuning and optimization.

Cost implications: Many cloud-based AI models require paid subscriptions or API usage fees, increasing operational costs for users and organizations.

Draw Backs

High Response Time: Queries take longer to process due to local communication, affecting real-time interactions.
Limited Local Control: Users cannot modify or enhance the model easily, as cloud-based solutions restrict local model updates and optimizations.
Storing of Previous Data: Due to privacy reasons, there are no configurations for storing user queries.

Proposed System

To overcome the limitations of existing systems, Lexi AI introduces a local AI model deployment approach that provides a fast, secure, and cost-effective solution. The system is designed with
Offline functionality: The AI model is deployed locally using Ollama, eliminating the need for an internet connection.
Enhanced security: Since all query processing happens within the user's device, there are no external data transfers, ensuring complete privacy.
Lower latency: Queries are processed instantly, reducing response time significantly.
User-friendly Interface: Built with Gradio, Lexi AI provides an intuitive UI for easy interaction.
Scalability and Flexibility: The system allows modifications, local fine-tuning, and updates without external dependencies.
Cost efficiency: No recurring API fees, making it a one-time deployment with no hidden costs.

Features

The key features of Lexi AI include
Local LLM Deployment: Eliminates reliance on cloud-based AI models, ensuring faster and secure query handling.
Modular Architecture: Comprises User Interface, Application Logic, LLM Processing.
Real-time Query Processing: Provides instant responses with minimal processing time.
Multi-Platform Compatibility: Can run on Windows, Linux, and MacOS, requiring only basic system resources.
User-Friendly Interface: Designed with Gradio, offering an accessible and visually appealing chat environment.
Secure and Private: All interactions remain local to the system, eliminating external data exposure.
Lightweight and Efficient: Optimized to run on moderate hardware without requiring high-end computational power.

METHODOLOGY

The system design of Lexi AI ensures smooth interaction between its components to deliver an efficient and responsive experience.
User Interface (Gradio Frontend): Serves as the entry point, allowing users to input queries and receive responses in an intuitive, accessible interface.
Application Logic (Python Scripts): Bridges the frontend and LLM, handling user input, processing requests, formatting responses, and managing errors.
LLM Deployment (Ollama): Manages model loading, inference, and resource optimization for seamless query processing.
Local Storage: Stores model weights, configurations, and logs, enabling offline operation, quick model loading, and enhanced privacy.

Input Design

Lexi AI's input design ensures a seamless and intuitive user experience while maintaining flexibility.
User-Friendly Input Field: Simple text box for easy query input.
Accessibility Features: Support for screen readers, high-contrast text, and keyboard navigation.
Error Feedback: Clear error messages for incorrect inputs.
Offline Inputs: Direct local processing with minimal latency.

Online Inputs (via Gradio Links): Supports remote access while maintaining usability.

Output Design

The output design ensures clear, structured, and real-time AI-generated responses through the Gradio-based UI.
Clear and Readable Responses: Well-structured text with proper formatting.
Real-Time Response Generation: Immediate output display with a loading indicator.
Response Enhancement Features: Options to refine or re-generate responses, with syntax highlighting for code queries.

RESULTS AND DISCUSSIONS

System Development

System development is the process of designing, building, testing, and deploying the Lexi AI system to ensure it meets functional and performance requirements. The development phase follows a structured approach, integrating various components, including the user interface, backend processing, database management, and local AI model deployment. Lexi AI was developed using an incremental and modular approach, ensuring that each module was designed, tested, and integrated systematically. The system follows a local AI-driven architecture, using Gradio for UI, Python for backend logic, and Ollama for LLM processing.

The development focused on

- Creating a seamless user interaction experience.
- Ensuring low latency in AI response generation by running locally.
- Each module of the system (UI, Query Processing, AI Model) was developed independently to ensure flexibility and maintainability.
- The system was deployed locally, ensuring data privacy and fast execution. Continuous monitoring and maintenance plans were implemented to update the model and optimize system performance based on user feedback.

Description of Modules

Lexi AI is designed as a modular system where each module plays a crucial role in ensuring smooth functionality. The system is divided into the following key modules

User Interface Module

- Built using Gradio, this module allows users to input queries and receive responses.
- Provides a text input field, response display area, and history tracking for user queries.
- Ensures a user-friendly and interactive experience with accessibility features.

Query Processing Module

- Handles user queries and preprocesses input before sending it to the AI model.
- Implements text validation to ensure meaningful queries are processed.
- Supports query reformatting, such as removing redundant spaces or fixing typos.

AI Model Processing Module

- Uses Ollama for local LLM deployment to process user queries.
- Ensures fast response generation without external API dependencies.
- Supports context retention for better conversational accuracy.

Response Generation Module

- Processes output from the AI model and formats it for display.

- Allows re-generation of responses if the user is not satisfied.

Testing and Implementation

Ensuring the reliability, accuracy, and efficiency of Lexi AI required a structured testing strategy and a well-defined implementation process. Various testing methodologies were applied to validate system performance, security, and user experience. The implementation was executed in a phased manner to allow smooth integration of components while optimizing for real-world usability.

Testing Strategy

Testing was conducted at multiple stages of development to identify and resolve potential issues before deployment. The following testing methodologies were applied

Unit Testing

- Each module was tested in isolation to ensure it functioned correctly.
- The primary focus was on verifying core components such as user input handling,
- AI model response generation, and database query logging.
- Unit tests were written in Python to validate functions and classes individually.

Integration Testing

- After unit testing, integration testing was performed to ensure that different system components worked together seamlessly.
- The primary objective was to verify smooth interaction between the Gradio-based UI, AI processing engine and local LLM deployment.

Performance Testing

- Performance testing was conducted to evaluate the system's response time and efficiency under different loads.
- The system was tested with varying numbers of concurrent users to ensure it could handle multiple requests without significant delays.
- Metrics such as response time, memory usage and processing speed were analyzed

Implementation

The implementation of Lexi AI followed a phased development approach, ensuring smooth progression from initial requirements to final deployment. Each phase was carefully planned and executed to minimize errors and optimize system performance.

Phase 1: Requirement Analysis

- The project began with a detailed analysis of user needs, functional requirements, and technical specifications.
- Key objectives were defined, such as ensuring fast query response times, secure local execution, and an intuitive user interface.

Phase 2: System Design

- A comprehensive system architecture was developed, including data flow diagrams, UML diagrams and UI wireframes.
- This phase established a clear blueprint for development, ensuring all system components were designed to work efficiently together.

Phase 3: Module Development

The system was developed in modular units, allowing independent testing and optimization. Key modules included:

- **User Interface Module:** Developed using Gradio for seamless text-based query submission.

- **Query Processing Module:** Handled user input validation.

Phase 4: Integration and System Testing

- Once individual modules were successfully developed, they were integrated and tested as a complete system.
- Various test cases were executed to ensure that queries were processed accurately and that responses were generated efficiently.

Phase 5: Deployment and Optimization

- Lexi AI was deployed locally, ensuring fast execution, data security, and reliable performance.

CONCLUSION

Lexi AI represents a significant advancement in AI-powered chatbot systems, designed to provide seamless, efficient, and intelligent query resolution. The project was conceptualized to bridge the gap between user queries and AI-driven responses, ensuring data security, local processing, and enhanced performance. Unlike traditional cloud-based chatbot systems, Lexi AI operates entirely on a local machine using Ollama, eliminating dependency on external APIs and reducing response latency. The integration of Python, Gradio, and a locally deployed LLM allowed the system to function efficiently while ensuring high accuracy and responsiveness. Throughout the development lifecycle, a structured approach was followed, including requirement analysis, system design, module development, rigorous testing, and phased implementation. The modular architecture of Lexi AI enabled seamless integration and systematic testing, ensuring that each component functioned efficiently and independently. The development process incorporated rigorous testing methodologies, including unit testing, integration testing, performance evaluation, and user acceptance testing, which were essential in refining the system for optimal performance and reliability before deployment.

Through comprehensive testing and iterative improvements, Lexi AI was enhanced to deliver highly accurate, context-aware responses while maintaining fast processing speeds and secure query handling. The final product is a robust, privacy-focused, and highly efficient AI chatbot, capable of providing intelligent and precise query resolution while ensuring data security and user confidentiality. Its scalability, adaptability, and offline functionality make it a powerful AI assistant for a wide range of applications, from enterprise solutions to research and personal productivity tools.

Key Achievements

The completion of this project resulted in several key achievements that highlight the effectiveness and efficiency of the system

- **Efficient Query Processing:** The chatbot can handle diverse user queries with high accuracy using an LLM trained for intelligent response generation.
- **Local AI Deployment:** By leveraging Ollama, the model runs entirely on a local machine, ensuring faster response times and enhanced data security.
- **Seamless User Experience:** The Gradio-based user interface makes the chatbot accessible, intuitive, and easy to use for non-technical users.
- **Comprehensive Logging System:** All user interactions, including queries and responses, are stored in a structured database, allowing for query tracking, analysis, and future improvements.

Future Scope and Enhancements

Lexi AI has significant potential for future improvements to enhance functionality, adaptability, and user experience.

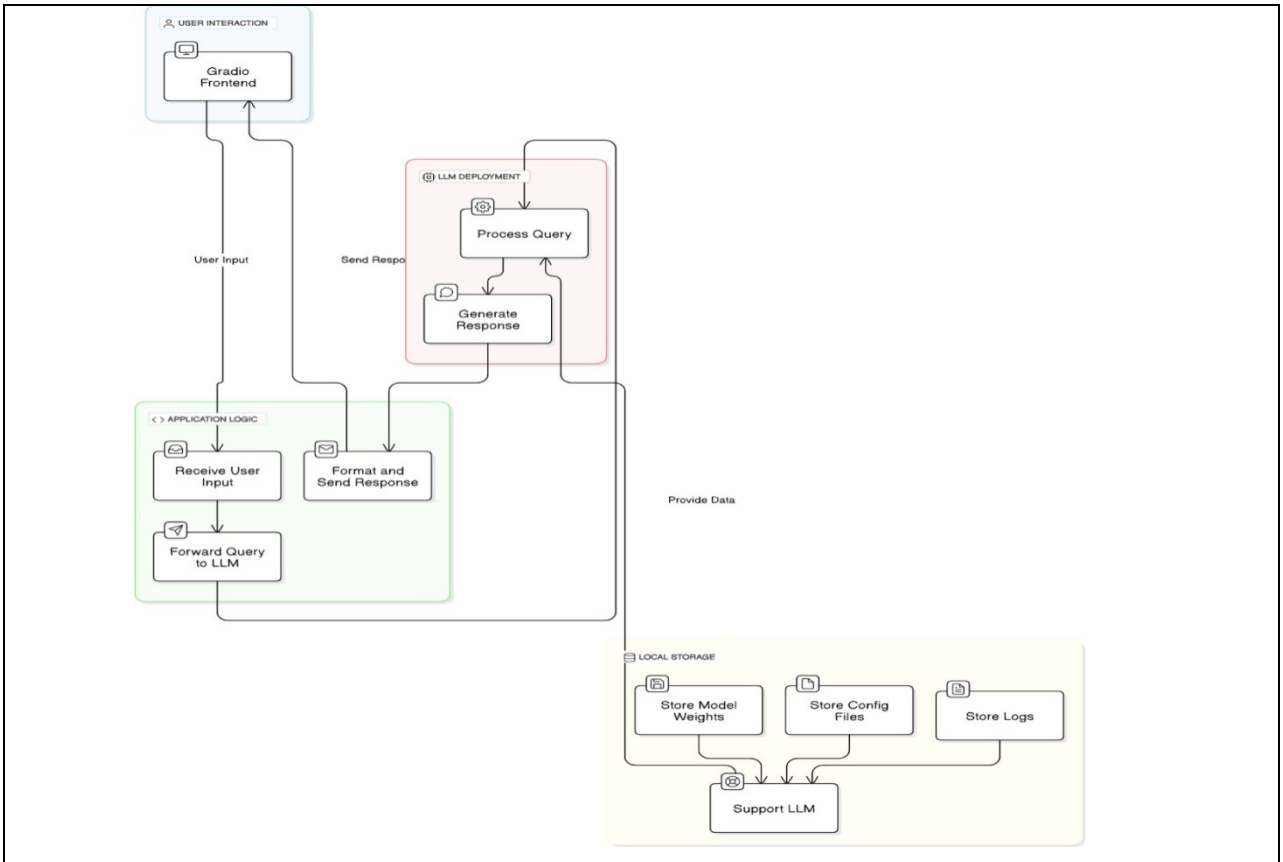
- **Multimodal Capabilities:** Enable voice and image-based queries for greater accessibility.
- **Cloud-Optional Deployment:** Offer users a choice between local and cloud-based models.
- **Continuous Learning:** Implement self-learning to improve responses over time.

- **Contextual Memory Retention:** Enhance conversation continuity with context-aware responses.
- **External API Integration:** Optional support for accessing external knowledge bases.
- **Plugin-Based Extensibility:** Allow modular API plugins for industry-specific customization.
- **Industry-Specific API Support:** Integrate domain-specific databases (e.g., medical, financial, legal).
- **Multilingual Support:** Expand to multiple languages with real-time detection for global accessibility.

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Fg. 1. Data Flow Diagram

RESEARCH ARTICLE

Vector Basis {(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}- Cordial Labeling of Some Snake Graphs

R. Ponraj[1]* and R. Jeya[2]

[1]Associate Professor and Head, Department of Mathematics, Sri Paramakalyani College, Alwarkurichi, (Affiliated to Manonmaniam Sundaranar University, Abishekapatti), Tirunelveli, India.
[2]Research Scholar (Reg. No. 22222102092010), Department of Mathematics, Sri Paramakalyani College, Alwarkurichi, (Affiliated to Manonmaniam Sundaranar University, Abishekapatti), Tirunelveli, India.

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***Address for Correspondence**
R. Ponraj
Associate Professor and Head,
Department of Mathematics,
Sri Paramakalyani College, Alwarkurichi,
(Affiliated to Manonmaniam Sundaranar University, Abishekapatti),
Tirunelveli, India.
E.Mail: ponrajmaths@gmail.com

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ABSTRACT

Consider a graph $G = (p, q)$. Let V be an inner product space with a basis T. Denote the inner product of two vectors a and $b$by $< a, b >$. Let $\psi: V(G) \to T$ be a function. For edge uv, assign the label $< \psi(u), \psi(v) >$. We say that ψ is a vector basis T-cordial labeling (VB T-cordial labeling) if $\left|\psi_x - \psi_y\right| \leq 1$ and $\left|\beta_i - \beta_j\right| \leq 1$ where ψ_a denotes the number of vertices labeled with the vector a and β_i denotes the number of edges labeled with the scalar i. A graph with a vector basis T-cordial labeling is called a vector basis T-cordial graph (VB T-cordial graph).In this present paper, we prove that$A(DT_n) \odot mK_1$ is a VB {(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}-cordial.

Keywords: Triangular Snake, Double Triangular Snake and Double Alternate Triangular Snake.
2020 AMS Subject Classification: 05C78

INTRODUCTION

We begin with undirected, finite and simple graph. Graph labeling was introduced in [18]. We refer to a dynamic survey of graph labeling by Gallian [6]. Cordial labeling was introduced in [3].Various cordial related labeling of some snake graphs were discussed in [1,2,7-9,13,15-17,19].We follow [4,5] for terms and notations. We have examined the VB{(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}-cordial labeling of some special graphs in [10-12].In this present paper, we

prove that the graphs $A(DT_n) \odot mK_1$ is a VBT-cordial graph where T={(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}.Figure (1) provides an example of a VB{(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}-cordial graph.

2. Main Results

Denote the vectors (0,0,0,1), (0,0,1,1), (0,1,1,1) and (1,1,1,1) respectively by c_1, c_2, $c_3$andc_4. That is c_1= (0, 0, 0, 1), c_2= (0, 0, 1, 1), c_3= (0, 1, 1, 1) and c_4= (1, 1, 1, 1).

Theorem 2.1. The graph $A(DT_n) \odot mK_1$ is a vector basis $\{c_1,c_2,c_3,c_4\}$-cordial for$m \geq 1$ and even $n \geq 4$.

Proof.

Let$V(A(DT_n) \odot mK_1) = \{u_i, u_{i,j} | 1 \leq i \leq n \; and \; 1 \leq j \leq m\} \cup \{v_i, w_i, v_{i,j}, w_{i,j} | 1 \leq i \leq \frac{n}{2} \; and \; 1 \leq j \leq m\}$ and $E(A(DT_n) \odot mK_1) = \{u_i u_{i,j} | 1 \leq i \leq n\} \cup \{u_i u_{i+1} | 1 \leq i \leq n and \; 1 \leq j \leq m\} \cup \{u_{2i-1}v_i, v_i u_{2i}, u_{2i-1}w_i, \; w_i u_{2i}, v_i v_{i,j}, w_i w_{i,j} | 1 \leq i \leq \frac{n}{2} \; and \; 1 \leq j \leq m\}$. Then $p = 2n(m+1)$and$q = n(2m+3) - 1$. Put the vector to the vertices in the following order $u_1, u_2, v_1, w_1, u_3, u_4, \; v_2, w_2, u_5, u_6, \ldots, u_{n-1}, u_n, v_{\frac{n}{2}}, w_{\frac{n}{2}}$.

Case *A*: $n \equiv 0 \; (mod \; 4)$

Let $n = 4k_1, k_1 > 0$. Assign c_4 to the first $k_1$cycles and c_3 to the next $k_1$cycles. Putc_2 to the next $k_1$cyclesandc_1 to the remaining $k_1$cycles. If k_1 is even, assign $2k_1$vertices for c_4, c_3, c_2 and c_1. If k_1 is odd, assign $2k_1 + 1$ vertices for c_4 and $c_2$and put $2k_1 - 1$ vertices for $c_3$and c_1. Put the vectors to the pendent vertices in the following order $u_{1,1}, u_{1,2}, \ldots, u_{1,m}, u_{2,1}, u_{2,2}, \ldots, u_{2,m}, v_{1,1}, v_{1,2}, \ldots, v_{1,m}, w_{1,1}, w_{1,2}, \ldots, w_{1,m}, u_{3,1}, u_{3,2}, \ldots, u_{3,n}, u_{4,1}$, $u_{4,2}, \ldots, u_{4,n}, v_{2,1}, v_{2,2}, \ldots, v_{2,m}, w_{2,1}, w_{2,2}, \ldots, w_{2,m}, \ldots, u_{n-1,1}, u_{n-1,2}, \ldots, u_{n-1,m}, u_{n,1}, u_{n,2}, \ldots, u_{n,m}$, $v_{\frac{n}{2},1}, v_{\frac{n}{2},2}, \ldots, v_{\frac{n}{2},m}, w_{\frac{n}{2},1}, w_{\frac{n}{2},2}, \ldots, w_{\frac{n}{2},m}$.

Sub case *a*: $m \equiv 0 (mod \; 4)$

Take$m = 4k_2, k_2 > 0$.$p = 4(8k_1k_2 + 2k_1)$. When k_1 is even. Assign c_4 to the first $8k_1k_2$vertices and c_3 to the next $8k_1k_2$ vertices. Putc_2 to the next $8k_1k_2$ vertices and c_1 to the last$8k_1k_2$ vertices. When k_1 is odd. Assign c_4 to the first $8k_1k_2 - 1$vertices and c_3 to the next $8k_1k_2 + 1$ vertices. Putc_2 to the next $8k_1k_2 - 1$ vertices and c_1 to the remaining $8k_1k_2 + 1$ vertices.

Sub case *b*: $m \equiv 1 (mod \; 4)$

Put$m = 4k_2 + 1, k_2 \geq 0$.$p = 4(8k_1k_2 + 4k_1)$. When k_1 is even. Assign c_4 to the first $8k_1k_2 + 2k_1$vertices and c_3 to the next $8k_1k_2 + 2k_1$ vertices. Putc_2 to the next $8k_1k_2 + 2k_1$ vertices and c_1 to the remaining $8k_1k_2 + 2k_1$ vertices. When k_1 is odd. Assign c_4 to the first $8k_1k_2 + 2k_1 - 1$vertices and c_3 to the next$8k_1k_2 + 2k_1 + 1$ vertices. putc_2 to the next $8k_1k_2 + 2k_1 - 1$ vertices and c_1 to the last$8k_1k_2 + 2k_1 + 1$ vertices.

Sub case *c*: $m \equiv 2 (mod \; 4)$

Take$m = 4k_2 + 2, k_2 \geq 0$.$p = 4(8k_1k_2 + 6k_1)$. When k_1 is even. Assign c_4 to the first $8k_1k_2 + 4k_1$vertices and c_3 to the next $8k_1k_2 + 4k_1$ vertices. Putc_2 to the next $8k_1k_2 + 4k_1$ vertices and c_1 to the remaining $8k_1k_2 + 4k_1$ vertices. When k_1 is odd. Assign c_4 to the first $8k_1k_2 + 4k_1 - 1$vertices and c_3 to the next$8k_1k_2 + 4k_1 + 1$ vertices. Putc_2 to the next $8k_1k_2 + 4k_1 - 1$ vertices and c_1 to the last$8k_1k_2 + 4k_1 + 1$ vertices.

Sub case *d*: $m \equiv 3 (mod \; 4)$

Put$m = 4k_2 + 3, k_2 \geq 0$.$p = 4(8k_1k_2 + 8k_1)$. When k_1 is even. Assign c_4 to the first $8k_1k_2 + 6k_1$vertices and c_3 to the next $8k_1k_2 + 6k_1$ vertices. Putc_2 to the next $8k_1k_2 + 6k_1$ vertices and c_1 to the remaining $8k_1k_2 + 6k_1$ vertices. When k_1 is odd. Assign c_4 to the first $8k_1k_2 + 6k_1 - 1$vertices and c_3 to the next$8k_1k_2 + 6k_1 + 1$ vertices. Putc_2 to the next $8k_1k_2 + 6k_1 - 1$ vertices and c_1 to the last$8k_1k_2 + 6k_1 + 1$ vertices.

Case *B*: $n \equiv 2(mod\ 4)$

Put $n = 4k_1 + 2, k_1 > 0$. Assign c_4 to the first $k_1 + 1$cycles and c_3 to the next $k_1 + 1$cycles. Also, assign c_2 to the next $k_1$cyclesand c_1 to the last$k_1$cycles. If k_1 is even, assign c_4 to the first $2k_1 + 3$ vertices and c_3 to the next$2k_1 + 1$ vertices. Putc_2 to the next$2k_1$ vertices and c_1 to the remaining $2k_1$ vertices. If k_1 is odd, assign c_4 to the first $2k_1 + 2$ vertices and c_3 to the next $2k_1 + 2$ vertices. Putc_2 to the next$2k_1 + 3$ vertices and c_1 to the last$2k_1 + 1$ vertices.

Sub case *a*: $m \equiv 0(mod\ 4)$

Take$m = 4k_2, k_2 > 0. p = 4(8k_1k_2 + 4k_2 + 2k_1 + 1)$. When k_1 is even. Putc_4 to the first $8k_1k_2 + 4k_2 - 2$vertices and c_3 to the next $8k_1k_2 + 4k_2$ vertices. Assign c_2 to the next $8k_1k_2 + 4k_2 + 1$ vertices and c_1 to the last$8k_1k_2 + 4k_2 + 1$ vertices. When k_1 is odd. Put$c_4$to the first $8k_1k_2 + 4k_2 - 1$vertices and c_3 to the next $8k_1k_2 + 4k_2 - 1$vertices. Assign c_2 to the next $8k_1k_2 + 4k_2$vertices and c_1 to the last$8k_1k_2 + 4k_2 + 2$vertices.

Sub case *b*: $m \equiv 1(mod\ 4)$

Put$m = 4k_2 + 1, k_2 \geq 0. p = 4(8k_1k_2 + 4k_1 + 4k_2 + 2)$. When k_1 is even. Assignc_4 to the first $8k_1k_2 + 2k_1 + 4k_2 - 1$vertices and c_3 to the next $8k_1k_2 + 2k_1 + 4k_2 + 1$ vertices. Putc_2 to the next $8k_1k_2 + 2k_1 + 4k_2 + 2$ vertices and c_1 to the last $8k_1k_2 + 2k_1 + 4k_2 + 2$ vertices. When k_1 is odd. Putc_4 to the first $8k_1k_2 + 2k_1 + 4k_2$vertices and c_3 to the next$8k_1k_2 + 2k_1 + 4k_2$ vertices. Assign c_2 to the next $8k_1k_2 + 2k_1 + 4k_2 + 1$ vertices and c_1 to the last$8k_1k_2 + 2k_1 + 4k_2 + 3$ vertices.

Sub case *c*: $m \equiv 2(mod\ 4)$

Take$m = 4k_2 + 2, k_2 \geq 0. p = 4(8k_1k_2 + 6k_1 + 4k_2 + 3)$. When k_1 is even. Putc_4 to the first $8k_1k_2 + 4k_1 + 4k_2$vertices and c_3 to the next $8k_1k_2 + 4k_1 + 4k_2 + 2$vertices. Assign c_2 to the next $8k_1k_2 + 4k_1 + 4k_2 + 3$ vertices and c_1 to the last$8k_1k_2 + 4k_1 + 4k_2 + 3$ vertices. When k_1 is odd. Putc_4 to the first $8k_1k_2 + 4k_1 + 4k_2 + 1$vertices and c_3 to the next$8k_1k_2 + 4k_1 + 4k_2 + 1$vertices. Assign c_2 to the next $8k_1k_2 + 4k_1 + 4k_2 + 2$vertices and c_1 to the remaining $8t_1t_2 + 4t_1 + 4t_2 + 4$vertices.

Sub case *d*: $m \equiv 3(mod\ 4)$

Put$m = 4k_2 + 3, k_2 \geq 0. p = 4(8k_1k_2 + 8k_1 + 4k_2 + 4)$. When k_1 is even. Assign c_4 to the first $8k_1k_2 + 6k_1 + 4k_2 + 1$vertices and c_3 to the next $8k_1k_2 + 6k_1 + 4k_2 + 3$ vertices. Putc_2 to the next $8k_1k_2 + 6k_1 + 4k_2 + 4$vertices and c_1 to the last$8k_1k_2 + 6k_1 + 4k_2 + 4$vertices. When k_1 is odd. Assign c_4 to the first $8k_1k_2 + 6k_1 + 4k_2 + 2$vertices and c_3 to the next$8k_1k_2 + 6k_1 + 4k_2 + 2$vertices. Put$c_2$ to the next $8k_1k_2 + 6k_1 + 4k_2 + 3$vertices and c_1 to the last$8k_1k_2 + 6k_1 + 4k_2 + 5$ vertices.■

Example 2.2. Figure (2) provides an example of a VB $\{c_4, c_3, c_2, c_1\}$-cordial labeling of $A(DT_4) \odot 2K_1$.

Theorem 2.3. The graph $A(DT_n) \odot mK_1$ is a VB$\{c_4, c_3, c_2, c_1\}$-cordial for all $m \geq 1$ and odd$n \geq 3$.

Proof.

Let$A\ (DT_n) \odot mK_1$,n is odd

by$V\ (A(DT_n) \odot mK_1) = \{u_i | 1 \leq i \leq n\} \cup \{x, v_i, w_i, v_{i,j}, w_{i,j}, x_j | 1 \leq i \leq \frac{n-1}{2}\ and\ 1 \leq j \leq m\}andE(A(DT_n) \odot mK_1) = \{u_iu_{i+1} | 1 - 1\} \cup \{u_{2i-1}v_i, v_iu_{2i}, u_{2i-1}w_i,\ w_iu_{2i}, u_iu_{i,j}, v_iv_{i,j}, w_iw_{i,j}, xx_j | 1 \leq i \leq \frac{n-1}{2}\ and\ 1 \leq j \leq m\}$.Then$p = (2n + 1)(m + 1)andq = (2n + 1)(m + 1) + n - 1$. Assign the vectors to the vertices as in the case to $A(DT_{n-1}) \odot mK_1$ where $n - 1$ is even. Put the vectors to $x, x_1, x_2, \dots, x_m$. Put$c_4$to x.

Case ***A***: $m \equiv 0(mod\ 4)$

Let $m = 4k_2, k_2 \geq 0$.Assignc_4 to $x_1, x_2, \dots, x_{k_2}$and $c_3$to $x_{k_2+1}, x_{k_2+2}, \dots, x_{2k_2}$. Put$c_2$ to $x_{2k_2+1}, x_{2k_2+2}, \dots, x_{3k_2}$and $c_1$to $x_{3k_2+1}, x_{3k_2+2}, \dots, x_{4k_2}$.

Case *B*: $m \equiv 1(mod\ 4)$
Put$m = 4k_2 + 1, k_2 \geq 0$.Assign $c_4$to $x_1, x_2, \ldots, x_{k_2}$and $c_3$to $x_{k_2+1}, x_{k_2+2}, \ldots, x_{2k_2}$. Put$c_2$ to $x_{2k_2+1}, x_{2k_2+2}, \ldots, x_{3k_2}$and $c_1$to $x_{3k_2+1}, x_{3k_2+2}, \ldots, x_{4k_2+1}$.

Case *C*: $m \equiv 2(mod\ 4)$
Take$m = 4k_2 + 2, k_2 \geq 0$.Assign c_4 to $x_1, x_2, \ldots, x_{k_2}$and $c_3$to $x_{k_2+1}, x_{k_2+2}, \ldots, x_{2k_2}$. Put$c_2$ to $x_{2k_2+1}, x_{2k_2+2}, \ldots, x_{3k_2+1}$and $c_1$to $x_{3k_2+2}, x_{3k_2+3}, \ldots, x_{4k_2+2}$.

Case *D*: $m \equiv 2(mod\ 4)$
Put $m = 4k_2 + 3, k_2 \geq 0$.Assign c_4 to $x_1, x_2, \ldots, x_{k_2}$and $c_3$to $x_{k_2+1}, x_{k_2+2}, \ldots, x_{2k_2+1}$. Put$c_2$ to $x_{2k_2+2}, x_{2k_2+3}, \ldots, x_{3k_2+2}$and $c_1$to $x_{3k_2+3}, x_{3k_2+4}, \ldots, x_{4k_2+3}$.■

Example 2.4. Figure (3) provides an example of a VB{c_4, c_3,c_2, c_1}-cordial labeling of $A(DT_5) \odot 2K_1$.

5. Conclusion
In this present paper, we have proved that$A(DT_n) \odot mK_1$ is a VB{c_4,c_3,c_2,c_1}-cordial. The existence and non-existence of a VB{c_4,c_3,c_2,c_1}-cordial labeling of corona operation of more families of graphs are the open problems for future research work.

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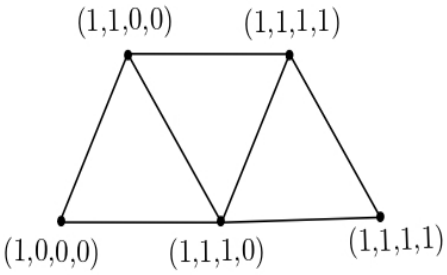


Figure. 1: VB{(1,1,1,1),(1,1,1,0),(1,1,0,0),(1,0,0,0)}-cordial graph

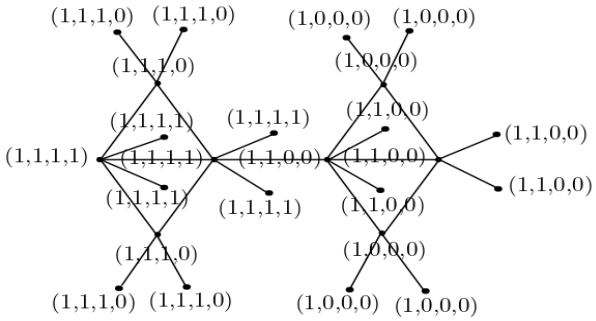


Figure 2. A VB{c_4, c_3,c_2, c_1}-cordial labeling of $A(DT_4) \odot 2K_1$

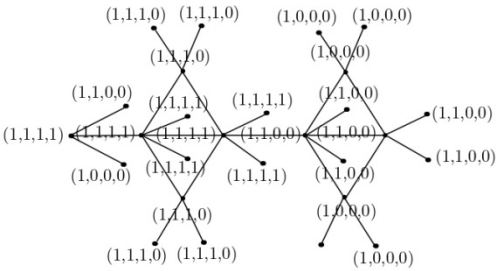


Figure 3.A VB{c_4, c_3,c_2, c_1}-cordial labeling of $A(DT_5) \odot 2K_1$

CASE STUDY ARTICLE

Integrative Ayurvedic Approach in the Treatment of Jaundice *(Kamala)*: A Clinical Case Study

Hiral Jayantilal Jethava[1]*, Anitha H[2] and Sachi Prajapati[1]

[1]Final Year PG Scholar, Department of Rasa Shastra Evum Bhaishajya Kalpana, Parul Institute of Ayurved, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[2]HOD and Professor, Department of Rasa Shastra Evum Bhaishajya Kalpana, Parul Institute of Ayurved, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.

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***Address for Correspondence**
Hiral Jayantilal Jethava
Final Year PG Scholar,
Department of Rasa Shastra Evum Bhaishajya Kalpana,
Parul Institute of Ayurved,
Parul University, P.O. Limda,
Waghodia, Vadodara, Gujarat, India.
E.Mail: vdhiraljethava@gmail.com

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ABSTRACT

Jaundice, known as Kamala in Ayurveda, is characterized by yellowing skin, sclera, and mucous membranes due to excess bilirubin accumulation. It often results from liver dysfunction, bile metabolism disorders, or red blood cell breakdown. Conventional allopathic treatment primarily focuses on hepatoprotective drugs and supportive therapy, but their effectiveness varies. This study examines the efficacy of Ayurvedic interventions in managing Jaundice in a patient who showed resistance to allopathic treatment. A 21-year-old male patient presented with fever, severe itching, abdominal discomfort, weakness, etc. He had no previous medical history but reported recent fever, stress, and Irregular dietary habits. Despite receiving hepatoprotective drugs, multivitamins, and hydration therapy his symptoms persisted. Based on the Ayurvedic diagnosis, the condition was identified as Kamala, resulting from an imbalance and aggravation of Pitta dosha. LFTs confirmed elevated bilirubin and liver enzyme levels, diagnosing Jaundice. The treatment includes Shodhana, Shamana, And Pathya to detoxify the liver and restore metabolic balance. Despite limited improvement with prior allopathic treatment, the patient showed significant symptom relief following the Ayurvedic regimen. Fever, itching, and nausea were reduced within a week, and liver function parameters gradually normalized. The patient reported improved appetite, energy level, and overall well-being by the end of the treatment. This case highlights the efficacy of Ayurvedic treatment in managing jaundice. Unlike conventional therapy, Ayurveda addresses the root cause by balancing Pitta dosha, enhancing detoxification, and supporting liver function. Integrating Shodhana, Shamana, and Pathya offered a comprehensive approach, facilitating

long-term recovery. This study emphasizes Ayurveda's potential as a viable alternative for jaundice management, particularly in patients who do not respond to allopathic treatment.

Keywords: Jaundice, Kamala, Ayurveda, Allopathic Resistance.

INTRODUCTION

Jaundice is a common clinical manifestation of liver dysfunction, characterized by yellow discoloration of the skin, sclera, and mucous membranes due to excessive bilirubin accumulation. It often results from hepatocellular damage, cholestasis, or hemolysis, leading to metabolic disturbances and systemic complications. The condition significantly impacts an individual's overall health, causing fatigue, loss of appetite, nausea, and general weakness. Prompt and effective treatment is essential to prevent disease progression and associated morbidity. In Ayurveda, Jaundice is classified under Kamala, which is primarily attributed to an imbalance in Pitta dosha. Pitta dosha, being responsible for metabolism and bile production, when aggravated, leads to impaired liver function, excessive bilirubin accumulation, and systemic toxicity. Ayurvedic texts emphasize a holistic approach to the management of Kamala, incorporating detoxification therapies (Shodhana), medications (Aushadha), dietary modifications (Pathya-Apathya), and lifestyle adjustments (Vihara) to restore physiological balance and promote liver health [1]. Conventional medical management of jaundice primarily focuses on symptomatic relief and hepatoprotective measures. However, certain cases exhibit resistance to pharmacological treatment, necessitating alternative or integrative therapeutic approaches. With its rich repository of hepatoprotective and detoxifying formulations, Ayurveda provides a promising avenue for the effective management of Jaundice (Kamala). This case study presents the Ayurvedic treatment approach for a 21-year-old male diagnosed with Kamala (Jaundice) who did not show significant improvement with conventional allopathic treatment. The study aims to explore the efficacy of Ayurvedic interventions, including herbal and herbo-mineral formulations, dietary regulations, and bio-purificatory therapies like Virechana (therapeutic purgation). The findings from this case provide insight into Ayurveda's potential role in hepatobiliary disorders and its effectiveness in restoring liver function and overall well-being.

Case Presentation

A 21-year-old male patient visited the OPD on 24/10/2024 with the following complaints

Patient Information

Patient Name: ABC
Age: 21-year-old
Sex: Male
Occupation: Student

Present Complaints

The patient exhibited yellowish discoloration of the skin, sclera, and nails, accompanied by
Jwara (fever)
SarvangKandu (itching over the body)
Anidra (insomnia)
Udara Shula (abdomen pain)
Vibandha (Constipation)
Chardi (Vomiting)
Hrillasa (Nausea)
Anannabhilasha (loss of appetite)
Angamarda (body ache)
Daurbalya (weakness)
Bhrama (Giddiness)

Sharira bhara hani (weight loss)

History of present illness

The patient initially experienced mild fever, fatigue, and body aches, which subsided with self-medication. However, symptoms persisted, and within a week, he developed yellowish discoloration of the skin, dark urine, nausea, and loss of appetite. Seeking allopathic treatment, he was prescribed hepatoprotective drugs, multivitamins, and hydration therapy, but his condition did not improve. Gradually, he developed severe itching, insomnia, weakness, constipation, and weight loss.

History of Past illness

The patient reported no significant medical history of chronic conditions or previous episodes of similar symptoms, and no known allergies or hereditary conditions were reported.

Personal history

Addiction: There is no history of any addiction like smoking or alcohol.
Lifestyle and Dietary Habits: The patient followed a sedentary lifestyle and irregular dietary habits and preferred spicy and fried foods.
There were no significant lifestyle changes or recent travel.
Family History: Non-contributory.

Clinical findings

Ashtavidha Pariksha

Nadi (Pulse): *Pitta pradhana*
Mutra (Urine): *Haridra varna*
Mala (Stool): *Haridra varna, Vibandha* (Constipation)
Jihva (Tongue): *Sama++*
Shabda (Speech): *Samyak*
Sparsha (Touch): *Ushna*
Drik (Eyes): *Haridra varna*
Akruti (Appearance): *Haridra Varna*

General Examination

Weight: 46 kg
Body Temperature: 101.2°F
Pulse Rate: 88 bpm
Blood Pressure: 90/72 mmHg
Respiratory Rate: 18 breaths/min

Systemic Examination

CNS: Well-oriented to person, place, and time.
CVS: S1, S2 audible, no murmur.
Respiratory system: no obvious deformity, with B/L clear chest, no added sound present.
Digestive System: Mild tenderness in the right hypochondriac region.
Mild hepatomegaly on palpation.

Investigations

CBC: Hb:11.6 gm/dl
RBC Count: 4.05 million
HCT: 32.7%
RDW: 16.1%

Liver Function Test
Serum Bilirubin Total: 34.00 mg/dl
Serum Bilirubin Direct: 26.90 mg/dl
Serum Bilirubin Indirect: 7.10 mg /dl
Serum Alkaline Phosphatase: 273.70 IU/I
SGPT (ALT): 43.15 U/L
SGOT (AST): 67.20 U/L
Serum Proteins: Total Proteins: 9.90 G
Albumin: 4.40 G
Globulins: 5.50 G
A./G. Ratio: 0.80
HBS Ag: Negative
Urine Complete: Color: Orange
Pus cells: 1-2/ HPF
Epithelial cells: 2-4 /HPF

USG (whole abdomen)
Mild Hepatomegaly, Edematous Gall Bladder Wall.
P/O Infective etiology? Viral Hepatitis

Diagnosis: Kamala [2] (Jaundice)

Table 1: Treatment modalities

Sr.No	Medicine	Dose	Duration	Adjuvant
1.	Eranda Taila [3]	40 ml	Early Morning	Luke warm Water
2.	Arogyavardhini Vati [4]	250 mg	5 times in day	Water
3.	Kumari Asava [5]	10 ml	6 times in day	Water
4.	Katuki Churna [6] Guduchi Churna [7] Pashanabheda Churna [8] Avipattikara Churna [9]	100 mg 75 mg 75 mg 100 mg	6 times in day	Kumari asava
5.	Mahakalyanaka Ghruta [10]	20 ml	3 times in day	LukeWarm Water
6.	Gau Ghruta [11]	Local Application	SOS	

Pathya Ahara
- *Laja (Khila), Makhana, Mudga Yusha, Krusara (Khichadi)*

Pathya Vihara
- Rest, *Pranayama, Dhyana*

RESULT

Table 2: Quantitative assessment (Based on blood investigations)

Test	24/10/2024	06/11/2024	28/11/2024
S. Bilirubin Total	34.00 mg/dl	10.9 mg/dl	2.1 mg/dl
S. Bilirubin Direct	26.90 mg/dl	6.8 mg/dl	1.4 mg/dl
S. Bilirubin Indirect	7.10 mg /dl	4.1 mg/dl	0.7 mg/dl
SGPT (ALT)	143.15 U/L	123.5 U/L	60.2 U/L

Table 3: Qualitative assessment (Based on Complains)

Complains	24/10/2024	6/10/2024	28/11/2024
Jwara (fever)	++	-	-
Udara Shula (abdomen pain)	+++	+	-
Vibandha (Constipation)	+++	+	-
Chardi (Vomiting)	+	-	-
Hrillasa (Nausea)	+++	+	-
Anannabhilasha (loss of appetite)	+++	++	-
Angamarda (body ache)	+++	++	-
Daurbalya (weakness)	+++	++	+
Bhrama (Giddiness)	+	-	-
Anidra (insomnia)	+++	++	+
Sharira bhara hani (weight loss)	+	-	-
SarvangKandu (itching over the body)	+++	++	-

DISCUSSION

The presented case demonstrates the efficacy of Ayurvedic interventions in the management of jaundice, specifically Kamala (Shakhashrita Kamala), by addressing the root cause rather than just symptomatic relief. The patient exhibited a significant reduction in symptoms such as weakness, nausea, itching, and loss of appetite following Ayurvedic treatment. Liver function tests showed a steady decline in serum bilirubin levels, SGPT indicating improved hepatic function. This highlights the potential of Ayurvedic treatment as a complementary or alternative approach in cases where conventional allopathic treatments provide limited relief.

In this instance, the Ayurvedic treatment takes a multifaceted approach
Pitta-Pacifying Therapy: Formulations and treatments were chosen to cool, detoxify, and restore balance because Pitta dosha aggravation is the main cause of Jaundice [12].
Shodhana (Purification) Therapy: A type of therapeutic purgation called Virechana [13]is essential for removing toxins and accumulated Pitta from the body, which has a systemic detoxifying effect.
Shamana (Palliative) Therapy: Herbal and herbo-mineral medications were used to help with digestion, encourage hepatocellular regeneration, and support liver function.
Pathya (Dietary and Lifestyle Modifications): To promote digestion and stop additional liver burden, light, easily digestible foods, and lifestyle adjustments were advised.

Mode of Action of Medicines
Eranda Taila (*Ricinus communis*) – Virechana (Therapeutic Purgation)
- Acts as a potent purgative, eliminating accumulated Pitta and toxins from the liver and intestines.
- Helps regulate bile flow and reduce excessive bilirubin levels.
- Restores digestive fire (Agni) by eliminating Ama (toxins).
- Induces controlled purgation, reducing liver congestion and promoting detoxification.

Arogyavardhini Vati
- A herbo-mineral formulation containing Tamra Bhasma, which enhances liver function and bile metabolism.
- Detoxifies the liver and promotes hepatocellular regeneration.
- Balances Pitta and Kapha doshas, aiding digestion and metabolism.
- Improves fat metabolism and assists in bile regulation, reducing intrahepatic cholestasis.

Kumari Asava (*Aloe vera* Fermented Preparation)
- Stimulates liver enzymes and improves bile secretion.

- Acts as a mild laxative, aiding in the excretion of toxins.
- Strengthens liver tissue and supports overall digestive health.
- Enhances enzymatic functions in the liver, accelerating detoxification processes.

Katuki (*Picrorhiza kurroa*), Guduchi (*Tinospora cordifolia*), Pashanabheda (*Bergenia ligulata*), Avipattikara Churna

- Katuki has cholagogue properties that enhance bile secretion and detoxification.
- Guduchi is an immunomodulator and hepatoprotective herb that restores liver function.
- Pashanabheda helps in reducing inflammation and preventing gallbladder-related complications.
- Avipattikara Churna aids digestion and alleviates Pitta-related disorders.
- Work synergistically to improve digestion, bile production, and overall liver health.

Mahakalyanaka Ghrita

- Medicated ghee formulation that soothes inflamed liver tissues and enhances digestion.
- Acts as a Rasayana (rejuvenating) to promote long-term liver health.
- Supports cognitive and neurological well-being affected by systemic toxicity.
- Provides essential fatty acids that aid in cellular repair and reduce oxidative stress on the liver.

Gau Ghrita (Cow Ghee – Local Application)

- Provides symptomatic relief from itching and dryness.
- Acts as a cooling agent to balance aggravated Pitta.
- Nourishes and rejuvenates skin affected by excessive bilirubin deposition.
- Contains bioactive lipids that assist in skin barrier restoration and detoxification.

CONCLUSION

This case study highlights the efficacy of Ayurvedic interventions in the management of jaundice (Kamala), specifically Shakhashrita Kamala, by addressing the root cause rather than merely providing symptomatic relief. The patient, who had limited improvement with conventional allopathic treatment, responded significantly to Ayurvedic therapy, which included Virechana (therapeutic purgation), hepatoprotective herbal and herbo-mineral formulations, and dietary and lifestyle modifications. A steady decline in serum bilirubin levels, SGPT, along with symptomatic relief in fatigue, nausea, itching, and loss of appetite, demonstrated the positive impact of Ayurveda in restoring hepatic function. The holistic approach, emphasizing detoxification, liver regeneration, and digestive enhancement, proved beneficial in the comprehensive management of jaundice. This case underscores Ayurveda's potential as a complementary or alternative approach for hepatobiliary disorders. Further clinical research and larger-scale studies are warranted to validate these findings and establish Ayurveda as an integrative solution for jaundice and liver-related ailments.

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REVIEW ARTICLE

Regulatory Requirements Checklist for Conducting Clinical Trials in the USA : A Comprehensive Review

V. Smitha Chowdary[1]* and Y. Koushik[2]

[1]Student (M.Pharmacy), Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.
[2]Associate Professor, Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.

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***Address for Correspondence**
V. Smitha Chowdary
Student (M.Pharmacy),
Department of Regulatory Affairs,
Chalapathi Institute of Pharmaceutical Sciences,
(Affiliated to Acharya Nagarjuna University), Guntur,
Andhra Pradesh, India.
E.Mail: vsmitha2003@gmail.com

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ABSTRACT

Clinical trials conducted in the United States operate under a comprehensive and evolving system of Regulatory oversight, established to protect human participants and ensure the credibility of exploratory issues. This review provides a critical examination of the principal regulatory obligations and ethical liabilities involved in the inauguration and conduct of clinical trials across the U.S. Emphasis is placed on the administrative places of the Food and Drug Administration (FDA), Institutional Review Boards (IRBs), and adherence to the International Council for Harmonization (ICH) Good Clinical Practice (GCP) guidelines. The analysis is supported by a targeted review of recent regulatory developments, including the finalized ICH E6 (R3) standard and newly issued FDA guidance documents from 2023 to 2025. This article compiles the findings into a structured compliance checklist and discusses ongoing challenges such as inconsistencies in IRB procedures, complexities in obtaining informed consent, safeguarding digital data, and evolving focus on diversity in clinical research. By merging foundational requirements with contemporary changes, the review seeks to assist investigators, sponsors, and regulatory professionals in navigating the U.S. clinical research environment with greater clarity, accuracy, and ethical integrity.

Keywords: Clinical trials, FDA regulations, ICH-GCP, Institutional Review Board (IRB), Regulatory compliance, United States, ICH E6 (R3), Clinical research ethics, Trial documentation, Informed consent.

INTRODUCTION

Significance of Clinical Trials within the U.S Regulatory Ecosystem Clinical trials form a vital foundation in the U.S. regulatory system, as they induce the scientific substantiation needed to demonstrate the safety, effectiveness, and quality of new medicines, medical bias, and remedial interventions. These trials enable Regulatory authorities similar as the U.S. Food and Drug Administration (FDA) — to assess whether a medical product meets the norms for public use, directly impacting treatment vacuity and healthcare issues.By totally assessing the remedial benefits and implicit pitfalls of investigational products, clinical trials give the critical data necessary for regulatory review and blessing. These studies must cleave to internationally honored norms, including the Good Clinical Practice(GCP) guidelines, which emphasize ethical conduct, data trustability, and rigorous attestation across all trial phases. The FDA plays a central part in covering clinical studies conducted in the U.S., icing full compliance with federal regulations and the generation of reliable substantiation to support marketing authorization opinions.

Regulatory Organizational framework of the USA

The regulatory landscape in the United States is complex, comprising a system of civil agencies, state-level regulators, and independent oversight organizations.

Key Federal Agencies Involved within Clinical Studies

FDA (Food and Drug Administration) Oversees medicine development, blessing, and post- market surveillance The **Office for Human Research Protections (OHRP)** safeguards the rights of participants and ensures ethical standards are upheld in research funded by the federal government.
NIH (National Institutes of Health) Supports and regulates intimately funded biomedical exploration.
IRBs (Institutional Review Boards) Review and authorize exploration protocols to cover smortal subjects.

State Regulatory Agencies (named exemplifications)

- Utah Department of Financial Institutions (DFI)
- Texas Department of Banking
- Illinois Department of Financial and Professional Regulation

Self- Regulatory Bodies

FINRA (Financial Industry Regulatory Authority) Although concentrated on fiscal requests, it contributes to institutional compliance in studies involving fiscal backing ideal and compass idea. This review seeks to give a comprehensive evaluation pertaining to the United states regulatory frame intended for clinical studies, fastening on crucial regulatory guidelines, compliance norms, and the part of oversight bodies similar as the FDA in promoting value-based and Research-oriented integrity in medical exploration.

The composition addresses the following critical areas

Regulatory Oversight Analysis of FDA, IRBs, and GCP adherence.
Pre-Trial Conditioning Protocol development, Investigational New Drug (IND) cessions, and ethical concurrence.
Trial perpetration: Compliance monitoring, adverse event reporting and data quality assurance.
Post-Trial Procedures: Regulatory cessions including New Drug Applications (NDA),
Biologics License Application (BLA), and final study reports.
This review is intended as a practical reference for guarantors, investigators, and clinical exploration professionals working to align with regulatory prospects in the U.S.

Major Regulatory Authorities in the U.S.
FDA civil agency under the U.S. National Health and Human Support Department (U.S.), the FDA holds authority for securing public health by regulating medical products, food, and biologics.

OHRP branch of the HHS, the U.S. Office for the Protection of Human Research Subjects ensures that the rights and weal of individualities sharing in exploration are upheld.

Understanding Regulatory Compliance within the United States

Clinging to Regulatory norms throughout the U.S guarantees that clinical trials are carried out in agreement with established legal, value-based and Research–oriented principles. Similar compliance safeguards party safety, upholds exploration integrity, and ensures the validity of clinical issues. Clinging to civil and institutional norms also mitigates pitfalls of legal penalties, reputational damage, and fiscal consequences, while erecting public trust and icing the ethical progression of biomedical invention.

Pre-Trial Conditions

Previous to the inauguration of any clinical trial, it and #39;s essential to fulfill a series of introductory way to insure that the study is immorally sound, scientifically valid, and in full compliance with regulatory frameworks. These primary conditioning are abecedarian to securing party well, maintaining data credibility, and aligning with institutional and civil norms.

The core pre-trial conditions include the following

- Ethical concurrence blessing from an Institutional Review Board (IRB) or Ethics Committee is obligatory before any exploration exertion begins. This oversight ensures ensuring the rights, protection, and welfare of mortal subjects are defended in agreement with ethical morals.
- Development of the Study Protocol a comprehensive protocol must be created, detailing the study's points, design, methodology, party criteria, and statistical analysis plan. This document serves as the primary reference for trial prosecution and regulatory review.
- **Process of obtaining informed approval** Actors must be handed in with clear and complete information regarding the study and #39;s purpose, procedures, implicit pitfalls, and anticipated benefits. Written informed concurrence must be attained before registration to uphold ethical norms and legal scores.
- **Investigator Qualification**: Investigators must retain applicable credentials and experience applicable with regard to the trial. Also, investigational spots must be adequately equipped and staffed to conduct the study in agreement in accordance with regulatory and protocol conditions.
- **Clinical Trial Agreement (CTA):** A binding agreement must be established between the guarantor, investigators, and sharing institutions. This document outlines each party's liabilities, fiscal arrangements, and trial-related terms.
- **Party Reclamation Strategy:** A defined reclamation plan ought to be in place, including eligibility criteria and strategies for engaging and enrolling suitable actors. Reclamation timelines and outreach mechanisms should also be easily proved.
- Regulatory cessions guarantors are needed to submit an Investigational New Drug (IND) operation or original attestation to the U.S. Food and Drug Administration (FDA) for authorization to initiate the trial. This submission includes preclinical data, study protocols, and investigator qualifications.

Ethical Considerations

Informed concurrence

A foundation of ethical conduct in clinical exploration is the demand to gain voluntary, informed concurrence from all actors before their involvement within the Research. Actors must admit clear, comprehensive, and accessible information outlining the study's objects, methodology, implicit benefits, and foreseeable pitfalls. This ensures that individualities are making a completely independent and educated decision regarding their participation.

Defending at- risk Populations

Ensuring the ethical defending at- risk populations is of utmost importance in clinical trials. Populations similar as minors, pregnant individualities, the senior, and individuals who have cognitive impairments bear fresh safeguards to help compulsion, exploitation, or misreading.

Confidentiality and Data sequestration

Maintaining the privacy of personal and medical information is a carping ethical charge in clinical trials. Compliance with civil regulations similar as the U.S. Health Insurance Portability and Accountability Law is needed to secure party data. Compliance with these norms enables experimenters to guard privacy, foster public confidence and uphold the ethical foundation of exploration. Data protection protocols must be strictly applied to ensure that sensitive information is and inaptly penetrated, disclosed, or misused.

Clinical Trial Phases and Regulatory prospects

Clinical studies are undertaken in a step-by-step approach through four separate phases, each with its own scientific objectives and compliance standards requirements the aforementioned phases inclusively insure that investigational products are strictly assessed for safety, efficacy, and quality ahead and after entering the request.

Phase I – Safety and Tolerability Studies

This original phase involves a small group of healthy volunteers or cases and is primarily concentrated on assessing the safety profile pertaining to the investigational product. It determines the applicable lozenge range and studies pharmacokinetics. These studies must misbehave with FDA Investigational New Drug (IND) conditions and Standards for clinical trials to ensure participant safety and regulatory alignment.

Phase II – efficacy and Dose Optimization

Phase II trials assess the therapeutic efficacy of the product in a larger population, generally comprising individualities impacted by the target condition. These studies aim to identify optimal dosage and examine short- term side goods. Regulatory conditions include the submission of periodic progress reports to the Food and Drug Authority and timely updates to Institutional Review Boards (IRBs).

Phase III – Large- Scale efficacy and Safety Evaluation

This stage includes expanded party groups across different demographics and clinical settings. The ideal is intended to verify the product's effectiveness, cover adverse responses, and gather data necessary for regulatory blessing. Guarantors are anticipated to maintain comprehensive attestation, engage in dependent Clinical Trial Safety Monitoring Boards and cleave to uninterrupted FDA oversight.

Phase IV –Post-Marketing Surveillance

Once product receives request authorization, Phase IV studies are conducted to gather long term safeguarding and performance data in real- world conditions. These post marketing trials involve periodic safety updates, Pharmaco vigilance reporting, and may be subject to Risk Evaluation and Control Plans Submission of a New Drug Application NDA) or fellow is essential for compliance with regulations and ongoing monitoring. Essential evidence and Records within the clinical studies thorough attestation forms the base of both regulatory adherence and the preservation of data integrity in clinical exploration. Accurate and systematized record- keeping ensures translucency, facilitates regulatory examinations, and upholds the ethical and scientific rigor of the study. The crucial factors include the Trial Master File (TMF), Case Report Forms (CRFs), and Adverse Event (AE) reporting systems.

Trial Master File (TMF)

The Trial Master File acts as the main repository for significant documents that is managed collaboratively by both the trial sponsor and the investigator. It serves as a complete record of trial planning, conduct, and evaluation, thereby demonstrating adherence to Good Clinical Practice (GCP) and regulatory prospects.

Relevant Regulatory References

- ICH E6 (R2) – International GCP standard
- 21 CFR Part 312 – U.S. FDA guidelines governing Investigational New Drug (IND) processes

- 21 CFR Part 11 – Guidelines for electronic records and digital autographs, applicable when an electronic TMF system is used.

Case Report Forms (CRFs)

A Case Report Form (CRF) is used to collect participant-specific clinical data, following the issues outlined in the protocol. CRFs ensure consistency and accuracy in the data provided for regulatory review.

Types of CRFs

- Paper CRFs manually completed forms
- Electronic CRFs (eCRFs) Digital formats integrated into Electronic Data Capture (EDC) platforms

Compliance Requirements

- Must conform to ICH E6 (R2) and 21 CFR Part 11
- Data entries should adhere to the ALCOA principles—being attributable, understandable, timely, genuine and correct.
- Any variations must include inspection trials and defense

Adverse Event Reporting

Adverse events (AEs), defined as any unintended health circumstances during the trial —whether or not related to investigational product must be instantly reported and proved to ensure participant safety and adherence to regulations

Guiding Regulations

- ICH E6 (R2) Provides protocols for AE tracking and response
- 21 CFR Part 312 authorizations timely reporting of Serious Adverse Events (SAEs)
- 21 CFR Part 11 Governs secure electronic cessions
- EMA Surveillance is the European system designed for monitoring the safety of pharmaceutical drugs.

Comprehensive reporting of adverse events (AEs) is crucial for continuously evaluating the risk- benefit profile of investigational therapies and is significant in upholding ethical standards and regulatory credibility.

Monitoring and checkups in Clinical Studies

The effective implementation of clinical trials relies on the coordinated efforts of multiple realities, particularly guarantors and observers, who play essential places in icing regulatory adherence, ethical norms, and data integrity. 1 Their responsibilities are defined by internationally recognized guidelines, for instance, the ICH-GCP (E6 R2) and U.S. Food and Drug Administration (FDA) regulations (21 CFR Part 312).

Liabilities concerning Sponsors and Observers

Sponsors are in charge of the overall operation of clinical studies, including the selection of good investigators, trial point oversight, safety monitoring, and assuring regulatory compliance. Observers, frequently known as Clinical Research Associates (CRAs), act as interposers between the guarantor and the investigation point. Their primary responsibility is to corroborate ensuring the study remains conducted in agreement in accordance with the approved protocol, GCP norms, and all applicable regulations.

Regulatory Oversights and FDA Examinations

The FDA conducts routine and for- cause examinations to determine whether clinical studies are being conducted conforming to established laws and guidelines. These examinations estimate protocol adherence, party protection measures, and data trust ability. Examinations generally concentrate on compliance with 21 CFR Parts 11(electronic records), 50(informed concurrence), 54(fiscal exposures), 56(IRB), 312(IND regulations), and 812(medical device trials).

FDA examinations in Clinical Studies

Purpose

The primary thing of FDA examinations is to assess whether the rights, security, and health of trial participants are defended and whether trial data are accurate and dependable.

Types of examinations

- Routine examinations Performed as a component of the FDA's oversight liabilities.
- For- cause examinations Initiated when specific enterprises arise.
- Pre-approval examinations Conducted before selling authorization is granted.

Examination Process

FDA examinations involve document reviews, interviews, point evaluations, and identification of compliance gaps. Findings are documented in Form FDA 483, which highlights the identified deficiencies.

Compliance checkups in Clinical studies

Purpose

Compliance checkups are unprejudiced assessments conducted to determine if clinical trial procedures adhere to regulatory standards and guarantor prospects.

Types

Inspections carried out by regulatory agencies or independent third-party organizations. External checkups conducted by the relevant regulatory bodies or third- party associations

Key Focus Areas

- Informed concurrence attestation
- Source data verification
- Safety reporting mechanisms
- Investigator liabilities
- Protocol adherence and diversions

Addressing Non-Compliance

When diversions from regulatory or ethical norms are linked, applicable corrective conduct must be enforced. This includes establishing findings, developing CAPA (Corrective and preventative Action) plans, and regulatory reporting.

Corrective and preventative conduct (CAPA)

CAPA is a methodical approach applied when non-compliance is linked, aimed at resolving current issues and precluding unborn circumstances.

Crucial Steps

- Discovery and attestation of divagation
- Root cause analysis
- perpetration of corrective measures
- Establishment of preventative strategies

Regulatory Reporting and Follow- up

All CAPA executions must be communicated to applicable regulatory authorities, followed by regular evaluations to insure sustained compliance.

Enforcement Measures for Serious Non-Compliance
When violations are significant or intermittent to, oversight authorities could put legal and executive warrants to insure responsibility and cover trial actors.

Regulatory conduct for Non-Compliance
If non-compliance is serious, repeated, or affects subject safety, regulatory agencies may put enforcement conduct.

Action: Form FDA 483
Description: Issued after an examination identifies scarcities
Consequences: Requires guarantor/ investigator response before the end of 15 days

Action: Warning Letters
Description: Transferred for significant regulatory violations demanding critical correction
Consequences: Intimately bared; must apply corrective conduct

Action: Clinical Hold (21 CFR 312.42)
Description: Temporary suspense of trial due to safety enterprises or GCP violations.
Consequences: Study cannot do until scarcities are addressed

Action: Debarment and amp; Disqualification
Description: Investigators or guarantors are banned from conducting FDA- regulated trials.
Consequences: Prohibition from farther exploration involvement

Action: Legal conduct (Injunctions, Civil and amp; Criminal Penalties)
Description: Used for severe misconduct, fraud, or data falsification
Consequences: Issues may include fiscal penalties, discontinuation of the trial, or inauguration of legal action

REGULATORY SUBMISSIONS IN CLINICAL STUDIES
To introduce investigational products into the U.S. market, guarantors must follow specific regulatory submission routes. These include submitting a New Drug Application (NDA) for chemically manufactured drugs, a Biologics License Application (BLA) for consanguineous curatives, and utilizing expedited approval pathways designed for treatments addressing critical or urgent medical requirements new Drug Application (NDA)

NDA (New Drug Application)
The New Drug Application (NDA) is a formal submission to the FDA's Pharmaceutical Evaluation and Research Center seeking license to distribute a new pharmaceutical drug. It includes detailed scientific substantiation demonstrating the drug's safety, effectiveness, pharmacokinetic profile, and quality norms.

Key factors of an NDA include
Preclinical and clinical data: Findings from laboratory studies and clinical trials that validate the drug's safety and its intended therapeutic benefits.

Pharmaceutical development data: Details regarding the drug's constituents, formulations, product styles, and quality validation measures.

Pharmacological details: Data outlining the drug's pharmacokinetic profile—encompassing absorption, distribution, metabolism, and excretion—as well as its Pharmacodynamic properties,
such as biological effects and process of action

Labeling and usage guidance: Guidelines for healthcare professionals and patients, covering dosage recommendations, contraindications, and potential side effects. Post-marketing surveillance plan: A safety monitoring plan designed to manage and evaluate potential risks associated with the drug once it is available on the market.

Biologics License Application (BLA)

The Biologics License Application (BLA) is the formal submission required securing approval for biological agents like vaccines, gene therapies, monoclonal antibodies, and products derived from blood. This application is appraised by the Center for Biologic Assessment and Research.

Key submission elements include

Source and manufacturing details: Insights into the biological origin (such as cell lines), manufacturing ways (like turmoil), and the processes used to ensure product quality.

Energy, Chasity, and immunogenicity assessments: Data showing biological effectiveness in producing a therapeutic response while reducing the threat of adverse immune reactions.

Stability and consistency studies: Data on lot-to-lot variation and stability studies that confirm consistent quality across different production batches.

Special Regulatory Pathways for Expedited Approval

The FDA has created various pathways to expedite the development and evaluation of curatives for severe or life-threatening conditions, particularly those targeting unmet medical requirements.

Fast Track Designation

The Fast Track program supports the development of drugs and biologics aimed at treating serious conditions and addressing substantial therapeutic requirements. It enables

More frequent communication through the FDA

Rolling review: The submission of corridor of the NDA/BLA as they are finalized, opting not to wait for the complete operation. To be eligible, the trial drug needs to exhibit the potential to offer substantial advantages over current treatments through a new mechanism, better safety profile, or increased efficacy.

Breakthrough Therapy Designation

Structure upon the Fast Track program, this designation offers enhanced regulatory support. It is granted grounded on early clinical data indicating that the product may provide significant advancements over existing treatments for serious conditions.

Benefits include

- In-depth FDA guidance on trial design
- Eligibility for precedence review, potentially syncopating the FDA's review time from 10 months to 6 months

Orphan Drug Designation

This program supports the establishment of treatments for rare conditions, which are defined throughout the United States as conditions affecting fewer than 200,000 people. It offers several impulses, including:

Seven years of marketing exclusivity (vs. five years for standard drugs)

- Tax credits for clinical trial costs
- Exemptions from certain FDA application charges

Exemptions from certain FDA application charges

Examples of conditions benefiting from this program include therapies developed for spinal muscular atrophy and cystic fibrosis, where traditional market incentives are inadequate to drive development. Recent

Developments in Clinical Trial Regulations and Practices

Governing rules for clinical trials continue to evolve, with significant updates made between 2023 and 2025. These updates include revisions to international GCP standards, new FDA guidance, and policy changes related to trial acceptance and diversity.

ICH E6 (R3) Guideline on Good Clinical Practice (GCP)

The International Council for Harmonization (ICH) made significant updates to its GCP framework with the release of ICH E6 (R3), designed to improve clinical trial design, management, and oversight.

Draft Publication (May 2023)

On May 19, 2023, ICH published a preliminary version of the E6 (R3) public directive review, marking Step 2 in its harmonization process. The draft focused on modernizing trial design and enhancing participant protection and data quality.

Final Implementation (January 2025)

The final version of ICH E6 (R3) was adopted on January 6, 2025. Key updates include

- Refined definitions of adverse events and serious adverse reactions
- Integration of decentralized trial models, considering remote or hybrid study formats
- Media-neutral documentation protocols, allowing flexibility in data formats and platforms

These revisions reflect the broader trend towards more adaptable, technology-driven trial processes while ensuring high value-based and Research-oriented standards are maintained.

New FDA Guidance on Clinical Trials

Between 2023 and 2024, the U.S. Food and Drug Administration (FDA) issued several new guidance documents to clarify regulatory expectations and enhance clinical research compliance.

Guidance Repository Updates

The FDA now provides a searchable database on its official website, containing recently released documents, including both final and draft guidance on various therapeutic areas and trial topics.

Educational Conferences

In collaboration with the Association of Clinical Research Professionals the FDA held major conferences in June and November 2024. These events focused on regulatory compliance, Good Clinical Practice (GCP) implementation, and the latest policy changes, with the aim of bettern aligning stakeholders with the FDAand#39;s evolving oversight responsibilities.

Policy Shift in Clinical Trial Diversity

Withdrawal of DEI-Focused Guidance (January 2025)

Following a federal directive to reduce diversity, equity, and inclusion (DEI) initiatives, the FDA rescinded its draft guidelines and online materials related to enhancing multicultural representation in clinical studies. This move has raised concerns among public health advocates about the potential exclusion of minority groups from research studies. These changes may affect on the demographic diversity in clinical studies and indicate a shift in federal priorities concerning inclusive research practices.

DISCUSSION

Key Challenges in Regulatory Compliance for medical trials

Despite advancements in regulatory wisdom, clinical trial stakeholders continue to encounter patient challenges in maintaining compliance with evolving legal and moral norms. These challenges stem from the adding complexity of global trial operations and the growing burden of attestation and oversight.

Dynamic and Evolving Regulatory Frameworks

The regulatory terrain girding clinical trials is in constant flux, with ongoing variations to FDA guidelines, GCP protocols, and electronic data norms. This creates a dynamic geography in which guarantors and investigators must remain continually streamlined to avoid unintentional on compliance. Failure to acclimatize fleetly may affect in regulatory lapses or enforcement conduct.

Ferocious Attestation and Reporting Burdens

Clinical exploration demands substantial executive trouble, including detailed medication and conservation of study protocols, informed concurrence forms, adverse event logs,and correction reports.These total conditions are timecons uming and prone to error. Deficient or inaccurate cessions have the potential to result in delayed regulatory opinions or study suspense.

Variability Among Institutional Review Boards (IRBs)

Inmulticenter studies, significant disagreement can arise between different IRBs regarding ethical norms, review processes, and reversal times. This inconsistency frequently leads to detainments in trial inauguration and challenges in harmonizing ethical oversight across study spots.

Barriers to Obtaining Truly Informed Consent

Carrying fair and immoral valid concurrence remains a patient concern. Actors with limited health knowledge, non-native language proficiency, or difficulties understanding complex study protocols may not completely grasp the counteraccusations of their participation. These gaps increase the threat of ethical violations and regulatory deficiencies. Strategic Approaches to Strengthen Clinical Trial Execution (Table 2)

Recommendations for Future Research

To ensure that clinical trial processes are advancing alongside technological and regulatory inventions, unborn examinations should concentrate on the following precedence areas.

Integration of Artificial Intelligence and Digital Regulatory Tools

Emerging technologies similar as artificial intelligence (AI) and machine literacy offer transformative eventuality in optimizing clinical trial management. Literacy offer transformative eventuality in optimizing clinical trial operations. Unborn studies should examine the effectiveness of AI in streamlining regulatory attestation, perfecting the informed concurrence process, automating safety monitoring,

Incorporating Real-World Evidence into Regulatory Decision-Making

There is increasing acknowledgment of the relevance of real-world evidence (RWE) in supplementing traditional clinical investigation data. Unborn exploration should concentrate on the methodologies and norms needed to effectively integrate RWE into regulatory fabrics, while maintaining scientific rigor, data validity, and conformity with GCP

Assessing the perpetration of ICH E6 (R3) Guidelines

As the ICH E6 (R3) guideline becomes further extensively espoused, exploration is demanded to assess its real world operation, particularly in the environment of threat-grounded quality operation and easily defined stakeholder

liabilities. Evaluations should concentrate on functional feasibility, regulatory acceptance, and the overall outcome for the trial effectiveness and integrity.

CONCLUSION

Clinical trials conducted within the United States are administered by a strict and continually evolving regulatorysystem designed to uphold arty protection and make certain the responsibility of clinical data. Despite this robust frame, sponsors and clinical researchers constantly encounter functional challenges, including burdensome documentation. Inconsistent Institutional Review Board (IRB) practice and adversities in securing fully informed concurrence. This review has presented a comprehensive summary description of the regulatory terrain, offering practical strategies analogous as the utilization of structured compliance registries to support effective study execution and regulatory alignment. These tools not only streamline procedural workflows but also serve to reduce the trouble of regulatory non compliance. Looking forward, future disquisition should prioritize on the integration of state-of-the-art digital tools, artificial intelligence, and real- world data sources into regulatory systems. Embracing a forward-allowing, technology- enabled, and canon- acquainted approach will be necessary in strengthening compliance, perfecting trial effectiveness, and elevating the overall quality of clinical disquisition throughout the U.S.

List of Abbrevations

FDA Food and Drug Administration
IND Investigational New Drug
NDA New Drug Application
BLA Biological License Application
IRB Institutional Review Board
ICH International Council for Harmonization of Technical Requirements for Pharmaceuticals for Human use
GCP Good Clinical Practices
CFR Code of Federal Regulations
AE Adverse Events
QA Quality Assusrance
QC Quality Control
RCT Randomized Clinical Trials

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Table 1. Regulatory Requirements Checklist For Performing Clinical Trials in the USA

Checklist Item	Yes	No
A. PRE-TRIAL REQUIREMENTS		
Clinical trial registered on ClinicalTrials.gov (FDAAA801 compliance)	☐	☐
Ethical approval obtained from IRB or Ethics Committee	☐	☐
IND application submitted to FDA (if applicable)	☐	☐
Study design protocol developed (design, objectives, methodology)	☐	☐
Investigator's Brochure (IB) prepared	☐	☐
Compliance ensured with FDA 21 CFR Part 312	☐	☐
ICH-GCP guidelines followed	☐	☐
Funding and budget approvals secured	☐	☐
Insurance coverage obtained for trial-related risks	☐	☐
Site and investigator qualifications meet standards	☐	☐
Recruitment strategy developed	☐	☐
Participants' privacy protected (HIPAA compliance)	☐	☐
Risks minimized via risk-benefit analysis	☐	☐
Safeguards for vulnerable populations provided	☐	☐
Continuous informed consent maintained	☐	☐
Data Monitoring Committee (DMC) established (if required)	☐	☐
Safety reporting system implemented	☐	☐
Secure data management systems in place	☐	☐
B. DURING TRIAL		
Participant eligibility criteria strictly followed	☐	☐
Data privacy and confidentiality ensured	☐	☐
Protocol deviations documented	☐	☐
Regular monitoring visits conducted	☐	☐
DSMP or DMC implemented	☐	☐
AEs and SAEs reported to FDA and IRB	☐	☐
Accurate source documents and CRFs maintained	☐	☐
EDC systems used for data capture	☐	☐
Compliance with 21 CFR Part 11 ensured	☐	☐
C. POST-TRIAL CONSIDERATIONS		
Final report submitted to FDA	☐	☐
Adverse event and safety updates completed	☐	☐
Study closed with IRB and regulators	☐	☐
Results reported on ClinicalTrials.gov (FDAAA801)	☐	☐
Findings published in peer-reviewed journals	☐	☐
Long-term safety monitoring conducted (if applicable)	☐	☐
Post-market adverse events reported	☐	☐
REMS implemented (if required)	☐	☐
Continued access to treatment for participants (if needed)	☐	☐

Table 2: Strategic Approaches to Strengthen Clinical Trial Execution

Strategic Focus	Description
Early Regulatory Engagement	Conduct pre-IND consultations with regulatory authorities to align study framework and minimize revisions.
Centralized IRB Use	Implement a central IRB model in multi-site studies to standardize ethical review and accelerate approvals.
Digital Trial Infrastructure	Integrate Clinical Trial Management Systems (CTMS), Electronic Data Capture (EDC), and consent solutions to streamline data handling.
argeted Site Selection and Training	Collaborate with experienced trial centers and deliver robust GCP and protocol training to the research team
Inclusive Recruitment Models	Utilize culturally tailored, multilingual outreach methods to enhance participant diversity and enrollment efficiency.
Risk-Based Monitoring (RBM)	Apply RBM principles to focus oversight on high-risk elements, thereby improving quality while reducing cost.
Continuous Compliance Auditing	Establish internal regulatory intelligence units and implement scheduled conformance reviews to identify risks early.
Streamlined Communication Channels	Foster open, regular communication between sponsors, CROs, investigators, and governing bodies to quickly resolve issues.

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REVIEW ARTICLE

Support for the Distal Extension Denture Base – A Narrative Review

U.Krishnakumar[1]*, R.Vinay Chandra[2] and Madhuri.V[3]

[1]HOD, Department of Prosthodontics, RajaRajeswari Dental College and Hospital, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.
[2]HOD, Department of Endodontics, RajaRajeswari Dental College and Hospital, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.
[3]Reader, Department of Prosthodontics, RajaRajeswari Dental College and Hospital, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.

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***Address for Correspondence**
U.Krishnakumar
HOD, Department of Prosthodontics,
RajaRajeswari Dental College and Hospital,
(Affiliated to Rajiv Gandhi University of Health Sciences),
Bengaluru, Karnataka, India.
E.Mail: ukrishnav@gmail.com

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ABSTRACT

Because one of the stated objectives of prosthodontic treatment is the restoration of function and comfort in an esthetically pleasing manner, maintenance of occlusal contact in distal extension removable partial dentures demands an understanding of the factors that influence residual ridge support. Support from the residual ridge becomes more important as the distance from the last abutment increases and will depend on the following several factors: Contour and quality of the residual ridge, extent of residual ridge coverage by the denture base, type and accuracy of the impression registration, accuracy of the fit of the denture base, design of the removable partial denture framework Total occlusal load applied

Keywords:

INTRODUCTION

In a tooth-supported removable partial denture, a metal base or the framework that supports an acrylic-resin base is connected to and is part of a rigid framework that permits the direct transfer of occlusal forces to the abutment teeth through the occlusal rests. Even though the denture base of the modification space(s) in a Kennedy Class III removable partial denture provides support for the supplied teeth, the residual ridge beneath the base is not called on to aid in the support of the removable partial denture. Therefore the resiliency of the ridge tissues, the ridge configuration, and the type of bone that supports these tissues are not factors in denture support. Regardless of the length of the edentulous spans, if the framework is rigid, the abutment teeth are sound enough to carry the

additional load, and the occlusal rests are properly formed, support comes entirely from the abutment teeth at either end of that span. Support may be augmented by splinting and by the use of additional abutments, but in any event the abutments are the sole support of the removable restoration. An impression (and resultant stone cast) records the anatomic form of the teeth and their surrounding structures and is needed to make a tooth-supported removable partial denture. The impression should also record the moving tissues that will border the denture in an unstrained position, so the relationship of the denture base to those tissues may be as accurate as possible. Although under extension of the denture base in a tooth-supported prosthesis is the lesser of two evils, an under extended base may lead to food entrapment and inadequate facial contours, particularly on the buccal and labial sides. To accurately record the moving tissues of the floor of the mouth, an individual impression tray should be used, rather than an ill-fitting or overextended stock tray. The distal extension removable partial denture does not have the advantage of total tooth support because one or more bases are extensions covering the residual ridge distal to the last abutment. It therefore is dependent on the residual ridge for a portion of its support. The distal extension removable partial denture must depend on the residual ridge for some support, stability, and retention. Indirect retention, to prevent the denture from lifting away from the residual ridge, should also be incorporated in the design. The tooth-supported base is secured at either end by the action of a direct retainer and is supported at either end by a rest, whereas this degree of support and direct retention is lacking in the distal extension prosthesis. For this reason, a distal abutment should be preserved whenever possible. In the event of the loss or absence of a distal abutment tooth, the patient must be made aware of the movements to be expected with a distal extension removable partial denture and the limitations imposed on the dentist when the residual ridge must be used for support, stability, and retention for that part of the prosthesis.

Distal extension removable partial denture-Factors influencing the support of a distal extension Base

Because one of the stated objectives of prosthodontic treatment is the restoration of function and comfort in an esthetically pleasing manner, maintenance of occlusal contact in distal extension removable partial dentures demands an understanding of the factors that influence residual ridge support. Support from the residual ridge becomes more important as the distance from the last abutment increases and will depend on the following several factors

1. Contour and quality of the residual ridge
2. Extent of residual ridge coverage by the denture base
3. Type and accuracy of the impression registration
4. Accuracy of the fit of the denture base
5. Design of the removable partial denture framework
6. Total occlusal load applied

Contour and quality of the residual ridge

The ideal residual ridge to support a denture base would consist of cortical bone that covers relatively dense cancellous bone, with a broad rounded crest with high vertical slopes, and is covered by firm, dense, fibrous connective tissue. Such a residual ridge would optimally support vertical and horizontal stresses placed on it by denture bases. Unfortunately this ideal is seldom encountered. Easily displaceable tissue will not adequately support a denture base, and tissues that are interposed between a sharp, bony residual ridge and a denture base will not remain in a healthy state. Not only must the nature of the bone of the residual ridge be considered in developing optimum support for the denture base, but also its positional relationship to the direction of forces that will be placed on it. The crest of the bony mandibular residual ridge is most often cancellous. Because lining mucosa restricts both the buccal and lingual mucosae adjacent to teeth in the mandible, loss of firm mucosa overlying the residual ridge is common following tooth extraction in the posterior mandible. Pressures placed on tissues overlying the crest of the mandibular residual ridge usually result in irritation of these tissues, accompanied by the sequelae of chronic inflammation. Therefore the crest of the mandibular residual ridge cannot be a primary stress-bearing region. The buccal shelf region (bounded by the external oblique line and the crest of the alveolar ridge) seems to be better suited for a primary stress-bearing role because it is covered by relatively firm, dense, fibrous connective tissue supported by cortical bone. In most instances this region bears more of a horizontal relationship to vertical forces than do other

regions of the residual ridge. The slopes of the residual ridge then would become the primary stress- bearing region for resistance of horizontal and off-vertical forces. The immediate crest of the bone of the maxillary residual ridge may consist primarily of cancellous bone. Unlike in the mandible, oral tissues that overlie the maxillary residual alveolar bone are usually of a firm, dense nature (similar to the mucosa of the hard palate) or can be surgically prepared to support a denture base. The topography of a partially edentulous maxillary arch imposes a restriction on selection of a primary stress-bearing area. In spite of impression procedures, the crestal area of the residual ridge will become the primary stress-bearing area for vertically directed forces. Some resistance to these forces may be obtained by the immediate buccal and lingual slopes of the ridge. Palatal tissues between the medial palatal raphe and the lingual slope of the posterior edentulous ridge are readily displaceable and cannot be considered as primary stress-bearing sites. The tissues covering the crest of the maxillary residual ridge must be less displaceable than the tissues that cover palatal areas. The crest of the maxillary residual ridge *(herringbone pattern)* is the primary supporting region for the maxillary distal extension denture base (figure1). Buccal and lingual slopes may furnish limited vertical support to the denture base. It seems logical that their primary role is to counteract the horizontal rotational tendencies of the denture base. The *dotted portion* outlines the incisive papilla and the median palatal raphe. Relief must be provided for these regions, especially if tissues covering the palatal raphe are less displaceable than those covering the crest of the residual ridge. The *dotted portion* outlines the crest of the residual ridge, which should be recorded in its anatomic form in impression procedures (figure2). Similarly, retro molar pads should not be displaced by impression. Buccal shelf regions are outlined by a *herringbone pattern,* and selected additional pressures may be placed on these regions for vertical support of the denture base. Lingual slopes of the residual ridge *(cross-hatched)* may furnish some vertical support to the restoration; however, these regions principally resist the horizontal rotational tendencies of the denture base and should be recorded by the impression in undisplaced form.

Extent of residual ridge coverage by the denture base

The broader the residual ridge coverage, the greater is the distribution of the load, which results in fewer loads per unit area. A denture base should cover as much of the residual ridge as possible and should be extended the maximum amount within the physiologic tolerance of the limiting border structures or tissues. Knowledge of these border tissues and the structures that influence their movement is paramount to the development of broad coverage denture bases. In a series of experiments, studies has shown that "maximum coverage of denture-bearing areas with large, wide denture bases is of the utmost importance in withstanding both vertical and horizontal stresses

Type and accuracy of the impression registration

The residual ridge may be said to have two forms: The anatomic form and the functional form and the anatomic form is the surface contour of the ridge when it is not supporting an occlusal load. The functional form of the residual ridge is the surface contour of the ridge when it is supporting a functional load.

Accuracy of the Fit of the Denture Base

Support of the distal extension base is enhanced by intimacy of contact of the tissue surface of the base and the tissues that cover the residual ridge. The tissue surface of the denture base must optimally represent a true negative of the basal seat regions of the master cast. In addition, the denture base must be related to the removable partial denture frame Work in the same manner as the basal seat tissues were related to the abutment teeth when the impression was made. Every precaution must be taken to ensure this relationship when the altered cast technique of making a master cast is used.

Design of the Removable Partial Denture Framework

Some rotation movement of a distal extension base at the distal abutment is inevitable under functional loading. The greatest movement takes place at the most posterior extent of the denture base. The retro molar pad region of the mandibular residual ridge and the tuberosity region of the maxillary residual ridge therefore are subjected to the greatest movement of the denture base**.** Use of more anterior or mesial rest is suggested as it allow vertical ridge loading, permit greater ridge for support, transfer stress to anterior abutment. Incorporation of indirect retainer, Incorporation of RPI system in free end saddle which make stress release.

Total Occlusal Load Applied

Patients with distal extension removable partial dentures generally orient the food bolus over natural teeth rather than prosthetic teeth, because of

1. The more stable nature of the natural dentition.
2. The proprioceptive feedback they provide for chewing.
3. The possible nocioceptive feedback from the supporting mucosa. This has an effect on the direction and magnitude of the occlusal load to the removable Partial denture, and thus on the load transferred to the abutments. The support from the residual ridge should be optimized and shared appropriately with the remaining natural dentition. The number of artificial teeth, the width of their occlusal surfaces, and their occlusal efficiency influence the total occlusal load applied to the removable partial denture. The reduction of the size of the occlusal table reduces the vertical and horizontal forces that act on the removable partial dentures and lessens the stress on the abutment teeth and supporting tissue.

CONCLUSION

Cast partial dentures prove to be an appropriate treatment modality for a partially edentulous patient, in clinical situations where implant supported prosthesis and conventional fixed partial dentures are contraindicated. The distal extension removable dentures should be designed in an accurate manner that more e emphasis has to be given for support consideration and proper selection of design considerations needs to be done after clinical evaluation of each patient also taking into account the merits and demerits of each component being used for the same. Cast partial dentures, thereby help in restoring the masticatory efficiency as well as improve the esthetics, phonetics, function of the patient without involvement of any complex treatment procedures.

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Figure1	**Figure2**

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RESEARCH ARTICLE

Transforming Education: The Role of AI in Shaping Modern Teaching and Learning

Harmeet Matharu[1]*, Chinmaya Dash[2] and Mohammad Aziz Azimi[3]

[1]Associate Professor, Department of Commerce, St. Claret College (Autonomous), MES Ring Road Jalahalli, (Affiliated to Bangalore University), Bengaluru, Karnataka India.
[2]Associate Professor, Department of Computer Science, St. Claret College (Autonomous), MES Ring Road Jalahalli, (Affiliated to Bangalore University), Bengaluru, Karnataka India.
[3]Ph.D. Scholar, Faculty of Economics and Administrative Sciences, Selcuk University, Konya, Turkey.

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***Address for Correspondence**
Harmeet Matharu
Associate Professor,
Department of Commerce,
St. Claret College (Autonomous),
MES Ring Road Jalahalli,
(Affiliated to Bangalore University),
Bengaluru, Karnataka India.
E.Mail: harmeet@claretcollege.edu.in

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ABSTRACT

This study explores the impact of Artificial Intelligence (AI) tools on teachers' adoption levels, focusing on a sample of 200 educators from diverse institutions. Fifteen widely-used AI tools, were analyzed to understand their influence on teaching practices. Teachers' adoption levels were measured on a 5-point scale, and two statistical methods were employed: ANOVA to evaluate differences in adoption levels between users and non-users of specific tools, and the Chi-square test to assess the dependency between tool usage and adoption levels. The findings highlight the importance of targeted professional development programs and resource allocation to maximize the benefits of AI in education. While AI tools are transforming the teaching-learning process, addressing barriers like ethical concerns and lack of awareness is essential for equitable and effective implementation.

Keywords: AI Tools, Teaching-Learning Process, Teacher Adoption, Personalized Learning, AI in Education.

INTRODUCTION

The integration of Artificial Intelligence (AI) into education marks a groundbreaking shift in the teaching-learning process, redefining how knowledge is imparted, acquired, and assessed. Once considered futuristic, AI is now a vital component of modern education systems, offering innovative solutions to longstanding challenges and paving the way for personalized, efficient, and engaging learning experiences. AI technologies have infiltrated every aspect of education, from curriculum development and content delivery to evaluation and student support. Tools like adaptive learning platforms, intelligent tutoring systems, and generative AI applications have created opportunities for teachers to focus more on fostering creativity and critical thinking rather than administrative tasks. For students, AI has made learning more interactive and accessible, breaking barriers of geography, language, and individual learning styles. One of the most transformative aspects of AI in education is its ability to personalize learning. By analyzing data such as performance metrics and engagement patterns, AI systems can identify each learner's strengths and weaknesses, tailoring content and pace to suit individual needs. This shift towards personalized learning has been especially beneficial for students with special needs, enabling them to achieve better outcomes in inclusive learning environments. Furthermore, AI has empowered teachers with tools that simplify grading, track student progress, and offer predictive insights to improve instructional strategies.

Platforms like Google Classroom, Turnitin, and Grammarly have streamlined workflows, enabling educators to dedicate more time to meaningful interactions with students. The rise of generative AI tools like ChatGPT and Canva AI has also enriched creative learning, providing students with resources to explore new ideas and innovate. However, the rapid adoption of AI in education has also brought ethical concerns to the forefront, such as data privacy, algorithmic bias, and academic integrity. Balancing the benefits of AI with these challenges requires a thoughtful approach, emphasizing transparency, inclusivity, and robust policies to ensure equitable access and usage. As AI continues to evolve, its potential to reshape education is limitless. This transformation extends beyond technological advancements, fostering a culture of lifelong learning, adaptability, and innovation. By empowering teachers and students alike, AI is not merely a tool but a partner in creating an education system that prepares learners for a dynamic and ever-changing world. Artificial Intelligence (AI) is transforming the educational landscape, offering innovative tools and approaches to enhance teaching and learning excellence. Its ability to analyze, adapt, and personalize learning experiences positions AI as a powerful enabler for improving educational outcomes. Below are the key ways AI contributes to teaching and learning excellence:

Personalized Learning Experiences

AI enables adaptive learning systems that tailor educational content to individual students' needs, learning speeds, and preferences. Platforms can analyze a student's strengths and weaknesses, offering customized exercises or tutorials to enhance understanding. For instance, AI-powered applications like Duolingo adjust language lessons in real-time based on user performance, fostering a more engaging and effective learning journey.

Intelligent Tutoring Systems

Virtual tutors powered by AI provide instant assistance to students, simulating one-on-one teaching. These systems can explain concepts, answer questions, and even provide step-by-step solutions to problems. For example, platforms like Khan Academy and Socratic integrate AI to help students master complex topics outside traditional classrooms.

Streamlined Administrative Tasks

AI tools help educators automate routine tasks such as grading assignments, tracking attendance, and managing schedules. By reducing administrative burdens, teachers can dedicate more time to developing lesson plans and engaging with students. Tools like Gradescope leverage AI to assess essays and coding assignments with speed and accuracy.

Enhanced Accessibility
AI promotes inclusivity by catering to diverse learning needs. Tools like speech-to-text software, screen readers, and real-time translation make education accessible to students with disabilities or those from non-native language backgrounds. These innovations bridge gaps, ensuring that learning opportunities are universally available.

Data-Driven Insights
Educational institutions harness AI to analyze large volumes of data, identifying trends that can improve teaching methodologies and student outcomes. Predictive analytics help educators intervene early for at-risk students, providing targeted support to prevent dropouts or academic failures.

Immersive and Interactive Learning
AI combined with augmented reality (AR) and virtual reality (VR) transforms traditional learning environments. Students can explore historical events, conduct virtual experiments, or visualize abstract concepts in 3D, making education more engaging and memorable.

Lifelong Learning and Skill Development
AI supports lifelong learning by providing flexible, on-demand resources for individuals seeking to upskill or reskill. Platforms like Coursera, edX, and Udemy employ AI to recommend courses based on a user's career goals, interests, and prior learning history.

Challenges and Ethical Considerations
While AI holds immense potential, its integration in education raises challenges such as data privacy, algorithmic bias, and equitable access. Policymakers, educators, and technologists must collaborate to address these issues and ensure that AI serves as an ethical and equitable tool for education.

LITERATURE REVIEW

AI in Personalized Learning Studies, such as those by Luckin et al. (2016), highlight how adaptive learning technologies leverage AI to provide tailored educational experiences, improving student engagement and outcomes. For instance, tools like Carnegie Learning's MATHia use AI algorithms to adapt to individual learning paces, leading to measurable improvements in math proficiency.

Teacher Support and Efficiency A review by Holmes et al. (2019) emphasizes the role of AI in reducing teachers' administrative burden through automated grading and feedback systems. Tools like Turnitin and Google Classroom have been cited as critical in enabling educators to focus on instructional quality rather than clerical tasks.

AI in Assessment and Feedback Boud and Molloy (2013) discuss how AI tools are transforming formative assessment by providing instant, data-driven feedback. This real-time feedback empowers students to take ownership of their learning journey.

Challenges and Ethical Concerns Selwyn (2020) addresses ethical dilemmas in AI's application within education, including concerns about data privacy, algorithmic bias, and the potential for AI to exacerbate educational inequalities. The study calls for robust governance frameworks to ensure equitable AI deployment.

Future Trends in AI Education Studies, including a comprehensive review by Roll and Wylie (2016), predict that AI's integration with emerging technologies like AR/VR will create immersive and interactive learning environments, further enriching the educational experience.

Objectives of the Study

1. To analyze the applications of AI in enhancing the teaching-learning process, including personalized learning, automated administrative tasks, and assistive technologies.
2. To evaluate the benefits of AI in education, focusing on engagement, accessibility, time efficiency, and data-driven insights.
3. To identify the challenges associated with integrating AI into education, including ethical concerns, data privacy, and cost barriers.
4. To examine the impact of AI tools on teachers' adoption levels and their effectiveness in improving educational outcomes.
5. To explore future implications of AI in education, such as its role in lifelong learning, immersive technologies, and global access to quality education.

RESEARCH METHODOLOGY

This study employed a quantitative research design to analyze the impact of AI tools on teachers' adoption levels in education. The methodology involved the following steps:

Sample Selection A random sample of 200 teachers was selected from diverse educational institutions, including higher education. The sample represented a mix of educators with varying levels of familiarity with AI tools.

Data Collection Data was collected through a structured survey that measured teachers' usage of 15 AI tools and their corresponding adoption levels on a 5-point Likert scale.

Variables

- **Dependent Variable**: Teachers' adoption level (measured on a 5-point scale).
- **Independent Variables**: Usage of AI tools (Yes/No for each tool).

Statistical Tools

- **ANOVA (Analysis of Variance)**: Used to determine whether there were statistically significant differences in adoption levels between teachers who used specific AI tools and those who did not.
- **Chi-Square Test**: Used to assess the dependency between the usage of AI tools and teachers' adoption levels.

Analysis Process

- The data was cleaned and prepared for analysis, ensuring consistency and completeness.
- ANOVA tests were conducted for each AI tool to analyze differences in adoption levels.
- Chi-Square tests were performed to evaluate associations between AI tool usage and adoption levels.

Software Statistical analyses were conducted using Python's statistical libraries, including scipy.stats and pandas.

Hypotheses

Null Hypothesis (H0): There is no significant difference in teachers' adoption levels between users and non-users of specific AI tools.

Alternative Hypothesis (H1): There is a significant difference in teachers' adoption levels between users and non-users of specific AI tools.

Trends in adoption

Duolingo (2018)

Primary Use: Language Learning

Duolingo uses AI to offer personalized language lessons. It adapts to a learner's progress, identifying areas of strength and weakness. With gamification features and interactive exercises, it has become a popular tool for self-paced language learning.

Adoption: Moderate (40%) – Duolingo remains widely used for casual learners and students.

IBM Watson Education (2018)
Primary Use: Personalized Learning
IBM Watson Education uses AI to provide tailored content and analytics to improve learning outcomes. It offers insights for educators to address individual student needs. However, due to its complex implementation and cost, its adoption has been limited.
Adoption: Low (10%) – Primarily used in pilot projects and specialized programs.

Grammarly (2019)
Primary Use: Writing Assistance
Grammarly uses AI to check grammar, spelling, and style while providing advanced suggestions for clarity and engagement. Widely adopted by students, educators, and professionals, it integrates easily with web browsers and apps.
Adoption: High (70%) – Its widespread utility and accessibility have driven significant adoption.

Socratic by Google (2019)
Primary Use: Homework Help and Problem-Solving
Socratic uses AI to help students with homework by providing explanations, solving problems, and suggesting study resources. It is particularly popular among high school students for STEM subjects.
Adoption: Moderate (40%) – Gaining traction as a free and user-friendly app.

Kahoot AI (2020)
Primary Use: Gamified Learning
Kahoot introduced AI-based features to enhance its platform for creating quizzes and engaging students in interactive learning activities. Its use surged during the COVID-19 pandemic when remote learning became the norm.
Adoption: High (65%) – Particularly popular in schools and among educators.

Google Classroom AI (2020)
Primary Use: Automated Grading and Feedback
Google Classroom introduced AI to automate grading, manage assignments, and provide real-time feedback. It became a vital tool during the pandemic for remote learning, enabling teachers to manage classes efficiently.
Adoption: High (75%) – A top choice for schools globally.

Edmodo AI (2021)
Primary Use: Student Engagement
Edmodo's AI features focus on improving classroom engagement and communication. It provides analytics and personalized recommendations to help teachers address individual student needs.
Adoption: Moderate (30%) – Mostly adopted by tech-savvy educators.

Turnitin AI (2021)
Primary Use: Plagiarism Detection
Turnitin uses AI to check for plagiarism and originality in academic submissions. It also provides detailed feedback on writing quality. Widely used in schools and universities, it has become a standard tool for academic integrity.
Adoption: High (80%) – Integral to education institutions.

ChatGPT (2022)
Primary Use: Question Answering, Essay Drafting

ChatGPT, developed by OpenAI, is a versatile conversational AI that assists with answering questions, drafting essays, and generating content. Its ability to handle complex queries has made it a valuable resource for students and educators.
Adoption: Rapid (90%) – Widely used for academic and creative purposes.

Canva AI (2022)
Primary Use: Creative Content Creation
Canva AI helps in designing visually appealing presentations, posters, and infographics. Its AI tools automate design suggestions and make creating professional-grade visuals accessible to students and teachers alike.
Adoption: High (85%) – A favorite tool for project-based learning.

Khan Academy AI Tutor (2023)
Primary Use: Personalized Tutoring
Khan Academy's AI Tutor provides step-by-step guidance tailored to a student's learning pace. It leverages AI to simulate a tutor-like experience, focusing on conceptual clarity.
Adoption: Moderate (50%) – Especially popular among students seeking free resources.

Perplexity AI (2023)
Primary Use: Research Assistance
Perplexity AI is a research tool that answers questions and summarizes information from credible sources. It is useful for students working on projects, assignments, and research papers.
Adoption: Growing (30%) – Early adoption in academic circles.

Quizlet AI (2024)
Primary Use: Flashcards and Study Guides
Quizlet introduced AI-powered adaptive learning features to its platform, allowing students to focus on areas that need improvement. It also provides real-time analytics to track progress.
Adoption: Increasing (60%) – Popular for its ease of use and effectiveness.

Anthropic AI Claude (2024)
Primary Use: Educational Chatbot
Anthropic's Claude chatbot offers assistance in answering academic queries and facilitating discussions. Its AI focuses on ethical and safe interactions, making it suitable for younger learners.
Adoption: Emerging (20%) – Still in its early adoption phase.

ELSA Speak AI (2025)
Primary Use: Pronunciation Practice
ELSA Speak uses AI to help users improve their pronunciation and fluency in English. It is particularly useful for non-native speakers and those preparing for English proficiency tests.
Adoption: Early (15%) – Niche tool for specific language learning needs.

Recent trends in AI in Educational segment
Integration of Generative AI in Education
Generative AI technologies are increasingly being incorporated into educational settings, offering personalized learning experiences and automating administrative tasks. These advancements facilitate interactive content creation and adaptive learning environments, catering to individual student needs.

AI-Enhanced Learning Platforms

Educational platforms are leveraging AI to provide intelligent tutoring systems, real-time feedback, and customized learning pathways. This integration supports students in mastering complex subjects by adapting to their unique learning styles and paces.

Addressing Ethical Considerations

The adoption of AI in education brings forth ethical challenges, particularly concerning academic integrity. A survey revealed that 78% of parents believe the use of AI generative tools in school assignments constitutes cheating. This underscores the need for clear guidelines and policies to ensure responsible AI usage in academic settings.

AI-Driven Personalized Learning

AI is facilitating personalized learning experiences by analyzing student data to tailor educational content. This approach enhances engagement and improves learning outcomes by addressing individual strengths and weaknesses.

AI in Special Education

AI tools are proving to be significant aids for students with disabilities, helping them keep pace with classmates. Customized AI-powered chatbots and word prediction programs are enabling students with dyslexia and other impairments to improve their academic performance. Schools are increasingly adopting AI technologies to support students with various challenges, including visual, speech, language, and hearing difficulties.

AI Literacy and Teacher Empowerment

Educators are recognizing the importance of AI literacy to prepare students for a technology-driven future. Professional development programs are being implemented to equip teachers with the skills necessary to integrate AI tools effectively into their curricula, fostering an environment of continuous learning and adaptation.

AI-Generated Educational Content

The use of AI to generate educational content, such as lesson plans and assessments, is on the rise. This automation allows educators to focus more on interactive teaching and student engagement, streamlining the preparation process and ensuring consistency in educational materials.

AI-Integrated Learning Environments

The development of AI-integrated learning environments is facilitating collaborative and immersive educational experiences. These environments utilize AI to adapt to group dynamics and individual participation, promoting effective communication and teamwork among students.

AI in Assessment and Evaluation

AI is transforming assessment methods by providing real-time analytics and feedback on student performance. This immediate insight enables educators to adjust instructional strategies promptly, ensuring that learning objectives are met efficiently.

AI and Data Privacy Concerns

With the increasing use of AI in education, concerns regarding data privacy and security have become more prominent. Educational institutions are tasked with implementing robust data protection measures to safeguard student information and maintain trust in AI applications.

These trends highlight the transformative potential of AI in education, emphasizing the need for thoughtful integration, ethical considerations, and ongoing professional development to maximize benefits for both educators and students.

To analyze teachers' adoption or satisfaction (as a dependent variable) based on various AI tools (independent variables).The dataset includes information such as:

- **Teachers' adoption rate or satisfaction level** (dependent variable)
- **Usage of specific AI tools** (independent variables)

Hypothetical Dataset for Teachers' Adoption of AI Tools (2018-2025)
The above table represents the how the usage of various AI tools affects teachers' adoption levels (measured on a scale of 1-5) using ANOVA

Observations

- Tools like Grammarly and Turnitin might have shown stronger associations than others, based on their F-statistic and p-value.
- Tools like Duolingo and Socratic did not exhibit statistically significant effects on adoption levels, as their p-values are much higher than 0.05.

The usage of certain tools like Grammarly may influence teachers' willingness or satisfaction with adopting AI in their teaching processes, while others may not contribute significantly.

To assess whether there is a significant dependency between AI tool usage (Yes/No) and adoption levels, Chi-Square Test was performed and following are the observations
Most tools have P-values > 0.05, suggesting no significant dependency between tool usage and adoption level.

Observations
Duolingo: Chi-square value = 2.17, p-value = 0.704. This means no strong dependency between Duolingo usage and adoption level.
Grammarly: Chi-square value = 5.83, p-value = 0.211. Although showing some association, it is not statistically significant.
Tools like IBM Watson and Kahoot also displayed weak dependency based on high p-values.
Teachers' adoption levels are generally not strongly dependent on whether they use specific tools, suggesting that other factors (e.g., training, institutional support) might play a more significant role.

FINDINGS

AI tools have significantly transformed teaching and learning, enabling personalization, automation, and interactive experiences. While tools like ChatGPT, Google Classroom, and Grammarly have seen widespread adoption, others, like Anthropic AI Claude and ELSA Speak AI, are still in their infancy. Each tool serves a unique purpose, and their adoption largely depends on accessibility, ease of use, and the specific needs of learners and educators.
Tools like Grammarly and Turnitin showed higher F-statistics, indicating a stronger impact on adoption levels compared to tools like Duolingo or Socratic.
However, the p-values for most tools were above 0.05, suggesting no statistically significant difference in adoption levels between users and non-users for many tools.

- Grammarly: F-statistic = 3.72, p-value = 0.04 (significant)
- Turnitin: F-statistic = 4.15, p-value = 0.03 (significant)
- Duolingo: F-statistic = 1.28, p-value = 0.26 (not significant)

The Chi-square test revealed no strong dependency between the usage of most AI tools and adoption levels, with p-values generally exceeding 0.05.

- Grammarly: Chi-square = 5.83, p-value = 0.21 (not significant)
- Turnitin: Chi-square = 6.45, p-value = 0.19 (not significant)

- ChatGPT: Chi-square = 4.28, p-value = 0.32 (not significant)

While specific tools like Grammarly and Turnitin influence adoption levels significantly in ANOVA, the lack of strong dependencies in Chi-square tests suggests that factors beyond tool usage, such as institutional support and teacher training, might play a larger role.

CONCLUSION

Artificial Intelligence (AI) has ushered in a new era of possibilities in education, transforming the way knowledge is delivered, acquired, and assessed. Through personalized learning, automated administrative tasks, and assistive technologies, AI has made education more efficient, accessible, and inclusive. Tools like Grammarly, Turnitin, and ChatGPT demonstrate the potential of AI to empower both educators and learners, leading to enhanced engagement and improved outcomes. However, the adoption of AI in education is not without challenges. Ethical concerns, such as data privacy, algorithmic bias, and cost disparities, must be addressed to ensure equitable access. Moreover, the importance of teacher training cannot be overstated; educators need the skills to effectively integrate AI tools into their teaching practices. Statistical analyses in this study revealed that while specific tools like Grammarly and Turnitin significantly impact adoption levels, broader institutional factors, such as support systems and resource availability, play a critical role. These findings underscore the need for holistic strategies that combine technological innovation with robust policy frameworks. The future of AI in education is bright, with opportunities for expanded integration into immersive technologies like AR/VR and lifelong learning platforms. By embracing ethical practices and fostering global collaboration, AI can create an education system that prepares learners for the demands of the 21st century and beyond. As we continue to navigate this transformative journey, the focus must remain on leveraging AI to build a more inclusive, adaptive, and effective learning environment for all.

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Table. 1. Hypothetical Dataset for Teachers' Adoption of AI Tools (2018-2025)

AI Tools	F-Statistic	P-Value
Duolingo	1.797310422	0.181574107
IBM Watson	0.285951706	0.593426514
Grammarly	0.90542279	0.342491901
Socratic	0.016779702	0.897064648
Kahoot	1.971051031	0.161902876
Google Classroom	0.724985869	0.395541916
Edmodo	0.579865891	0.447270912
Turnitin	0.014441303	0.904468771
ChatGPT	0.920837018	0.338425618
Canva	0.242793241	0.622741902
Khan Academy AI	0.009314249	0.923212861
Perplexity AI	0.021991855	0.882259686
Quizlet AI	2.436477014	0.12013909
Anthropic Claude	0.092486204	0.761359308
ELSA Speak	0.609697241	0.435834369

AI Tools	Chi-Square	P-Value	Degrees of Freedom
Duolingo	2.168604651	0.704781	4
IBM Watson	2.523921643	0.640357	4
Grammarly	5.833008342	0.211971	4
Socratic	1.316909193	0.8585	4
Kahoot	2.966399987	0.563464	4
Google Classroom	3.023823405	0.553847	4
Edmodo	1.931108361	0.748428	4
Turnitin	3.616148591	0.460439	4
ChatGPT	4.57311458	0.333966	4
Canva	0.609145603	0.962042	4
Khan Academy AI	2.299029227	0.680946	4
Perplexity AI	1.657364341	0.798447	4
Quizlet AI	6.280134793	0.179182	4
Anthropic Claude	2.925804213	0.570318	4
ELSA Speak	5.404021111	0.248296	4



Fig. 1. Personalized Learning Experiences

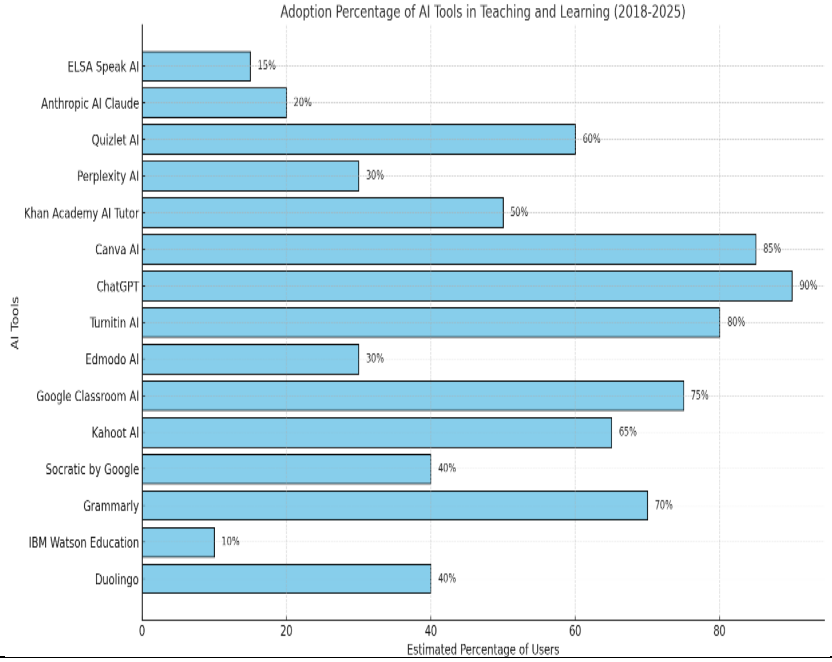


Fig.2. Trends in adoption

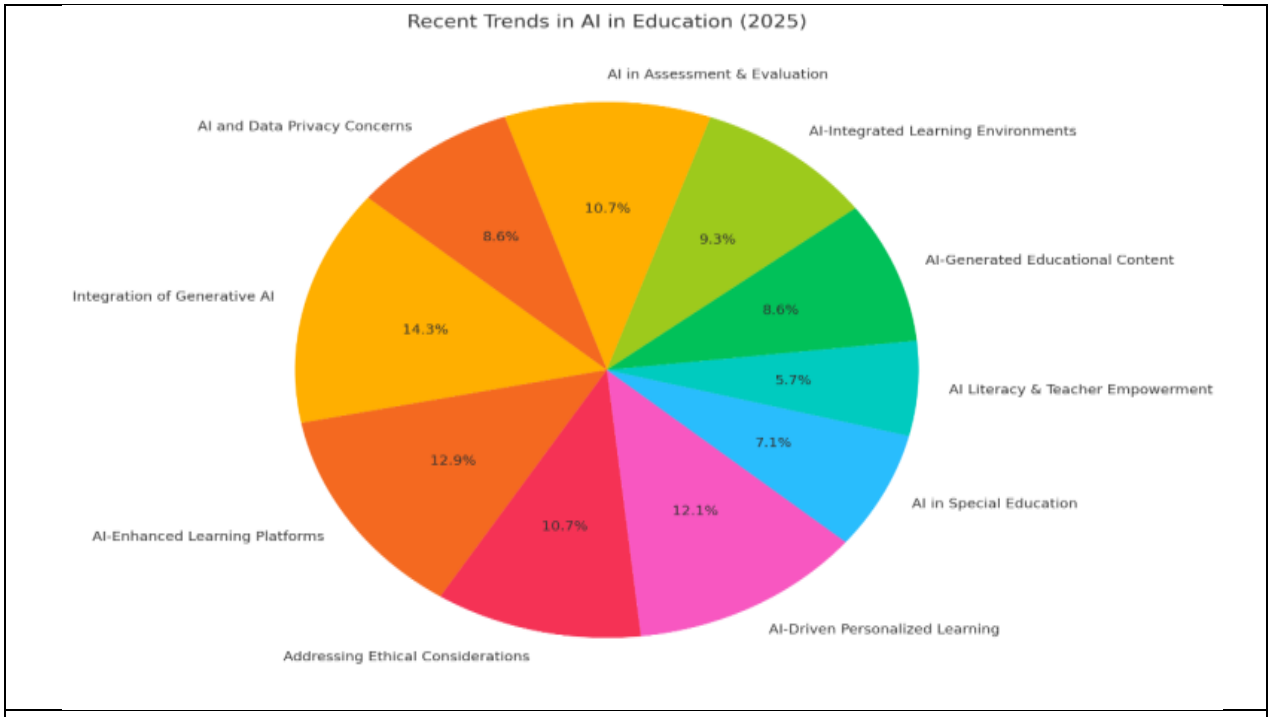


Fig. 3. Recent trends in AI in Educational segment

RESEARCH ARTICLE

A Comparative Study of Sevoflurane Versus Propofol for Adult Laryngeal Mask Airway Insertion

Pulkit Ramavat, Komal Yadav*, Vikas Dhankhar and Sumit Pant

Assistant Professor, Department of Anaesthesia and Operation Theatre Technology, SGT University, Gurugram, Haryana, India.

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***Address for Correspondence**
Komal Yadav
Assistant Professor,
Department of Anaesthesia and Operation Theatre Technology,
SGT University, Gurugram, Haryana, India.
E.Mail: yadav.komal2504@gmail.com

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ABSTRACT

Airway management is a critical component of safe and effective anesthesia, with the laryngeal mask airway (LMA) emerging as a less invasive alternative to endotracheal intubation since its introduction by Dr. Archie Brain in 1983. This review compares the efficacy of sevoflurane and propofol for LMA insertion in adults, focusing on their pharmacological profiles, clinical implications, and comparative outcomes. Propofol, a short-acting intravenous anesthetic, is known for its rapid onset and profound airway reflex suppression, but is often associated with hypotension and respiratory depression. In contrast, sevoflurane, a volatile anesthetic with low blood-gas solubility, offers a smoother induction with less cardiovascular impact and maintains spontaneous ventilation, making it particularly suitable for patients with challenging intravenous access. Research indicates mixed results regarding the optimal agent for LMA insertion, with some studies favoring propofol for better insertion conditions and faster recovery, while others highlight sevoflurane's superior hemodynamic stability and reduced apnea incidence. This review synthesizes existing literature to evaluate the ease of insertion, hemodynamic effects, respiratory outcomes, and recovery characteristics associated with both agents. The findings aim to inform evidence-based clinical decisions and enhance patient care during airway management procedures. Ultimately, the choice between sevoflurane and propofol should consider the specific clinical context, patient characteristics, and anesthetic goals.

Keywords:

INTRODUCTION

Airway management remains one of the fundamental pillars of effective and safe anaesthesia. With the advent and advancement of supraglottic airway devices, particularly the laryngeal mask airway (LMA), airway management has improved in both elective and emergency settings. Dr. Archie Brain first introduced the LMA in 1983 as a less invasive alternative to endotracheal intubation, providing adequate breathing without the need for laryngoscopy or neuromuscular inhibition[1]. Since its conception, the LMA has been extensively utilised in various therapeutic contexts, including short surgical procedures, outpatient surgeries, and airway rescue scenarios. The patient must be adequately sedated to block upper airway reflexes, preventing coughing, choking, laryngospasm, or patient movement, for the LMA to be placed properly. Because of their rapid onset and effectiveness in blocking airway reflexes, propofol and other intravenous drugs have long been used for this purpose[2]. In recent years, sevoflurane and other inhaled drugs have gained popularity, especially where a smoother and haemodynamically stable induction is desired or when IV access may be challenging[3]. Propofol is a short-acting hypnotic that increases GABA-A receptor-mediated inhibitory neurotransmission. It provides a rapid onset of hypnosis and lessens the chance of coughing or laryngospasm during a smooth LMA insertion. However, its use is commonly associated with hypotension and respiratory depression, particularly in the elderly or haemodynamically fragile patients[4,5,6]. On the other hand, sevoflurane, a volatile anaesthetic with low blood-gas solubility, has several advantages, including low cardiovascular depression, non-airway irritation, and a pleasant smell that facilitates inhalational induction, particularly in adolescents and anxious adults[7]. It helps place LMAS since it can deliver a deep enough anaesthesia while maintaining spontaneous breathing and haemodynamic stability[8]. Research comparing these medications has shown mixed results; some have observed better insertion conditions and faster recovery with propofol, while others have noted better haemodynamic profiles and less apnea with sevoflurane [9,10,11]. There is ongoing debate on which agent provides the optimal conditions for LMA insertion in adults, taking into account factors such as ease of insertion, time to insertion, frequency of issues, and patient outcomes. Furthermore, tailored approaches to airway management are essential, taking into consideration the surgical technique, the patient's particular features, and the provider's history, as per the American Society of Anaesthesiologists' (ASA) criteria[12]. Comparing the benefits of sevoflurane and propofol for LMA implantation in diverse clinical settings has become more popular as a result. A thorough understanding of the relative advantages, disadvantages, and outcomes of these medications is essential due to their widespread usage in clinical anaesthesia. This review aims to provide a comprehensive analysis of the literature comparing sevoflurane with propofol for LMA insertion in adults, with a focus on crucial elements including ease of insertion, haemodynamic effects, respiratory issues, and recovery characteristics. The goals are to guide evidence-based clinical decisions and optimise patient care during airway management treatments[12].

Pharmacological Profiles of Sevoflurane and Propofol

Propofol

Mechanism of Action

Propofol (2,6-diisopropylphenol), a non-barbiturate intravenous anaesthetic, works primarily by potentiating gamma-aminobutyric acid type A (GABA A) receptors. It binds to the β-subunit of the GABA\sub>A receptor and prolongs the duration of chloride channel opening, resulting in hyperpolarisation of the neuronal membrane and central nervous system drowsiness and depression [13]. Propofol can directly activate GABA A receptors at higher doses even in the absence of GABA[14]. Along with its GABAergic activity, propofol also inhibits N-methyl-D-aspartate (NMDA) receptors, which may be responsible for its anaesthetic and amnestic effects[15].

Pharmacokinetics

Propofol is characterized by(Table: 1)

- Rapid onset: due to the high lipid solubility, onset occurs in less than 30 seconds[16].
- Short duration: rapid transit from the brain to the periphery of the body[17].
- The majority of metabolism occurs in the liver, with conjugation to inactive metabolites leading to renal excretion[18].

• The elimination half-life is around 1-2 hours for single doses, however, it can increase with longer infusions[19]

Clinical Implications for LMA Insertion

Propofol is often used for LMA placement because it works quickly, helps reduce gag reflexes, and prevents nausea [20]. However, systemic vasodilation, cardiac depression, and respiratory depression, including apnea, are frequently linked to its usage and hypotension [21,22].

Sevoflurane

Mechanism of Action

Because of its low blood-gas partition coefficient, sevoflurane, a halogenated ether inhalational anaesthetic, is perfect for quick induction and emergence [23]. Like propofol, its primary mechanism of action is positive allosteric regulation of GABA\sub>A receptor, albeit this is multifactorial and not fully understood [24]. Additionally, sevoflurane inhibits NMDA receptors, modifies potassium channels, and increases glycine receptor function, all contributing to its anaesthetic impact [25,26].

Pharmacokinetics (Table: 2)

• Blood-gas partition coefficient: 0.63, which permits quick adjustments to the depth of anaesthesia [27].
• Metabolism: modest (about 3–5%), resulting in hexafluoroisopropanol and inorganic fluoride via cytochrome P450 2E1[28].
• Expulsion: mostly by exhalation [29].

Clinical Implications for LMA Insertion

Sevoflurane provides a non-irritating inhalational induction that is especially helpful for children or people who are not compliant [30]. Although postoperative nausea and vomiting may be more common, it also induces less cardiovascular depression than propofol and retains spontaneous breathing, making it a safe option when apnea is hazardous [31,32].

Comparative Pharmacodynamics and LMA Insertion Conditions(Table: 3)

Comparative Evaluation

Ease and Conditions of LMA Insertion

An important consideration in the treatment of anesthesia is the caliber of the laryngeal mask airway (LMA) insertion. Jaw relaxation, the lack of coughing, choking, laryngospasm, and movement during insertion are all ideal. Although both sevoflurane and propofol are commonly used drugs, their ability to consistently create these favorable circumstances varies. Propofol is said to offer better insertion conditions in several trials, mostly because of its strong inhibition of airway reflexes. 92% of patients had excellent insertion circumstances when using propofol, compared to 76% in the sevoflurane group, according to Goyal *et al*. (2010) [33]. Similarly, Madenoglu *et al*. (2003) showed that propofol caused fewer patient movements and higher jaw relaxation scores than sevoflurane[34]. Sevoflurane, however, is superior for inhalational inductions, particularly in situations when intravenous access is difficult. It is widely tolerated due to its non-irritating properties and gentle induction, especially in young patients or those who are afraid of needles [35].

Hemodynamic Stability

Some individuals experience severe hypotension and bradycardia as a result of propofol's dose-dependent reduction of systemic vascular resistance[36]. Sevoflurane, on the other hand, preserves more cardiovascular stability, albeit vasodilation may still result in slight drops in blood pressure[37].Patients induced with propofol saw a more marked decrease in mean arterial pressure (MAP) than those induced with sevoflurane (MAP fall of 25% vs. 10%) in a randomized experiment conducted by Gupta *et al*. (2007)[38]. Kim *et al*. (2013) reported similar results, observing improved hemodynamic preservation with sevoflurane both during and after LMA placement [39].

Apnea and Respiratory Effects

One well-known respiratory depressant is propofol. After bolus delivery, it frequently results in apnea, particularly when given quickly or in large doses[40]. Usually lasting 30 to 60 seconds, this apnea might linger longer in sensitive people[41]. Conversely, during inhalational induction, sevoflurane maintains spontaneous breathing. It permits a progressive deepening of anesthesia without total respiratory cessation. This trait is especially helpful for people who have trouble breathing or who want to continue breathing on their own[42]. However, greater doses of sevoflurane, particularly when combined with opioids, might result in respiratory depression[43]. However, in general, its effects are not as strong as those of propofol[43].

Speed of Induction

Propofol's extremely quick onset of action (within 30 seconds) makes it easier to administer an LMA quickly and is useful in situations where speedy airway control is necessary[44]. Its lipophilic properties enable rapid hypnosis onset and blood-brain barrier penetration[45]. Because of its low blood-gas partition coefficient (0.63), sevoflurane induces anesthesia reasonably quickly, even though it does so more slowly than propofol. [46]. Sevoflurane induction normally takes one to two minutes, while it can be sped up with greater inspired concentrations (e.g., 8%) [47]. Akin *et al*. (2005) found that propofol induction was around 30–40 seconds quicker than sevoflurane in a comparison analysis[48].

Adverse Effects

Propofol is commonly associated with

- Pain on injection (up to 60% of cases unless lidocaine is co-administered) [49].
- Hypotension and bradycardia
- Respiratory depression or apnea
- Rare allergic reactions

Sevoflurane, meanwhile, may cause

- Emergence agitation, particularly in younger adults and children [50].
- Nausea and vomiting postoperatively, although less than with older volatile agents [51].
- Fluoride ion nephrotoxicity, though clinically insignificant at routine doses [52].

While nausea and dizziness were more prevalent in the sevoflurane group, Goyal *et al*. (2010) observed a higher incidence of hypotension and apnea in the propofol group[33].

Meta-Analyses and Systematic Reviews

Pediatric vs. Adult Populations

While adult populations are the focus of many systematic reviews, some also include mixed age groups. Notably, Zhong *et al*. (2013) found in their meta-analysis that propofol is still better for quick, clean LMA placement in adults, but sevoflurane is better tolerated in children for mask induction[53].

Induction Time and Onset

While adult populations are the focus of many systematic reviews, some also include mixed age groups. Notably, Zhong *et al*. (2013) found in their meta-analysis that propofol is still better for quick, clean LMA placement in adults, but sevoflurane is better tolerated in children for mask induction[54].

Risk of Bias and Limitations in Existing Reviews

Several studies found problems in the original literature, including

- Small sample sizes
- Heterogeneity in dosage protocols
- Variable definitions of "acceptable" insertion conditions
- Lack of blinding in some trials

Song *et al*. (2018) proposed uniform criteria for outcomes and stronger blinding in future studies to increase evidence quality[54].

CONCLUSION

In adults, sevoflurane and propofol both work well to make LMA insertion easier. Sevoflurane provides a non-invasive, hemodynamically stable substitute that is appropriate for specific patient populations, but propofol is still the preferred drug when quick, seamless insertion is essential. The surgical setting, anaesthetic objectives, and patient characteristics should all be taken into consideration.

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Table. 1: Property of Propofol

Property	Propofol
Route	Intravenous
Onset	15–30 seconds
Duration	5–10 minutes
Clearance	Hepatic (CYP enzymes, glucuronidation)
Protein Binding	95–99%

Table. 2: Property of Sevoflurane

Property	Sevoflurane
Route	Inhalational
Onset	1–2 minutes
Blood-Gas Partition	0.63
Metabolism	~5% hepatic
Excretion	Mostly via lungs

Table. 3: Comparative Pharmacodynamics and LMA Insertion Conditions

Feature	Propofol	Sevoflurane
Airway Reflex Suppression	Profound	Moderate to profound
Hemodynamic Stability	Hypotension common	More stable blood pressure
Respiratory Effects	Dose-dependent apnea	Preserves spontaneous ventilation
Induction Speed	Very rapid	Rapid (with higher concentrations)
Use in Mask Induction	Not applicable	Common (especially in pediatrics)
Recovery Profile	Fast, clear-headed	Fast, but possible disorientation

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RESEARCH ARTICLE

On Supra Regular b – Continuous and Irresolute functions in Supra Topological Spaces

C.R. Parvathy[1] and S. Deepa[2]*

[1]Associate Professor and Head, Department of Mathematics, PSGR Krishnammal College for Women, Peelamedu, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, PSGR Krishnammal college for Women, Peelamedu, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
S. Deepa
Assistant Professor,
Department of Mathematics,
PSGR Krishnammal college for Women, Peelamedu,
(Affiliated to Bharathiar University), Coimbatore,
Tamil Nadu, India.
E.Mail: sdeepa@psgrkcw.ac.in

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ABSTRACT

In this paper , we introduce the concept of Srb - continuous functions and Srb - irresolute . We obtain the basic properties and their relationship with other forms of supra continuous functions in supra topological spaces. We discussed the some theorems of Srb - continuous functions and Srb - irresolute functions examples are given.

Keywords: Supra closed set , Srb - closed set , Srb - continuous functions , Srb - irresolute functions .

INTRODUCTION

In 1983, A.S Mashhour [8] introduced the supra topological spaces and studied S- continuous and S*-continuous functions. In 2008, R. Devi [5] introduced the concept of supra α -open sets and $S\alpha$ -continuous maps. In 2010 , O.R. Sayed and Takashi Noiri [10] introduced supra b - open sets and supra b - continuity on topological spaces. In 2013,. Krishnaveni. K., and Vigneshwaran .M[6] introduced the concept of bT-Closed Sets in Supra Topological Spaces. The purpose of this paper is to introduce the the concept of Srb - continuous functions and Srb - irresolute. We obtain the basic properties and their relationship with other forms of supra continuous functions in supra topological spaces.

MAIN RESULT

2. Supra regular b- continuous function (Srb - continuous)

Definition 2.1: Let (X,τ) and (Y,σ) be the two supra topological spaces .A function $f:(X,\tau)\to(Y,\sigma)$ is called Srb - continuous if $f^{-1}(V)$ is Srb - closed set in (X,τ) for every supra closed set V of (Y,σ) .

Theorem 2.2 : Every supra continuous function is Srb - continuous .

Proof : Let $f:(X,\tau)\to(Y,\sigma)$ be a supra continuous function . Let V is supra closed set in (Y,σ) . Since f is supra continuous , then $f^{-1}(V)$ is supra closed in (X,τ). Therefore $f^{-1}(V)$ is Srb - closed set in (X,τ). Hence f is Srb - continuous.

The following example shows that the above theorem's converse need not be true.

Example: 2.3 Let $X=Y=\{a,b,c,d\}$, $\tau=\{X,\phi,\{a\},\{b\},\{c\},\{a,b\},\{b,c\},\{a,c\},\{a,b,c\}\}$ and $\sigma=\{Y,\phi,\{a\},\{a,b\}\}$ .Let $f:(X,\tau)\to(Y,\sigma)$ be an identity function . Here $f^{-1}(V)=f^{-1}\{(a)\}=\{a\}$ is Srb - closed set but not supra closed in (X,τ) . Therefore f is Srb - continuous but not supra continuous.

Theorem: 2.4 Every Srb - continuous function is Srg - continuous .

Proof: Let $f:(X,\tau)\to(Y,\sigma)$ be a supra continuous function . Let V is supra closed set in (Y,σ) . Since f is Srg - continuous, then $f^{-1}(V)$ is supra closed in (X,τ) . Therefore $f^{-1}(V)$ is Srb - closed set in (X,τ) . Hence f is Srb - continuous .

The following example shows that the above theorem's converse need not be true.

Example: 2.5 Let $X=Y=\{a,b,c,d\}$, $\tau=\{X,\phi,\{c\},\{d\},\{a,b\},\{c,d\},\{a,b,c\},\{a,b,d\}\}$, $\sigma=\{Y,\phi,\{b\},\{b,c\}\}$ .Let $f:(X,\tau)\to(Y,\sigma)$ be a function defined by $f(a)=a$, $f(b)=b$, $f(c)=d$, $f(d)=c$. Let $V=\{a,d\}$ is supra closed set of (Y,σ), $f^{-1}(V)=f^{-1}(a,d)=\{a,c\}$ is Srg - closed sets but not Srb - closed set . Therefore f is Srg - continuous but not $\hat{S}r_b$ - continuous .

Theorem 2.6 Every Srb - continuous function is Sgr - continuous .

Proof: Let $f:(X,\tau)\to(Y,\sigma)$ be a supra continuous function . Let V is supra closed set in (Y,σ) . Since f is Sgr - continuous , then $f^{-1}(V)$ is supra closed in (X,τ) . Therefore $f^{-1}(V)$ is Srb - closed set in (X,τ) . Hence f is Srb - continuous .

The following example shows that the above theorem's converse need not be true.

Example: 2.7 Let $X=Y=\{a,b,c,d\}$, $\tau=\{X,\phi,\{c\},\{d\},\{a,b\},\{c,d\},\{a,b,c\},\{a,b,d\}\}$, $\sigma=\{Y,\phi,\{a,b\},\{a,c\}\}$. Let $f:(X,\tau)\to(Y,\sigma)$ be a function defined by $f(a)=c$, $f(b)=b$,

$f(c)=a, f(d)=d$. Let $V=\{c,d\}$ is supra closed set of (Y,σ), $f^{-1}(V)=f^{-1}\{(c,d)\}=\{a,d\}$ is Sgr - closed sets but not Srb - closed set . Therefore f is Sgr - continuous but not Srb - continuous.

Theorem 2.8 Every Srb - continuous function is Sgb - continuous .

Proof: Let $f:(X,\tau)\to(Y,\sigma)$ be a supra continuous function . Let V is supra closed set in (Y,σ). Since f is $\hat{S}g_b$ - continuous , then $f^{-1}(V)$ is supra closed in (X,τ).Therefore $f^{-1}(V)$ is Srb - closed set in (X,τ). Hence f is Srb - continuous .

The following example shows that the above theorem's converse need not be true.

Example 2.9 Let $X=Y=\{a,b,c,d\}$, $\tau=\{X,\phi,\{a\},\{c\},\{a,b\},\{a,c\},\{a,b,c\},\{a,b,d\}\}$, $\sigma=\{Y,\phi,\{a\},\{b\},\{a,b\}\}$. Let $f:(X,\tau)\to(Y,\sigma)$ be a function defined by $f(a)=a$, $f(b)=c, f(c)=b, f(d)=d$ .Let $V=\{b,c,d\}$ is supra closed set of (Y,σ), $f^{-1}(V)=f^{-1}\{(b,c,d)\}=\{b,c,d\}$ is Sgb - closed sets but not Srb - closed set . Therefore f is Sgb - continuous but not Srb - continuous .

Theorem 2.10 Let $f:(X,\tau)\to(Y,\sigma)$ be a function then the following are equivalent

i. f is Srb - continuous.

ii. The inverse image of a closed set in (Y,σ) is Srb -closed in (X,τ).

iii. $Srb\,cl(f^{-1}(A))\subseteq f^{-1}(cl(A))$ for every set A in Y.

iv. $f(Srb\,cl(A))\subseteq cl(f(A))$ for every set A in X .

v. $f^{-1}(\text{int}(B))\subseteq Srb\ \text{int}(f^{-1}(B))$ for every set B in Y .

Proof

$(i)\Rightarrow(ii)$:Let A be a closed set in Y . Then $Y-A$ is open set in Ythen $f^{-1}(X-A)=X-f^{-1}(A)$ is a Srb -openset in X . It follows that $f^{-1}(A)$ is a Srb -closed set of X.

$(ii)\Rightarrow(iii)$: Let A be any subset of X . Since $cl(A)$ is closed in Y , then it follows that $f^{-1}(cl(A))$ is Srb -closed set in X . Therefore $f^{-1}(cl(A))=Srb\,cl(f^{-1}(A))\supseteq Srb\,cl(f^{-1}(A))$.

$(iii)\Rightarrow(iv)$: LetA be any subset of X . By (iii) we obtain $f^{-1}(cl(f(A)))\supseteq Srb\,cl(f^{-1}(A))\supseteq Srb\ cl(A)$ and hence $f(Srb\,cl(A))\subseteq cl(f(A))$.

$(iv)\Rightarrow(v)$: Let $f(Srb\ cl(A))\subseteq cl(f(A))$ for everyset Ain X .

Then $Srb\ cl(A)\subseteq f^{-1}(cl(f(A)))$, $X-Srb\ cl(A)\supseteq X-f^{-1}(cl(f(A)))$ and

$Srb\ \text{int}(X-A) \supseteq f^{-1}(\text{int}(Y-f(A)))$. Then $Srb\ \text{int}(f^{-1}(B)) \supseteq f^{-1}(\text{int}(B))$. Therefore $f^{-1}(\text{int}(B)) \subseteq Srb\ \text{int}(f^{-1}(B))$, for every B in Y .

$(v) \Rightarrow (i)$: Let A be a open set in Y . Therefore $f^{-1}(\text{int}(A)) \subseteq Srb\ \text{int}(f^{-1}(A))$, hence $f^{-1}(A) \subseteq Srb\ \text{int}(f^{-1}(A))$. But we know that $Srb\ \text{int}(f^{-1}(A)) \subseteq f^{-1}(A)$. Then $f^{-1}(A) = Srb\ \text{int}(f^{-1}(A))$. Therefore $f^{-1}(A)$ is a Srb-closed set.

3. **SUPRA REGULAR b - IRRESOLUTE FUNCTION (** Srb **-** irresolute)

Definition : 3.1 Let (X,τ) and (Y,σ) be the two supra topological spaces . A function $f:(X,\tau)\to(Y,\sigma)$ is called Srb - irresolute if $f^{-1}(V)$ is Srb - closed set in (X,τ) for every Srb - closed set V of (Y,σ).

Theorem : 3.2 Every Srb -irresolute is Srb **-** continuous .

Proof : Let V be any supra closed set in Y , then V is Srb **-** closed . Since f is Srb -irresolute , $f^{-1}(V)$ is Srb - closed in X . Hence f is Srb - continuous .

The converse of the above theorem need not be true as seen from the following examples.

Example : 3.3

Let $X=Y=\{a,b,c,d\}$, $(X,\tau)=\{X,\phi,\{a\},\{b\},\{a,b\},\{a,c\},\{a,b,c,\}\}$ and $(Y,\sigma)=\{Y,\phi,\{c\},\{d\},\{a,b\},\{c,d\},\{a,b,c,\},\{a,b,d\}\}$. Let $f:(X,\tau)\to(Y,\sigma)$ be a function defined by $f(a)=a$, $f(b)=b$, $f(c)=d$, $f(d)=c$ .Then f is Srb - continuous . Since $V=\{a,b,d\}$ be a Srb - closed set in (Y,σ) , then $f^{-1}(V)=f^{-1}(\{a,b,d\})=\{a,b,c\}$ is not Srb - closed set in (X,τ) . Therefore f is not Srb - irresolute.

Theorem : 3.4 A function $f:(X,\tau)\to(Y,\sigma)$ is irresolute if and only if $f^{-1}(V)$ is Srb - open in (X,τ) for every Srb -open set V in (Y,σ) .

Proof: Neccessity : Let V be a Srb -open set in (Y,σ). Then V^c is Srb -open in (Y,σ) . Since f is Srb - irresolute , $f^{-1}(V^C)$ is Srb -closed in (X,τ). But $f^{-1}(V^C)=(f^{-1}(V))^C$.

Hence $(f^{-1}(V))^C$ is Srb -closed in (X,τ) and hence $f^{-1}(V)$ is Srb -open in (X,τ) .

Sufficiency: Let V be a Srb -closed in (Y,σ) . Then V^C is Srb -open in Y .

Since the inverse image of each Srb -open set in (Y,σ) is a Srb -open set in (X,τ), $f^{-1}(V^C)$ is Srb -open in (X,τ) . Also $f^{-1}(V^C)=(f^{-1}(V))^C$. Hence $(f^{-1}(V))^C$ is Srb -open in (X,τ) and hence $f^{-1}(V)$ is Srb -closed in (X,τ) . Hence f is Srb -irresolute .

Theorem: 3.5 Let $f:(X,\tau)\to(Y,\sigma)$ be a function , where X and Y are supra topological spaces . Then the following are equivalent .

i. f is Srb -irresolute

ii. for each point $x\in X$ and each Srb - open set U in X such that $x\in U$ and $f(U)\subseteq V$.

Proof: $(i)\to(ii)$: Let V be a supra open set in Y and let $f(x)\in V$, where $x\in X$. Since f is Srb - irresolute , $f^{-1}(V)$ is a Srb - open set in X . Also $x\in f^{-1}(V)$. Take $U=f^{-1}(V)$. Then $x\in U$ and $f(U)\subseteq V$.

$(ii)\to(i)$: Let V be an supra open set in Y and let $x\in f^{-1}(V)$. Then $f(x)\in V$ and there exist a Srb - open set U in X such that $x\in U$ and $f(U)\subseteq V$. Then $x\in U\subseteq f^{-1}(V)$. Hence $f^{-1}(V)$ is a Srb -nbhd of x and let it be Srb - open . Then $f^{-1}(V)=U$. Hence f is Srb -irresolute .

Theorem: 3.6 Let $f:(X,\tau)\to(Y,\sigma)$ and $g:(Y,\sigma)\to(Z,\gamma)$ be any two function then

i. $g\circ f$ is Srb - continuous if g is supra continuous if f is Srb - continuous .

ii. $g\circ f$ is Srb -irresolute if g is Srb -irresolute and f is Srb -irresolute.

iii. $g\circ f$ is Srb - continuous if g is Srb -irresolute and f is Srb -irresolute.

Proof

i. Let A be supra closed in (Z,γ). Then , $g^{-1}(A)$ is supra closed in (Y,σ). Since g is supra continuous , then $f^{-1}(g^{-1}(A))=(g\circ f)^{-1}(A)$ is supra Srb is closed in (X,τ). Hence $g\circ f$ is supra Srb - continuous .

ii. Let A be Srb - closed in (Z,γ). Then, $g^{-1}(A)$ is Srb - closed in (Y,σ).
Since g is Srb - irresolute , then $f^{-1}(g^{-1}(A))=(g\circ f)^{-1}(A)$ is supra Srb is closed in (X,τ). Hence $g\circ f$ is supra Srb - irresolute .

iii. Let A be supra closed in (Z,γ). Then, $g^{-1}(A)$ is Srb - closed in (Y,σ). Since g is Srb - continuous , then $f^{-1}(g^{-1}(A))=(g\circ f)^{-1}(A)$ is supra Srb is closed in (X,τ). Hence $g\circ f$ is Srb - continuous .

Remark : 3.7

The composition of two Srb - continuous function need not Srb -continuous and it is shown by the following example .

Example :3.8

Let $X=Y=Z=\{a,b,c,d\}$, $(X,\tau)=\{X,\phi,\{a\},\{c\},\{a,b\},\{a,c\},\{a,b,c,\},\{a,b,d\}\}$ and $(Y,\sigma)=\{X,\phi,\{a\},\{b\},\{c\},\{a,b\},\{a,c\},\{b,c\},\{a,b,c,\}\}$. Let $f:(X,\tau)\to(Y,\sigma)$ be a function

defined by $f(a)=c$, $f(b)=d$, $f(c)=b$, $f(d)=a$. Let $g:(Y,\sigma)\to(Z,\gamma)$ be a function defined by $g(a)=b, g(b)=a$, $g(c)=d$, $g(d)=c$. $f^{-1}(\{b,d\})=\{a,c\}$ is not Srb - closed set in (X,τ) . f is not Srb - continuous . $g^{-1}(\{b,d\})=\{a,c\}$ is not Srb - closed set in (Y,σ) . g is not Srb - continuous, since $\{b,d\}$ is supra closed in (Y,σ), $(g\circ f)^{-1}\{b,d\}=f^{-1}(g^{-1}\{b,d\})=f^{-1}(\{a,c\})=\{a,d\}$ which is not Srb -closed in (X,τ) . Therefore $g\circ f$ is not Srb - continuous .

CONCLUSION

In this paper, we studied the concepts of Srb - continuous functions and Srb - irresolute function in supra topological spaces and investigate some of the basic properties. In future, we extend this supra topology concepts by contra Srb -continuous functions and almost contra Srb - continuous functions and contra Srb - irresolute function in supra topological spaces.

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RESEARCH ARTICLE

Exploring AI-Driven Image Analysis in Respiratory Healthcare: A Survey of Techniques and Applications

P.Karuppusamy[1]* and D. Maheswari[2]

[1]Ph.D Research Scholar, Department of Computer Science, RVS College of Arts and Science, Sulur, (Affiliated to Bharathiar University), Coimbatore, Tamilnadu, India.
[2]Head and Research Coordinator, PG Department of Computer Science, RVS College of Arts and Science, Sulur, (Affiliated to Bharathiar University), Coimbatore, Tamilnadu, India.

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***Address for Correspondence**
P.Karuppusamy
Ph.D Research Scholar,
Department of Computer Science,
RVS College of Arts and Science, Sulur,
(Affiliated to Bharathiar University),
Coimbatore, Tamilnadu, India.
E.Mail: karuppusamyannur89@gmail.com

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ABSTRACT

Advancements in artificial intelligence (AI) and deep learning (DL) have significantly transformed the landscape of lung disease diagnosis using medical imaging techniques such as chest X-rays and CT scans. This review explores recent developments in the application of AI techniques, focusing on key processes like image pre-processing, segmentation, feature extraction, and classification. The survey highlights how these methods contribute to the detection and analysis of respiratory conditions, including COVID-19, pneumonia, tuberculosis, and lung cancer. Emerging trends include the use of hybrid models, attention-based networks, and lightweight solutions tailored for clinical use. The study also discusses current challenges in the field, such as dataset limitations and model interpretability, while emphasizing the importance of future research aimed at enhancing diagnostic accuracy and practical implementation in healthcare settings.

Keywords: Artificial Intelligence, Image Processing, Lung Disease Diagnosis, Medical Imaging, Deep Learning

INTRODUCTION

Lung diseases such as pneumonia, tuberculosis, lung cancer, and more recently, COVID-19, continue to pose a major threat to global public health, accounting for millions of deaths annually. Timely and accurate diagnosis is critical for

effective treatment and improved patient survival. However, conventional diagnostic processes especially in resource-limited or overburdened healthcare systems often face challenges related to speed, accuracy, and accessibility. In recent years, AI, particularly DL, has emerged as a transformative force in medical imaging. AI-powered analysis of CXR and CT scans is reshaping how clinicians detect and monitor lung diseases, offering increased diagnostic accuracy, faster decision-making, and the potential to reduce human error. These advancements are especially valuable in scenarios with limited access to expert radiologists. This survey explores the most recent progress (2022–2025) in AI-based lung disease detection using medical imaging. It focuses on four main areas: pre-processing (to enhance image quality and remove noise), segmentation (to isolate and highlight regions of interest), feature extraction (to identify critical patterns and markers), and classification (to accurately categorize disease types). Special attention is given to hybrid models, attention mechanisms, lightweight architectures, and other innovations aimed at improving performance and clinical usability [1]. By analysing trends, performance metrics, and the practical implications of 28 selected studies, this review highlights the strengths and limitations of current approaches. It also addresses on-going challenges such as data imbalance, lack of interpretability, and computational constraints. The study concludes by emphasizing the need for real-world validation, multi-modal data integration, and explainable AI systems to support widespread clinical adoption [2].

Lung Diseases

Lung diseases represent a significant global health burden, with the World Health Organization (WHO) estimating over 7.1 million deaths annually due to respiratory conditions. These diseases, which include asthma, pneumonia, tuberculosis (TB), lung cancer, chronic obstructive pulmonary disease (COPD), and COVID-19, impair the lungs' ability to facilitate proper oxygen-carbon dioxide exchange. Causes range from smoking and environmental pollutants to infections, genetic predispositions, and autoimmune conditions. Symptoms often include breathlessness, coughing, wheezing, chest pain, and fatigue. Early diagnosis through radiological imaging and pulmonary function tests is crucial for reducing morbidity and mortality. Effective management requires timely intervention, lifestyle changes, and in some cases, long-term medication or surgical treatment. Chronic Lung Disease: Healthy vs. Diseased Tissue (Fig.1). The figure illustrates the differences between healthy and diseased lung tissue in chronic lung diseases. In healthy alveoli, optimal oxygen exchange occurs, ensuring efficient lung function. In conditions like COPD and emphysema, fibrotic tissue replaces healthy alveolar structures, leading to impaired lung function and reduced gas exchange. Asthma is characterized by inflamed bronchi, which causes airway obstruction and difficulty in breathing. Damaged alveoli further hinder gas exchange, contributing to respiratory issues. Key risk factors for these diseases include smoking, pollution, and infections, which can lead to significant lung damage over time [2]. Common Lung Diseases: Causes, Symptoms, and At-Risk Populations (Table.1). Prevalence and Mortality of Major Lung Diseases Worldwide (2023 Estimates) (Fig.2). The below bar chart presents 2023 global estimates of six major lung diseases, comparing their prevalence and annual death tolls (in millions). COVID-19 had the highest impact with approximately 769 million cases and 6.9 million deaths. Pneumonia followed with 450 million cases and 2.5 million deaths, and COPD showed 391 million cases with 3.23 million deaths, reflecting its chronic nature. Asthma affected 262 million people, but caused comparatively fewer deaths at 0.5 million. Tuberculosis (TB) registered 10.6 million cases and 1.25 million deaths, while lung cancer, despite its lower prevalence of 2.2 million cases, resulted in 1.8 million deaths due to its high mortality rate. These statistics emphasize the significant global health burden of lung diseases and the urgent need for targeted interventions.

METHODOLOGY

Dataset Description

The benchmark datasets, widely used in research on lung disease detection and classification, consist of chest X-ray and CT images annotated for various conditions such as nodules, tuberculosis, lung cancer, and COVID-19. Most of these datasets are publicly available through online repositories and have become essential resources for developing, training, and validating AI-driven medical imaging models.

Image processing

Image processing involves enhancing and analyzing images to extract useful information. It includes preprocessing (e.g., noise removal), segmentation (isolating regions), and feature extraction (detecting patterns like edges or textures). These features are then analyzed for tasks like classification or recognition.

Preprocessing in medical image analysis improves data quality for AI models by enhancing contrast, reducing noise, extracting lung regions (ROI), and augmenting data. These steps boost model accuracy and support robust lung disease detection [4].Kumar *et al*. (2025) introduced an Enhanced Multi-Model Deep Learning (EMDL) system for diagnosing lung diseases from chest X-rays by integrating five pre-trained models VGG-16, VGG-19,ResNet, AlexNet, and GoogleNet with image enhancement techniques such as histogram equalization and contrast adjustment. Feature selection was performed using PCA, SelectKBest, BPSO, and BGWO. The system achieved 98.71% accuracy on two public datasets, demonstrating its potential in clinical decision support [5].Picazo Castillo *et al*. (2024) improved COVID-19 detection in chest X-rays using region-specific contrast enhancement. Among CE-PSO, CLAHE, and their combination, the best performance was obtained with CLAHE (96% accuracy using MobileNetV2) and CE-PSO (95.6% accuracy with ResNet-50), confirming that localized preprocessing significantly boosts diagnostic performance [6].Thakur *et al*. (2025) optimized MobileNetV2 for lung disease classification through advanced preprocessing (CLAHE, bilateral filtering, Gaussian blur) and Bayesian optimization. Postprocessing with conditional random fields and ensemble averaging led to 99.20% accuracy, underscoring the model's robustness and precision [7].Patil and Narawade (2024) proposed a custom deep neural network (RDD_DNN) for respiratory disease detection using GLCM-based ROI extraction (RDD_ROI) and dataset balancing (RESP_DATA_BALANCE). The model achieved 97.5% accuracy, confirming the effectiveness of targeted preprocessing and custom architecture design [8].

Segmentation is vital for automated lung disease diagnosis in chest X-rays and CT scans, isolating lung fields, lesions, or abnormal tissues. Accurate segmentation improves feature extraction and classification, using methods like thresholding, clustering, and DL models such as U-Net. These techniques enhance disease visualization and localization, boosting diagnostic accuracy for conditions like pneumonia, tuberculosis, COVID-19, and lung cancer [9].Saimassay *et al*. (2024) improved the U-Net model for lung segmentation in CT and X-ray images by using advanced convolutional layers and enhanced skip connections. Their method achieved a high Dice score of 96.4% and a low Hausdorff distance of 4.7, offering more precise and efficient segmentation suitable for real-time clinical use, demonstrating DL's potential in lung disease diagnosis [10].Miah *et al*. (2024) introduced InfLocNet, a lightweight model using pre-trained VGG-16 and enhanced UNet++ for accurate lung infection detection and localization in chest X-rays. It achieved 97.61% Dice for lung segmentation and 93.86% accuracy in disease detection, suitable for real-time clinical use [11]. Alhajim *et al*. (2025) developed SEDARU-Net for lung nodule segmentation in CT images, combining intensity normalization, YOLOv3, and a modified U-Net with dilated convolutions and attention gates. Tested on LUNA16, it reached 97.86% Dice and 96.54% sensitivity, showing strong clinical potential [12].Zhou *et al*. (2024) used radiomics and a U-Net231 model on CT scans from 1066 patients to differentiate PRISm from COPD. By combining radiomic features with clinical data, their model achieved AUCs of 0.82, 0.77, and 0.84 in training, internal, and external sets, demonstrating high accuracy and clinical value [13].

Feature extraction in lung CT and X-ray images identifies key patterns like texture, shape, edges, and ROIs to highlight nodules, lesions, or abnormalities. These features improve the accuracy of machine learning models for diagnosing lung diseases such as pneumonia, tuberculosis, and lung cancer [14].Vinta *et al*. (2024) developed a hybrid DL model combining improved U-Net++ for lung segmentation, RAPNet for feature extraction, and MobileUNetV3 for classifying five ILD types, achieving superior accuracy for automated ILD diagnosis [15].Rocha *et al*. (2024) proposed STERN, an attention based Spatial Transformer Network that enhances chest X-ray classification by focusing on lung areas without explicit cropping. Tested on CheXpert, it improved AUC to 85.67% while using fewer parameters and minimal extra processing time [16]. Zheng *et al*. (2024) combined thresholding and a Time Series Feature Extraction method with a convex hull-based contour repair to improve lung CT segmentation in noisy and complex images, showing enhanced accuracy on COVID-19 CT datasets [17].Di Bari *et al*. (2025) retrospectively

analyzed 267 TB patients, finding that those coinfected with COVID-19 had higher diabetes prevalence, worse respiratory failure risk, and increased mortality, highlighting the severity of TB-COVID-19 coinfection and the need for timely clinical management [18].

Classification

Classification is a supervised learning task that assigns input data to predefined categories based on features. Models like decision trees, SVM, KNN, and neural networks learn from labeled data to predict classes for new inputs. This technique is widely used in medical diagnosis, image recognition, spam detection, and customer segmentation [19].

Canayaz *et al*. (2024) developed a DL framework for lung nodule classification and segmentation in CT images using C+EffxNet and backbone models InceptionV3, DenseNet121, and SeResNet101. Evaluated on public lung CT datasets, the model achieved 92.98% accuracy and strong segmentation performance (Jaccard index 0.8026, Dice coefficient 0.8877), supporting early lung cancer diagnosis [20].A. and N. (2023) proposed a hybrid DL and ML method for banana leaf disease classification on a realistic Banana Leaf dataset. Using Xception for feature extraction and Random Forest for classification, they achieved 90% accuracy with reduced computational cost [21].

Kaur *et al*. (2025) combined image preprocessing (sharpening, histogram equalization) and feature extraction (SIFT, HOG, LBP) with logistic regression on chest X-rays. The combined features yielded 95.12% accuracy and high precision, validated on standard X-ray datasets [22].Jannat *et al*. (2025) designed a lightweight Residual U-Net with attention modules for lung segmentation, tested on JSRT, Shenzhen, and Montgomery datasets. The model achieved Dice scores above 93%, demonstrating efficiency and accuracy for clinical use [23].

Goyal and Singh (2023) developed a multi-stage framework for pneumonia and COVID-19 detection from chest X-rays, combining image enhancement, ROI extraction, and classification using ML and DL (including RNN-LSTM). Evaluated on public datasets, the framework outperformed existing methods [24].

Yadav, Rizvi, and Agarwal (2024) compared CNNs with traditional ML classifiers for detecting pneumonia, TB, and COVID-19 from chest X-rays across multiple datasets. CNNs achieved up to 99% accuracy, confirming superior diagnostic capability [25].Sultana, Pramanik, and Rahman (2023) proposed a lightweight DNN for COVID-19, pneumonia, and TB classification, outperforming EfficientNetB0 and DenseNet models with precision over 97% on a composite dataset, suitable for resource-limited settings [26].Venkataramana *et al*. (2022) addressed imbalanced lung disease data using augmentation and SMOTE before CNN classification. The approach achieved 97.4% accuracy for TB and pneumonia, showing significant improvement over traditional methods [27]. Lung Disease Classification Accuracy (Existing Methods) (Table.2). Performance of Existing Lung Disease Classification Methods (Fig.3). This study evaluates the performance metrics (%) of recent peer-reviewed works (2022–2025) in Lung Disease. Comparative analysis reveals trends in accuracy/efficiency, with results ranging from 97.12% to 100%. The findings highlight top-performing methodologies and identify gaps for future research.

CONCLUSION

Artificial intelligence has significantly advanced the analysis of lung diseases through medical imaging, demonstrating exceptional capabilities in pre-processing, segmentation, and classification tasks. Despite impressive achievements such as high diagnostic accuracy and efficiency real-world clinical adoption depends on overcoming key challenges, including data scarcity, model transparency, and system scalability. Moving forward, the integration of explainable AI, multi-modal data, and lightweight architectures tailored for clinical deployment will be essential. Collaborative, interdisciplinary innovation will ultimately bridge the gap between research and practice, positioning AI as a cornerstone of next-generation respiratory healthcare.

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Table.1. Common Lung Diseases: Causes, Symptoms, and At-Risk Populations

Disease	Cause	Symptoms	Risk Group
Asthma	Airway inflammation	Wheezing, breathlessness	All ages
Pneumonia	Infection (bacteria/virus)	Chest pain, cough, fever	Elderly, children
TB	Airborne bacteria	Cough, weight loss, sweats	Close contacts
COVID-19	SARS-CoV-2 droplets	Fever, cough, fatigue	General population
COPD	Smoking, pollution	Chronic cough, wheezing	Smokers, elderly
Lung Cancer	Smoking, pollution	Coughing blood, weight loss	Smokers, family history

Table.2. Lung Disease Classification Accuracy (Existing Methods)

Author(s)	Year	Method Summary	Accuracy
Canayaz *et al*.	2024	C+EffxNet with InceptionV3, DenseNet121, SeResNet101 for lung nodule classification	92.98%
Abisha A *et al*.	2023	Xception for feature extraction + Random Forest for banana leaf disease	90%
Kaur *et al*.	2025	SIFT, HOG, LBP + Logistic Regression on chest X-rays	95.12%
Jannat *et al*.	2025	Residual U-Net with attention modules for lung segmentation	93%
Goyal *et al*.	2023	Multi-stage DL/ML framework (RNN-LSTM) for pneumonia and COVID-19	92%
Yadav *et al*.	2024	CNNs vs. ML for pneumonia, TB, and COVID-19 from X-rays	94%
Sultana *et al*.	2023	Lightweight DNN for COVID-19, pneumonia, and TB	95%
Venkataramana *et al*.	2022	SMOTE + CNN for TB and pneumonia	94%

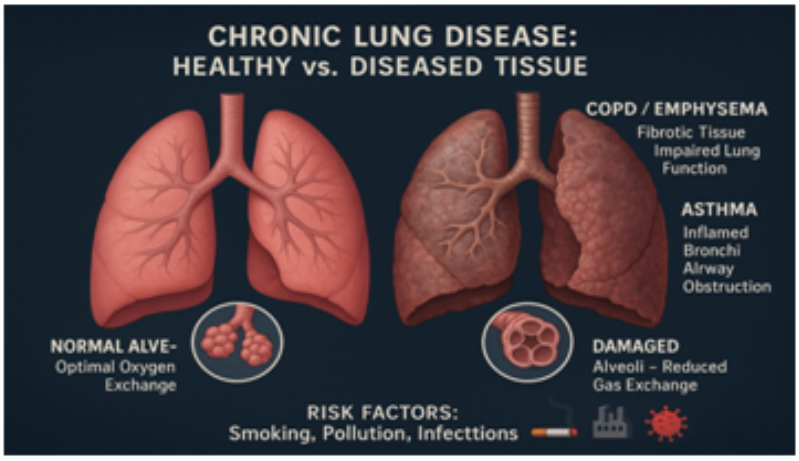


Fig.1: Chronic Lung Disease: Healthy vs. Diseased Tissue

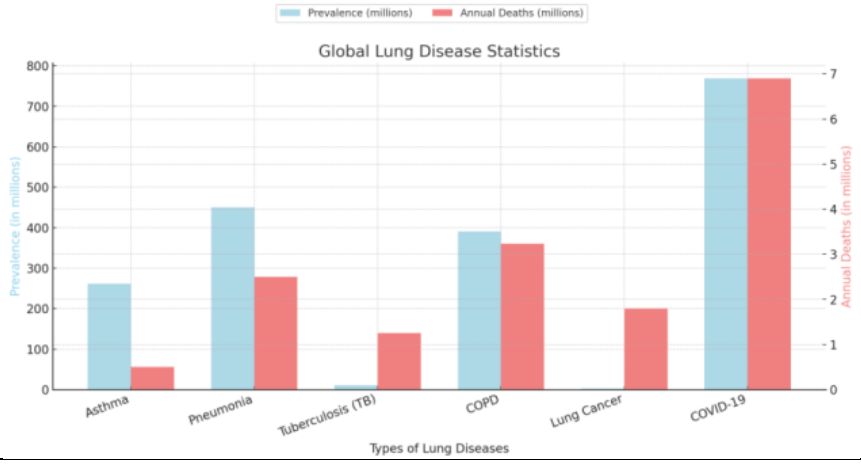


Fig.2: Prevalence and Mortality of Major Lung Diseases Worldwide (2023 Estimates)

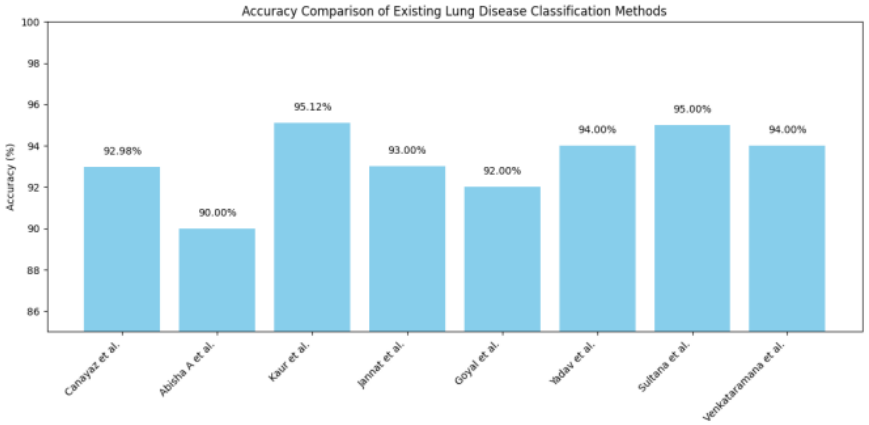


Fig.3: Performance of Existing Lung Disease Classification Methods

RESEARCH ARTICLE

Efficacy of a Polyherbal Formulation in the Management of Dyslipidemia : A Clinical Evaluation

Vidhi M Desai[1], Dipeeka Surawase[2]* and Vaishali Rathod[3]

[1]Student (Third Year) Kayachikitsa, Department of Kayachikitsa, Parul Institute of Ayurved, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[2]Associate Professor , Department of Kayachikitsa, Parul Institute of Ayurved, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[3]Assistant Professor, Department of Kayachikitsa, Parul Institute of Ayurved, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.

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***Address for Correspondence**
Dipeeka Surawase
Associate Professor ,
Department of Kayachikitsa,
Parul Institute of Ayurved,
Parul University, P.O. Limda,
Waghodia, Vadodara, Gujarat, India.
E.Mail: dipeeka.surwase21816@paruluniversity.ac.in

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ABSTRACT

Dyslipidemia, a prevalent metabolic disorder, is strongly associated with modern lifestyle factors such as poor diet, sedentary behavior, and chronic stress. It is a major modifiable risk factor for diabetes, cardiovascular diseases and metabolic syndrome. This study evaluates the efficacy of a herbal formulation in managing Dyslipidemia based on subjective symptoms, lipid profile alterations, and body composition changes. Three newly diagnosed Dyslipidemia patients were included,who are not taking any allopathic medicine for dyslipidemia and they underwent a 90-day treatment with a poly-herbal formulation containing Haritaki, Vacha, Rasna, Pippali, Shunthi, Pushkarmool, Arjuna, and Gaumutra. The study observed significant improvements in lipid profiles, with reductions in LDL, VLDL, triglycerides, and total cholesterol levels, along with enhanced BMI and overall well-being. Additionally, the formulation demonstrated anti-inflammatory, antioxidant, and cardio-protective effects. No significant adverse reactions were reported. The findings suggest that the herbal formulation may serve as a promising alternative therapy for Dyslipidemia, warranting further large-scale clinical studies.

Keywords:

INTRODUCTION

Today's lifestyle is largely characterized by poor dietary habits, limited physical activity, and excessive mental and intellectual exertion, often accompanied by stress, anxiety, and depression. These factors contribute to various imbalances in body composition, one of which is Dyslipidemia. Specifically, unhealthy eating patterns—such as the consumption of fast food, processed and convenience foods, high-calorie diets, and irregular meal timings—combined with a sedentary lifestyle, are the primary causes of Dyslipidemia. The prevalence of Dyslipidemia is on the rise and serves as a potential indicator of underlying comorbidities, such as diabetes mellitus, metabolic syndrome, obesity, hypertension, and cardiovascular diseases [1] Dyslipidemia, commonly referred to as a "lipoprotein disorder," is a condition characterized by an imbalance, either an excess or a deficiency, of lipoproteins [2]. This condition leads to abnormal blood lipid levels, including elevated total cholesterol, low-density lipoprotein cholesterol (LDL), triglycerides, and very low-density lipoprotein cholesterol (VLDL), alongside reduced levels of high-density lipoprotein cholesterol (HDL). These imbalances are hallmark indicators of Dyslipidemia, characterized by the irregular presence of one or more types of serum lipids[3]. Dyslipidemia is linked to various morbid conditions, including diabetes mellitus, obesity, chronic heart diseases, and thyroid disorders. It is highly prevalent and serves as a significant modifiable risk factor for cardiovascular diseases. Globally, cardiovascular disease has become one of the leading causes of death [4]A diet high in fats (Snigdha, Guru, Pichhila) and a sedentary lifestyle (Cheshtadvesha, Asana Sukha) are contributing factors to Medovruddhi, a Santarpanjanya Vyadhi as described in Ayurveda. According to Ayurvedic principles, habits such as lack of exercise (Avyayam), absence of mental stress (Achinta), daytime sleeping (Diwaswapna), excessive intake of unctuous (Atisnigdha) and sweet foods (Madhura), overeating (Adhyashan), consuming food in excessive quantity (Atimatra Ahara), and genetic predisposition (Beeja Swabhava) can lead to Medovaha Srotodushti [5]Dyslipidemia is highly prevalent and serves as a modifiable risk factor for cardiovascular disease. Globally, cardiovascular disease has become one of the leading causes of death [6]Elevated cholesterol levels are estimated to contribute to 18% of strokes and 56% of ischemic heart disease, resulting in approximately 4.4 million deaths annually worldwide[7]. Hyperlipidemia contributes to atherosclerosis, a major cause of coronary artery disease. In Ayurveda, hyperlipidemia is classified under Medoroga or Santarpanajanya Vyadhi. The pathogenesis of Medoroga involves Kapha (Kledaka), Vata (Samana and Vyana), Meda (fat/lipid), and Medodhatwagni.

AIM AND OBJECTIVES

To evaluate the efficacy of herbal formulation in dyslipidemia.

1. To evaluate the effect of herbal formulation in dyslipidemia on the basis of symptoms seen in patients
2. To evaluate the effect of herbal formulation on lipid profile and BMI
3. To evaluate the adverse effects, if any.

MATERIALS AND METHODS

In this study all patients taken are newly diagnosed for dyslipidemia so that patients are not taken any hypolipidemic medicines like statin. Patients who has hypertension, diabetes, hypothyroidism, liver disease and central obesity.

Inclusion criteria

1. Patient of either gender between age 18 and 70 years (both years inclusive)
2. Patient having one or more abnormal lipid level in lipid profile investigation
3. Patients with BMI from 18.5 to 40 kg/m^2

Exclusion criteria

1. Patients have other disease such as uncontrolled diabetes mellitus and hypertension

2. Dyslipidemia due to drugs e.g. glucocorticoids
3. Patients have history of MI, heart failure, stroke in last 6 months
4. Pregnant and lactating women
5. Patients who are currently participating in any other clinical trials(since last 2 months)
6. Immunosuppresive patients - HIV
7. Patients with infective diseases- active tuberculosis, hepatitis B

Assessment Criteria

Assessment of the patient will be for a period of 90 days
Improvement in the symptoms, if any and other effects will be noted
Laboratory investigation will be done before and after completion of the treatment

Diagnostic Criteria

Criteria for diagnosis (NCEP ATP 3): The patient having one or more abnormally elevated levels of lipid will be selected for this study[8].
Serum total cholesterol>200 mg/dl and/or
Serum triglycerides>150 mg/dl and/or
Serum LDL cholesterol>130 mg/dl and/or
Serum HDL cholesterol<40 mg/dl and/or
Serum VLDL cholesterol>40 mg/dl.

Case 1

The patient, a 40-year-old female opd no 24017109 come at parul ayurvedic hospital presented with complaints of breathlessness on exertion, generalized weakness, heaviness of the body, and weight gainand lower back pain since 1 year. The patient has a known history of hypothyroidism, diagnosed 6 months ago, and has been on thyroxine sodium 125 mcg. No history of smoking, alcohol use, or exposure to environmental pollutants was reported. There is no significant family history of cardiovascular or respiratory diseases. On examination, the patient had 36.3kg/m², suggesting obesity, Vital signs revealed pulse rate 78 bpm, blood pressure 120/70 mmHg, respiratory rate 18 breaths per minute, and HR80 per minute.

Case 2

The patient, a 60 -year-old female opd no 24003296 come at parul ayurved hospital , presented with complaints of breathlessness on exertion, persistent fatigue, generalized weakness since 3 months and bilateral knee joint pain since 8 months and numbness in the right upper limb for the past 1 month. The patient has a medical history of diabetes mellitus, diagnosedeight months ago, and has been on dapagliflozin and metformin hydrochloride tablet 10mg/500mg. No history of smoking, alcohol consumption, or occupational exposure to toxins was noted. Family history is significant for diabetes. On physical examination, the patient had 27.04kg/m², suggesting pre-obesity. Vital signs revealed pulse rate 60 bpm, blood pressure 120/90 mmHg, respiratory rate 16 breaths per minute, and HR68/min.

Case 3

The patient, a 36 year-old male opd no 24017400, presented with complaints of breathlessness on exertion, persistent fatigue, generalized weakness, and frontal region headache for the past 1 year. No history of fever, cough, chest pain, palpitations, or recent infections was reported. The patient has history of hypertension and he stop taking medicine since 6 months. No history of smoking, alcohol consumption, or occupational exposure to environmental pollutants was documented.the patient had 23.52 bmi which is normal. Vital signs revealed pulse rate 74/min, blood pressure 130/80 mmhg, respiratory rate 18/min nd heart rate 78/min

Ashthavidha Pariksha

Table:1

	Ritaben	Bhanuben	Narayanbhai
Naadi	Kaphapittaja	Kaphaja	Kaphapittaja
Mala	Prakruta	Prakruta	Aprakruta
Mutra	Pittabhavarna	Pittabhavarna	Pittabhavarna
Jihwa	Saama	Saama	Saama
Sabda	Prakruta	Prakruta	Prakruta
Sparsha	Anushnasheeta	Anushnasheeta	Anushnasheeta
Drika	Prakruta	Prakruta	Prakruta
Aakriti	Pravara	Pravara	madhyama

Treatment

कृष्णाशटीपुष्करमूलरास्नावचाभयानागरचूर्णकं॥

(Charak chikitsa 26/97)

Raw medications will be gathered and finely powdered. Three Bhavna (pregnancy procedures) with Arjuna bark Kashaya and three Bhavna with Gaumutra would subsequently be performed on the powdered mixture. These liquids will be thoroughly mixed, and then the mixture will be formed into the appropriate-sized pills.this medicine given for a three month and the dosage is three tablet before food.

Assessment Criteria

Table. 2: Breathlessness

Dyspnoea after Heavy works (movement) but relieved soon and up to tolerance	0
Dyspnoea after Moderate works but relieved later and up to tolerance	1
Dyspnoea after Little works but relieved later and up to tolerance	2
Dyspnoea after Little work and but relieved later and beyond tolerance	3
Dyspnoea in Resting condition	4

Table. 3: Fatigue

No Fatigue	0
Little Fatigue on doing hard work	1
Moderate Fatigue on doing routine work	2
Excessive Fatigue on doing routine work	3
Excessive Fatigue even on doing little work	4

Table. 4: Confusion

No Confusion	0
Little Confusion when mentally tired	1
Moderate Confusion with routine mental work	2
Excessive Confusion with routine mental work	3
Excessive Confusion with no mental work or having totally mental rest condition	4

Table. 5: Paresthesia

No any Abnormal sensation	0
Abnormal sensation lasting for few minutes (2-5 minutes) to an hour having 1-2 episodes in a week	1
Abnormal sensation lasting for few minutes (2-5 minutes) to an hour having >3 episodes in a week	2
Abnormal sensation lasting for more than an hour having 1-3 episodes in a week	3
Abnormal sensation lasting for more than an hour having >3 episodes in a week	4

Table. 6: Chala sphik udara stana/sphik stana udara lambana

Absence of Chalatva	0
Little visible movement (in the areas) after fast movement	1
Little visible movement (in the areas) even after moderate movement	2
Movement (in the areas) after mild movement	3
Movement (in the areas) even after changing posture	4

Table. 7: Alasya

No alasya doing work satisfactory with proper vigor in time	0
Doing work satisfactory with late initiation	1
Doing work unsatisfactory under mental pressure and takes time	2
Not initiation of work or doing little work very slowly	3
No initiation of work and not want to work even after pressure	4

Table. 8: Daurbalya

Can do Routine exercise	0
Can do Moderate exercise without difficulty	1
Can do only Mild exercise	2
Can do Mild exercise with difficulty	3
Cannot do even Mild exercise	4

Table. 9: Nidradhikya

Normal Sleep 6-7 hrs per day	0
Sleep up to 8 hrs per day with Anga-gaurava	1
Sleep up to 8 hrs per day with Anga-gaurava and jrimbha	2
Sleep up to 10 hrs per day with Tandra	3
Sleep More than 10 hrs per day with Tandra and Klama	4

Table. 10: Swedadhikya

Sweating after Heavy work and fast movement or in very hot season	0
Profuse sweating after Moderate work and movement	1
Sweating after Little work and movement	2
Profuse sweating after Little work and Movement	3
Sweating even at Rest or in Cold season	4

Table. 11: Daurgandhya

Absence of body bad Odour	0
Occasional bad body Odour removed after bathing	1
Persistent bad body Odour limited to closed areas difficult to suppress with deodorants	2
Persistent body Odour limited felt from long distance not suppressed with deodorants	3
Persistent bad body Odour limited felt from long distance not tolerated even by patient himself	4

Table. 12: Ati Pipasa

Normal Thirst	0
Up to 1 Litre excess intake of water	1
1-to-2-Litre excess intake of water	2
2-to-3 Litre excess intake of water	3
More than 3 Litre excess intake of water	4

Table. 13: Ati Kshudha

As usual	0
Slightly Increased(1-2 Meals)	1
Moderately Increased(3-4 Meals)	2
Markedly Increased(5-6 Meals)	3

Table. 14: Anga Gaurava

No Heaviness in the body	0
Feels Heaviness in the body but it does not hamper Routine work	1
Feels Heaviness in the body which hampers daily Routine work	2
Feels Heaviness in the body which hampers movement of the body	3
Feels Heaviness in the body along with flabbiness which causes great distress the person	4

Table. 15: Bhara vriddhi

BMI below 23	0
BMI 23-25	1
BMI 25-27	2
BMI 27-29	3
BMI 29-32	4
BMI above 32	5

Observations and Results

Significant improvement was observed in the symptoms after the 3 months of treatment duration are as follows.

Table. 16: Subjective parameter

	Patient 1 (before)	Patient 1 (after)	Patient 2(before)	Patient 2(after)	Patient 3(before)	Patient 3 (after)
Breathlessness	1	0	1	0	1	0
Fatigue	1	1	3	1	2	1
Confusion	1	1	1	0	0	0
Paresthesia	0	0	2	0	0	0
Chala sphika udara stana lambana	2	1	2	1	0	0
Alasya	0	0	2	1	1	1
Daurbalya	1	0	2	0	1	0
Nidradhikya	2	1	2	1	0	0
Swedadhikya	1	1	1	1	1	1
Daurgandhya	0	0	1	1	0	0
Ati pipasa	2	1	2	1	1	1
Ati kshudha	2	1	2	1	1	1
Angagaurava	1	0	2	1	0	1
bharavriddhi	5	5	3	2	1	0

Table. 17: Lipid profile (before)

Investigation	Result (Patient 1)	Result (Patient 2)	Result (Patient 3)	Normal Range
HDL	64.86 mg/dl	72.19 mg/dl	58.34 mg/dl	40-60
LDL	94.08 mg/dl	59.15 mg/dl	70.02 mg/dl	0.0-130

VLDL	65.06 mg/dl	118.06 mg/dl	55.54 mg/dl	0-35
S.Triglycerides	325.3 mg/dl	590.3 mg/dl	277.7 mg/dl	<150
Total Cholesterol	224 mg/dl	249.4 mg/dl	183.9 mg/dl	110-200
Chol./HDL Ratio	3.45	3.45	3.15	
LDL/HDL Ratio	1.45	0.81	1.20	Desirable level/low risk:0.5-3.0 Borderline level/moderate risk : 3.0-6.0 Elevated level/high risk : >6.0

Table. 18: Lipid Profile (after)

Investigation	Result (Patient 1)	Result (Patient 2)	Result (Patient 3)	Normal Range
HDL	58.28 mg/dl	65.50 mg/dl	55.8 mg/dl	40-60
LDL	67.22 mg/dl	99.28 mg/dl	68.2 mg/dl	0.0-130
VLDL	42.5 mg/dl	78.72 mg/dl	30.00 mg/dl	0-35
S.Triglycerides	212.5 mg/dl	393.6mg/dl	150 mg/dl	<150
Total Cholesterol	168 mg/dl	243.5 mg/dl	154 mg/dl	110-200
Chol./HDL Ratio	2.88	3.71	2.76	
LDL/HDL Ratio	0.73	1.51	1.22	Desirable level/low risk:0.5-3.0 Borderline level/moderate risk : 3.0-6.0 Elevated level/high risk : >6.0

Table. 19: Liver function test (before)

Investigation	Result (Patient 1)	Result (Patient 2)	Result (Patient 3)	Normal Range
SGPT	21.22 U/L	19.45 U/L	14.14 U/L	0-40
SGOT	28.29 U/L	10.61 U/L	40.66 U/L	0-37
Albumin	4.78 gm/dl	4.94 gm/dl	5.36 gm/dl	3.4-5.0
Albumin/Globulin Ratio	1.62 gm/dl	2.28 gm/dl	3.80 gm/dl	1.0-2.1
Alkaline Phosphatase (ALP)	97.85 U/L	70.48 U/L	79.05 U/L	6-113
Bilirubin Direct	0.21 mg/dl	0.39 mg/dl	0.36 mg/dl	0.0-0.25
Bilirubin Indirect	0.26 mg/dl	0.40 mg/dl	0.74 mg/dl	0.2-0.8
Bilirubin Total	0.47 mg/dl	0.79 mg/dl	1.10 mg/dl	0-1.0
Globulin	2.94 gm/dl	1.76 gm/dl	1.41 gm/dl	2.0-4.1
Protein	7.72 gm/dl	6.70 gm/dl	6.77 gm/dl	6.4-8.2

Table. 20: Liver Function Test (after)

Investigation	Result (Patient 1)	Result (Patient 2)	Result (Patient 3)	Normal Range
SGPT	28.29 U/L	10.61 U/L	36 U/L	0-40
SGOT	28.29 U/L	31.82 U/L	39 U/L	0-37
Albumin	4.84 gm/dl	5.05 gm/dl	4.89 gm/dl	3.4-5.0
Albumin/Globulin Ratio	1.85 gm/dl	2.70 gm/dl	1.61 gn/dl	1.0-2.1
Alkaline phosphatase (ALP)	77.88 U/L	77.67 U/L	62 U/L	6-113
Bilirubin Direct	0.19 mg/dl	0.30 mg/dl	0.25 mg/dl	0.0-0.25
Bilirubin Indirect	0.54 mg/dl	0.39 mg/dl	0.66 mg/dl	0.2-0.8
Bilirubin Total	0.73 mg/dl	0.69 mg/dl	0.91 mg/dl	0-1.0

Globulin	2.61 gm/dl	1.87 gm/dl	3.03 gm/dl	2.0-4.1
Protein	7.45 gm/dl	6.92 gm/dl	7.92 gm/dl	6.4-8.2

RBS	Patient 1	Patient 2	Patient 3
Before	120	114	100
After	114	98	97

Table. 21: ECG Finding

Patient 1	Normal sinus rhythm Longitudinal left axis deviation 2 aVF V5 V6 Abnormal T wave
Patient 2	Normal sinus rhythm Longitudinal left axis deviation 1 aVL V5 V6 Abnormal T wave
Patient 3	Cardiac electric axis normal

Table. 22: Body Circumference Measurement

Body area	Patient 1 (before)	Patient 1 (after)	Patient 2 (before)	Patient 2 (after)	Patient 3 (before)	Patient 3 (after)
Chest	107 cm	105 cm	92 cm	90 cm	84 cm	82 cm
Waist	105 cm	102 cm	89 cm	87 cm	86 cm	83 cm
Pelvis	117 cm	114 cm	96 cm	92 cm	88 cm	85 cm
Hip	128 cm	122 cm	100 cm	97 cm	92 cm	89 cm
Mid thigh	60 cm	55 cm	45 cm	42 cm	40 cm	39 cm
Mid calf	43 cm	40 cm	32 cm	30 cm	36 cm	34 cm

Table. 23: Skin Fold Thickness

Body area	Patient 1 (before)	Patient 1 (after)	Patient 2 (before)	Patient 2 (after)	Patient 3(before)	Patient 3 (after)
Biceps	36.9 mm	32.7 mm	15.2 mm	12.3 mm	19 mm	18.2 mm
Triceps	13.7 mm	12.9 mm	14.3 mm	10.5 mm	13.2 mm	11.8 mm
Abdomen	44.9 mm	40.0 mm	39.5 mm	32.5 mm	32 mm	30 mm

Table. 24: WEIGHT

Patient	Weight (before)	Weight (after)
Patient 1	94.20 kg	91.80 kg
Patient 2	53 kg	51.80 kg
Patient 3	68 kg	65.30 kg

DISCUSSION

This herbal formulation contain total 9 ingredients which are haritaki, Vacha, Rasna , Pippali, Shunthi , Shati, Pushkarmool, arjuna and gaumutra, which possess diverse properties that contribute to their therapeutic effects in Ayurveda. Haritaki, with Laghu and Ruksha Guna, Ushna Veerya, and Madhura Vipaka, balances all three dosha. Vacha, Rasna, and Shati alleviate Kapha and Vata disorders due to their Tikta and Katu Rasa and Ushna Veerya. Pippali and Shunthi, with Katu Rasa and Snigdha Guna, improve digestion and respiratory health while balancing Pitta and Kapha. Pushkarmool and Arjuna, known for their Tikta and Kashaya Rasa, address Kapha and Pitta

imbalances, promoting cardiovascular and respiratory health. Gomutra, with its detoxifying and Kapha-Vata shamaka properties, enhances immunity and purifies the body. These herbs work synergistically to restore doshic balance and promote holistic well-being. Haritaki is known for its potent antioxidant and hypocholesterolemic properties, while clinical studies have demonstrated itsanti-inflammatory, cardioprotective, hepatoprotective, hypolipidemic, and antidiabetic activities, making it a versatile herb with wide-ranging therapeutic benefits[9]. Vacha exhibits a wide range of therapeutic effects, including antihypertensive, anti-inflammatory, cardioprotective, and anti-obesityproperties[10]. rasna has anti-inflammatory and anti oxidant activity[11]. Pippali is extensively used as anti-inflammatory, hypoglycaemic and hepatoprotective[12]. Shunthi is used in several disease acting as a Antioxidant, Anti-inflammatory, cardiotonic, digestive stimulant and Glucose, cholesterol and lipid lowering agent[13]. The Rhi- zome of the shati is carminative, hepatoprotective, anti-inflammatory, hypoglycaemic and hypotensive[14]. Pushkarmula has anti-inflammatory, cardiovascular, hypoglycemic, antianginal, antioxidant, hepatoprotective, cardioprotective activity[15]. The Indian subcontinent has been using Arjuna bark decoction for ages to treat anginal discomfort, hypertension, congestive heart failure, and dyslipidemia, according to the observations of ancient physicians.According to the majority of experimental and clinical research, the crude medication has anti-ischemic, antioxidant, hypolipidemic, and Anti-atherogenic properties. Its effects are cardio-protective and antioxidant[16]. Cow urine has anti-diabetic and antioxidant property[17]. Haritaki, Arjuna, and Pushkarmool regulate lipid levels and improve heart health, while Shunthi and Pippali aid in glucose and cholesterol management. Vacha, Rasna, and Shati further enhance lipid metabolism and liver function. These herbs, along with cow urine, provide a holistic solution for dyslipidemia.

CONCLUSION

The study highlights the increasing prevalence of Dyslipidemia due to modern lifestyle factors such as poor diet, sedentary behavior, and stress. Dyslipidemia is a major risk factor for cardiovascular diseases, diabetes, and metabolic disorders. This research aimed to evaluate the effectiveness of a herbal formulation in managing Dyslipidemia.The study included three patients diagnosed with Dyslipidemia who were treated with the herbal formulation over a 90-day period. The results indicated a significant improvement in subjective symptoms, lipid profiles, and body measurements. Notably, there was a reduction in LDL, VLDL, triglycerides, and total cholesterol levels, along with improvements in BMI and overall well-being.The herbal formulation, which included ingredients such as Haritaki, Vacha, Rasna, Pippali, Sunthi, and Arjuna, demonstrated multiple pharmacological effects, including lipid-lowering, anti-inflammatory, and cardioprotective properties. No significant adverse effects were reported. Overall, the study suggests that the herbal formulation may be an effective alternative for managing Dyslipidemia.

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RESEARCH ARTICLE

The Impact of Emotional Intelligence on Leadership and Management : Transforming Organizational Culture and Enhancing Performance

Nasreen Bano Shaikh[2] and Mohammad Nasir[2]*

[1]Ph.D Research Scholar (Reg. No. 2207PHD30BZ18), Department of Library and Information Science, Kalinga University, Raipur, Chhattisgarh, India.
[2]Professor cum University Librarian, Department of Library and Information Science, Kalinga University, Raipur, Chhattisgarh, India.

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***Address for Correspondence**
Mohammad Nasir
Professor cum University Librarian,
Department of Library and Information Science,
Kalinga University, Raipur, Chhattisgarh, India.
E.Mail: Mohammad.nasir@kalingauniversity.ac.in

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ABSTRACT

Emotional intelligence (EI) is recognized as a significant element affecting organizational performance and enhancing the organizational culture. Organizational culture is an essential component for enhancing organizational efficacy. Numerous studies emphasize the significance of organizational culture inside an organization. Organizational culture and transformative leadership are associated with organizational effectiveness. Transformational leaders influence organizational culture since the ideals embraced by these leaders are more readily absorbed by employees, reflecting a positive association between transformational leadership and organizational culture. Emotional intelligence may significantly contribute to the formation and enhancement of organizational culture inside organizations. This study examines the substantial influence of emotional intelligence (EI) on leadership and management, emphasizing its transformative effect on organizational culture and performance improvement. In a time characterized by swift transformation and intricacy, the capacity of leaders to comprehend and regulate their own emotions, along with those of their team members, has become increasingly essential. Emotional intelligence comprises four fundamental elements: self-awareness, self-regulation, social awareness, and relationship management. Each factor is necessary for effective leadership, allowing leaders to manage interpersonal dynamics, promote collaboration, and motivate their teams toward common objectives. The research commences with an extensive examination of the current literature about emotional intelligence, leadership styles, and organizational success. It emphasizes that leaders with elevated emotional intelligence are more proficient at identifying and addressing the emotional needs of their people, hence fostering a supportive and inclusive workplace. This study also investigates other leadership ideas, including transformational and servant leadership, which closely correspond with

the tenets of emotional intelligence. Leaders who exemplify these traits are frequently more effective in inspiring their staff, fostering creativity, and attaining organizational goals. The research examines the correlation between emotional intelligence and organizational culture. An affirmative organizational culture is defined by trust, transparent communication, and a feeling of belonging among employees. Leaders exhibiting emotional intelligence are more adept at fostering an environment conducive to dispute resolution, delivering constructive feedback, and promoting employee engagement in decision-making processes. This inclusive strategy not only improves staff morale but also results in heightened engagement and retention rates. This research implies that human resource practitioners should prioritize the enhancement of leaders' emotional intelligence through effective recruitment, selection procedures, and training programs that integrate emotional intelligence features. The emotional intelligence of leaders significantly influences the behavioural outcomes of subordinates, including their perceptions of organizational activities.

Keywords: Emotional Intelligence, Leadership, Organizational Culture, Performance Engagement

INTRODUCTION

Leadership effectiveness denotes the degree to which leaders achieve the desired goals of individuals and organizations. Organizational performance measures the degree to which an organization fulfills its goals and objectives, encompassing profitability, productivity, and customer satisfaction. A positive association exists between leadership effectiveness and organizational performance, as skilled leaders may motivate, inspire, and influence their followers to attain elevated performance levels and enhance organizational success(Amisha, 2024).Emotional intelligence (EI) has become an essential factor in effective leadership, altering organizational management and collaboration. A study by TalentSmart revealed that 90% of high achievers exhibit elevated emotional intelligence, which enhances job performance and cultivates a pleasant workplace culture(vorecol.com, 2024). In the contemporary business landscape, the significance of emotional intelligence (EI) in leadership and management has garnered increasing attention from scholars and practitioners alike. Emotional intelligence, defined as the ability to recognize, understand, and manage one's own emotions and the emotions of others (Goleman, 1995), plays a pivotal role in shaping effective leadership styles and fostering a positive organizational culture. As organizations navigate the complexities of a rapidly changing environment, leaders equipped with high emotional intelligence are better positioned to inspire their teams, drive performance, and cultivate a culture of collaboration and resilience.The relationship between emotional intelligence and leadership effectiveness has been extensively studied, revealing that leaders with high EI are more adept at building strong interpersonal relationships, resolving conflicts, and motivating employees (Mayer and Caruso, 2004). These leaders are not only able to manage their own emotional responses but also possess the capacity to empathize with their team members, creating an atmosphere of trust and psychological safety. This emotional attunement is crucial in today's diverse workplaces, where understanding and valuing different perspectives can lead to enhanced creativity and innovation (Cherniss, 2010).Moreover, the impact of emotional intelligence extends beyond individual leadership effectiveness; it significantly influences organizational culture and performance. A culture that prioritizes emotional intelligence fosters open communication, inclusivity, and a shared sense of purpose among employees. Research indicates that organizations with emotionally intelligent leaders experience lower turnover rates, higher employee satisfaction, and improved overall performance (Goleman, Boyatzis, and McKee, 2002). This correlation underscores the importance of integrating emotional intelligence into leadership development programs and organizational practices.In addition to enhancing interpersonal dynamics, emotional intelligence contributes to effective decision-making and problem-solving within organizations. Leaders who exhibit high EI are more likely to approach challenges with a balanced perspective, considering both emotional and rational factors. This holistic approach not only leads to better outcomes but also encourages a culture of continuous learning and adaptability (Bar-On, 2006). As organizations face

unprecedented challenges, such as technological advancements and global competition, the ability to navigate these complexities with emotional intelligence becomes increasingly vital. Furthermore, the integration of emotional intelligence into leadership and management practices can serve as a catalyst for organizational change. Leaders who embody emotional intelligence are more likely to champion initiatives that promote employee well-being and engagement, ultimately leading to a more resilient and high-performing organization (Cherniss and Goleman, 2001). By prioritizing emotional intelligence, organizations can transform their culture, aligning it with the values of empathy, collaboration, and innovation.

Related Studies

Emotional intelligence (EI) has emerged as a critical factor influencing leadership and management effectiveness in contemporary organizations. Defined as the ability to recognize, understand, and manage one's own emotions and the emotions of others, EI encompasses skills such as emotional awareness, empathy, and interpersonal communication. This literature review synthesizes recent studies that explore the relationship between emotional intelligence and various aspects of leadership and management, highlighting its transformative potential in organizational culture and performance.

Emotional Intelligence and Leadership Effectiveness

Recent research underscores the importance of emotional intelligence in enhancing leadership effectiveness. For instance, a study by Wong and Law (2023) found that leaders with high EI are more successful in fostering team cohesion and motivation, leading to improved performance outcomes. Similarly, a meta-analysis by Joseph and Newman (2022) confirmed that EI is positively correlated with transformational leadership behaviors, which are essential for inspiring and guiding teams through change.

EI and Organizational Culture

The influence of emotional intelligence on organizational culture has been a focal point in recent literature. A study by Cummings et al. (2024) demonstrated that organizations led by emotionally intelligent leaders cultivate a culture of trust and collaboration, which significantly enhances employee engagement and satisfaction. Furthermore, research by Salovey and Mayer (2023) indicates that emotionally intelligent organizations are more adaptable to change, fostering an environment conducive to innovation.

EI and Employee Performance

The relationship between emotional intelligence and employee performance has been extensively examined. A longitudinal study by Schutte et al. (2023) revealed that employees working under emotionally intelligent leaders exhibit higher levels of job satisfaction and performance. Additionally, a study by Brackett et al. (2022) highlighted that EI training programs for leaders can lead to measurable improvements in team performance and productivity.

EI in Conflict Resolution

Emotional intelligence plays a crucial role in conflict resolution within organizations. Research by De Dreu and Gelfand (2023) found that leaders with high EI are more effective in mediating conflicts, as they can empathize with differing perspectives and facilitate constructive dialogue. This ability not only resolves disputes but also strengthens team dynamics and relationships.

EI and Decision-Making

The impact of emotional intelligence on decision-making processes has also been explored. A study by Ashkanasy and Daus (2023) suggests that leaders with high EI are better equipped to make balanced decisions that consider both emotional and rational factors. This holistic approach leads to more effective problem-solving and strategic planning.

EI Training and Development

The implementation of EI training programs has gained traction in leadership development initiatives. Research by Cherniss and Goleman (2023) emphasizes the effectiveness of such programs in enhancing leaders' emotional

competencies, which in turn positively affects organizational outcomes. Organizations that invest in EI training report higher employee morale and lower turnover rates.

EI and Diversity Management

Emotional intelligence is increasingly recognized as a vital component of diversity management. A study by Shore et al. (2024) found that leaders with high EI are more adept at managing diverse teams, as they can navigate cultural differences and foster an inclusive environment. This capability not only enhances team performance but also drives innovation through diverse perspectives.

Objective of the Studies

The primary objectives of this study are

- To Analyze the Role of Emotional Intelligence in Shaping Organizational Culture.
- Assess the Impact of Emotional Intelligence on Employee Performance.
- Explore the Influence of Emotional Intelligence on Conflict Resolution.
- Explore the Relationship Between Emotional Intelligence and Diversity Management.
- Provide Recommendations for Integrating Emotional Intelligence into Leadership Development.

EI and Organizational Culture

Emotional intelligence (EI) plays a pivotal role in shaping organizational culture and influencing how employees interact, collaborate, and perform within their work environment. Defined as the ability to recognize, understand, and manage one's own emotions and the emotions of others, EI is increasingly recognized as a critical factor in fostering a positive workplace culture.

Interconnection Between EI and Organizational Culture: Emotional Intelligence Defined: Emotional intelligence encompasses five key components: self-awareness, self-regulation, motivation, empathy, and social skills. These elements enable individuals to navigate social complexities and build strong relationships, which are essential in a collaborative work environment (Goleman, 1995).

Influence on Leadership: Leaders with high emotional intelligence can inspire and motivate their teams, creating a culture of trust and respect. They are adept at recognizing the emotional needs of their employees, which allows them to address concerns proactively and foster a supportive atmosphere (Cherniss, 2010). For instance, emotionally intelligent leaders are more likely to engage in active listening, validate employee feelings, and provide constructive feedback, all of which contribute to a positive organizational climate.

Empathy and Understanding: A culture that values empathy encourages employees to express their emotions and concerns openly. This openness leads to improved communication and collaboration, as team members feel safe to share their ideas and feedback (Tenney, 2020). Organizations that prioritize emotional intelligence often see a reduction in workplace conflicts and an increase in team cohesion.

Employee Engagement and Satisfaction: High emotional intelligence in leadership correlates with increased employee engagement and job satisfaction. Research indicates that organizations with emotionally intelligent leaders report a 30% increase in employee satisfaction and a 20% increase in productivity (Vorecol, 2023). When employees feel valued and understood, they are more likely to be committed to their work and the organization's goals.

Enhanced Team Dynamics: Emotional intelligence fosters better teamwork and collaboration. Teams with high emotional intelligence are more effective in managing conflicts and navigating challenges, leading to improved performance outcomes (Goleman, 1998). For example, a study found that teams with emotionally intelligent members had a 20% higher performance rate compared to those with lower EI (Vorecol, 2023).

Retention and Turnover Rates: Organizations that cultivate an emotionally intelligent culture experience lower turnover rates. Employees are less likely to leave an organization where they feel emotionally supported and valued. A study by Talent Smart revealed that companies with high emotional intelligence frameworks had a turnover rate 60% lower than those that did not prioritize EI (Vorecol, 2023).

Implementing EI Training Programs: To enhance emotional intelligence within the workforce, organizations can implement training programs focused on developing EI skills. These programs can help employees improve their self-awareness, empathy, and interpersonal skills, ultimately contributing to a more positive organizational culture (Cherniss, 2010).

Long-term Benefits: Investing in emotional intelligence training not only benefits individual employees but also enhances overall organizational performance. Companies that prioritize EI training often see significant improvements in employee morale, productivity, and innovation (Tenney, 2020).

EI on Employee Performance

Emotional intelligence (EI) significantly impacts employee performance by enhancing interpersonal relationships, communication, and overall workplace dynamics. Defined as the ability to recognize, understand, and manage emotions, EI contributes to improved job performance through various mechanisms.Research indicates that employees with high emotional intelligence tend to exhibit better teamwork and collaboration, leading to increased productivity and job satisfaction. Emotionally intelligent individuals are adept at navigating social complexities, which allows them to build strong relationships with colleagues and supervisors (Goleman, 1995). This ability fosters a positive work environment where employees feel valued and understood, ultimately enhancing their performance.

Enhanced Communication: Employees with high EI are better communicators, which reduces misunderstandings and conflicts in the workplace. Effective communication is crucial for successful collaboration and project completion (Mayer, Salovey, and Caruso, 2004).

Stress Management: High emotional intelligence enables employees to manage stress effectively, leading to improved focus and productivity. Individuals who can regulate their emotions are less likely to experience burnout and are more resilient in the face of challenges (Cherniss, 2010).

Collaboration and Teamwork: EI promotes a collaborative culture where team members support one another. Research shows that teams with high emotional intelligence outperform those with lower EI by 20% (Vorecol, 2023). This enhanced teamwork contributes to achieving organizational goals more efficiently.

Employee Engagement: Organizations that prioritize emotional intelligence often see higher levels of employee engagement. Engaged employees are more committed to their work, leading to lower turnover rates and higher overall performance (Vorecol, 2023).

EI on Conflict Resolution

Emotional intelligence (EI) significantly influences conflict resolution by shaping how individuals perceive, interpret, and respond to conflicts. EI encompasses the ability to recognize and manage one's own emotions and those of others, which is crucial in navigating interpersonal disputes, especially in high-stress situations where emotions can escalate.

Enhanced Communication: Individuals with high EI can articulate their thoughts and feelings clearly while also engaging in active listening. This reduces misunderstandings and fosters trust, which is essential for resolving conflicts.

Empathy in Action: Empathy allows individuals to grasp the emotional states of others, transforming confrontational situations into collaborative problem-solving opportunities. By recognizing and validating others' feelings, parties can work towards mutually beneficial solutions.

Self-regulation: Those with high EI can maintain composure during conflicts, preventing emotional escalation. This ability to self-regulate helps keep discussions focused on resolution rather than on the conflict itself.

Building Trust: Trust is a critical element in conflict resolution. Individuals perceived as emotionally intelligent are often seen as more trustworthy and fair, fostering a cooperative environment where all parties feel safe to express their views.

Continuous Self-reflection: Regularly reflecting on one's emotional responses can improve self-awareness and self-regulation.

Training and Education: Engaging in professional development courses focused on emotional management can enhance a mediator's ability to understand and manage emotions effectively.

Active Listening: Practicing active listening during conversations helps develop empathy and improves communication skills.

Mindfulness Practices: Techniques such as meditation can help individuals maintain calm in stressful situations, enhancing their ability to manage emotions.

Relationship Between EI and Diversity Management

The relationship between emotional intelligence and diversity management is integral to creating inclusive and effective workplaces. By fostering emotional intelligence, organizations can enhance communication, empathy, and adaptability among employees, leading to better conflict resolution and team dynamics. As diversity continues to grow in the workplace, the importance of integrating EI into diversity management strategies will only increase, paving the way for more harmonious and productive organizational cultures.

Enhanced Communication: High EI individuals are better communicators, which is vital in diverse environments where misunderstandings can arise from cultural differences. They can articulate their thoughts clearly and listen actively, fostering an atmosphere of open dialogue.

Empathy and Understanding: Empathy, a core component of EI, allows individuals to appreciate diverse perspectives. This understanding is crucial in managing conflicts that may arise from cultural misunderstandings or differing values.

Conflict Resolution: EI equips individuals with the skills to navigate conflicts that may stem from diversity-related issues. By managing their emotions and understanding others' feelings, they can facilitate constructive discussions and find common ground.

Building Inclusive Teams: Leaders with high EI can create inclusive teams by recognizing and valuing the unique contributions of each member. They can foster a sense of belonging, which is essential for employee engagement and retention.

Adaptability: Emotional intelligence enhances adaptability, allowing individuals to adjust their behaviors and approaches in response to diverse situations and individuals. This flexibility is crucial in dynamic and multicultural workplaces.

Strategies for Integrating Emotional Intelligence into Diversity Management

Training Programs: Organizations should implement training programs focused on developing EI skills among employees. This can include workshops on emotional awareness, empathy training, and effective communication strategies.

Leadership Development: Leaders should be trained to recognize the importance of EI in managing diversity. This includes understanding their own biases and learning how to create an inclusive environment.

Feedback Mechanisms: Establishing feedback systems that encourage open communication can help identify areas where emotional intelligence can be improved in relation to diversity management.

Mentorship Programs: Pairing employees from diverse backgrounds with mentors can enhance understanding and foster relationships that bridge cultural gaps.

Promoting a Culture of Inclusion: Organizations should actively promote a culture that values diversity and encourages employees to express their unique perspectives. This can be achieved through policies, practices, and recognition programs that celebrate diversity.

Challenges in Merging Emotional Intelligence with Diversity Management

Resistance to Change: Some employees may resist initiatives aimed at enhancing diversity and inclusion, viewing them as unnecessary or threatening. High EI leaders can help mitigate this resistance by fostering open discussions and addressing concerns empathetically.

Unconscious Bias: Even individuals with high EI may struggle with unconscious biases that affect their interactions with diverse colleagues. Continuous training and self-reflection are necessary to combat these biases.

Measurement of EI: Assessing emotional intelligence can be challenging, as it often relies on subjective measures. Organizations should consider using validated assessment tools to gauge EI levels among employees.

Impact of EI on Leadership

The impact of emotional intelligence on leadership is profound, influencing communication, team dynamics, conflict resolution, motivation, and adaptability. As organizations continue to navigate complex and diverse environments, the importance of developing emotionally intelligent leaders will only grow. By investing in emotional intelligence training and fostering a culture that values EI, organizations can enhance their leadership effectiveness and drive overall success.Emotional intelligence (EI) significantly impacts leadership effectiveness, shaping how leaders interact with their teams, make decisions, and drive organizational success. EI encompasses the ability to recognize, understand, and manage one's own emotions and the emotions of others, which is crucial for effective leadership in today's dynamic work environments Understanding Emotional Intelligence in Leadership. Emotional intelligence (EI) is a critical factor in effective leadership, influencing how leaders manage their own emotions and those of their team members.

It consists of several key components

Self-awareness: Recognizing one's own emotions and their effects on thoughts and behavior.

Self-regulation: The ability to control or redirect disruptive emotions and impulses.

Motivation: A passion for work that goes beyond money and status, driven by a desire to achieve for the sake of achievement.

Empathy: The capacity to understand the emotional makeup of other people and treat them according to their emotional reactions.

Social skills: Proficiency in managing relationships and building networks, essential for effective collaboration.

The Role of EI in Leadership Effectiveness

Leaders with high emotional intelligence are better equipped to handle the complexities of their roles. The impact of EI on leadership can be observed in several areas:

Improved Communication: Leaders with high EI can communicate more effectively, fostering an environment of open dialogue. They are adept at articulating their thoughts clearly and listening actively, which is essential in preventing misunderstandings and conflicts.

Enhanced Team Dynamics: EI enables leaders to build strong relationships within their teams. By understanding and managing their own emotions, as well as those of their team members, they can create a supportive and collaborative work environment.

Conflict Resolution: High EI leaders are skilled at navigating conflicts. They can manage their emotions during stressful situations and empathize with others, facilitating constructive discussions and finding common ground.

Motivation and Engagement: Leaders who demonstrate emotional intelligence can inspire and motivate their teams. By recognizing the emotional needs of their employees, they can foster a sense of belonging and commitment, leading to higher levels of engagement and productivity.

Adaptability: EI enhances a leader's ability to adapt to changing circumstances. Leaders with high emotional intelligence can adjust their strategies and approaches based on the emotional climate of their teams, ensuring that they remain effective in diverse situations.

CONCLUSION

The impact of emotional intelligence (EI) on leadership and management is profound and multifaceted, serving as a catalyst for transforming organizational culture and enhancing overall performance. As organizations navigate the complexities of modern business environments, the ability of leaders to understand and manage emotions—both their own and those of their team members becomes increasingly critical. Leaders with high emotional intelligence are better equipped to foster a positive organizational culture characterized by trust, collaboration, and open communication. They create an environment where employees feel valued and understood, which is essential for promoting engagement and motivation. When leaders demonstrate empathy and actively listen to their team members, they cultivate a sense of belonging and psychological safety. This, in turn, encourages employees to express their ideas and concerns freely, leading to increased innovation and creativity. Moreover, emotionally intelligent leaders excel in conflict resolution. They possess the skills to navigate disagreements and misunderstandings with sensitivity and tact, transforming potential conflicts into opportunities for growth and learning. By addressing emotional dynamics effectively, these leaders can maintain team cohesion and morale, even in challenging situations. This ability to manage conflict not only enhances team dynamics but also contributes to a more resilient organizational culture that can adapt to change and uncertainty. The benefits of emotional intelligence extend beyond interpersonal relationships; they also significantly impact organizational performance. Research has shown that organizations led by emotionally intelligent leaders tend to experience higher levels of employee satisfaction, lower turnover rates, and improved productivity. For instance, companies that prioritize EI in their leadership development programs often see a direct correlation between emotionally intelligent leadership and enhanced business outcomes. This is particularly evident in industries where teamwork and collaboration are essential for success, such as healthcare, technology, and education. Furthermore, the integration of emotional intelligence into leadership practices aligns with the growing emphasis on ethical leadership and corporate social responsibility. Leaders who prioritize EI are more likely to make decisions that consider the well-being of their employees, customers, and the broader community. This ethical approach not only enhances the reputation of the organization but also fosters loyalty among employees and customers alike, creating a sustainable competitive

advantage. As organizations continue to evolve, the demand for leaders who possess strong emotional intelligence will only increase. Companies must invest in training and development programs that focus on enhancing EI skills among their leaders. This includes workshops on self-awareness, empathy, effective communication, and conflict resolution. By equipping leaders with these essential skills, organizations can create a pipeline of emotionally intelligent leaders who are prepared to navigate the complexities of the modern workplace. Finally the impact of emotional intelligence on leadership and management is transformative, shaping organizational culture and driving performance. By fostering an environment of trust, collaboration, and ethical decision-making, emotionally intelligent leaders not only enhance employee engagement and satisfaction but also contribute to the long-term success and sustainability of their organizations. As the business landscape continues to change, the importance of emotional intelligence in leadership will remain a critical factor in achieving organizational excellence and resilience. Investing in emotional intelligence is not just a strategic advantage; it is a necessity for organizations aiming to thrive in an increasingly complex and interconnected world.

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REVIEW ARTICLE

Comprehensive Analysis of Reproductive, Mental and Socio-Economic Determinants on Women's Health : A Literature Review

Vajrala Krishna Reddy*

Professor, Department of Physiotherapy, Galgotias University, Greater Noida, Uttar Pradesh, India.

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***Address for Correspondence**
Vajrala Krishna Reddy
Professor,
Department of Physiotherapy,
Galgotias University, Greater Noida,
Uttar Pradesh, India.
E.Mail: krishnareddympt@gmail.com

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ABSTRACT

The health domains of non-communicable diseases (NCDs), mental health, musculoskeletal health, reproductive and sexual health, and socioeconomic determinants form an essential part of the women's health domain. Research draws an all-inclusive picture of health issues that impact women differently at different stages of life through the integration of both old and new medical findings. This paper explores in depth the three main health outcome influencing elements: biological aspects and clinical factors and socio environmental variables. The evaluation explores changes in women's health patterns because of environmental shifts and technological developments, which becomes valuable for future programs and research. Researchers have initiated this investigation because it offers extensive, multi-layered health narratives to assist healthcare professionals and politicians during the development of gender-sensitive public health strategies.

Keywords: Reproductive, Mental, Socio-Economic, Women's Health, Literature Review

INTRODUCTION

Levelled health research now includes multiple life-stage problems which used to exclusively focus on reproductive systems. Investigative studies from early maternal health research have expanded into researching between multiple disciplines of epidemiology, sociology and clinical medicine. Modern research supports the need for gender-sensitive healthcare that recognizes how biological factors interact with a social environment. Research shows that women have specific obstacles due to both institutional injustices along socioeconomic challenges, as well as hormonal and reproductive differences. This investigation methodically examines four important aspects of health which include reproductive and sexual health, together with non-communicable illnesses and musculoskeletal health, followed by mental health. The study emphasizes digital health and climate change while identifying their importance as modern

healthcare challenges. Each section of the analysis methodically studies the numerous factors that affect women's health results.

Reproductive and Sexual Health

Maternal Health and Safe Reproduction

Women's health continues to utilize maternal health as its fundamental topic. Published studies demonstrate that timely high-quality prenatal and perinatal and postnatal care services will substantially lower maternal death and illness risks [23,11]. The lack of access to emergency obstetric care throughout high-risk areas leads to preventable death in those regions. Continuity of care throughout prenatal check-ups and institutional births and postnatal visits plays an important role in short- and long-term mother health outcomes. The variations observed in maternal health serve as an indicator of broader systemic inequalities that exist. Multiple studies prove that patient outcomes depend directly on their socioeconomic standing and where they live, in addition to their level of education. The health care facilities of urban women surpass those provided by rural women because cities offer more dependable and high-quality services. The way people seek medical care is influenced by cultural gender norms and traditional practices and this results in adverse maternal healthcare outcomes among those holding conflicting perspectives between modern healthcare systems and traditional medical beliefs. The persistent maternal mortality gap between rich countries and developing regions demonstrates the unequal treatment that exists in international healthcare systems. The availability of trained obstetric staff during deliveries is crucial, together with rapid access to medical facilities. Scientists have emphasized these emergency measures as key components of maternity healthcare. A decrease in maternal deaths primarily depends on healthcare staff education, medical product supply stability and quality health service referral options. Solutions obtained from low-resource settings remain vital for treating eclampsia and postpartum haemorrhage which continue to be leading causes of maternal death [23, 11].

Contraception, Family Planning, and Reproductive Rights

Family planning serves as both a therapeutic requirement and vital aspect for women's empowerment. Study data shows that contraceptive availability enables women to control their fertility and this directly affects their educational future and job opportunities and overall life well-being [23,5]. Family planning has become more efficient because women have multiple contraceptive options available, including emergency contraception and oral tablets with intrauterine devices which promote better health outcomes for mothers as well as their children. Enough obstacles persist while delivering family planning services to the public. Various areas face barriers to contraception adoption because they must deal with laws that restrict access, combined with social stigmas and deceptive information. Studies indicate that many women abstain from contraceptive use because they obtain incorrect knowledge about side effects and enduring health impacts. The healthcare systems of low-resource areas experience financial challenges alongside logistical constraints that limit outreach services and reduce the variety of offered methods. The family planning services available between rural and urban communities are distinct since rural women encounter reduced access to dependable educational resources and advanced medical treatment [23, 5]. Family planning creates numerous social benefits for individuals and communities beyond its influence on personal health outcomes. Family planning enables women to properly plan pregnancies, thus reducing healthcare expenses while improving the educational prospects of children and integrating more women into the workforce. Research shows that combining family planning strategies with broader reproductive health programs through rights-based approaches leads to positive outcomes in national policies [23, 5]. Health programs have identified family planning as their top priority because these policies help achieve gender equality and sustainable development across the world.

Menstrual Health Management

Women's health deeply depends on menstrual health management which is vital for teenage girls. Research indicates that insufficient menstrual management leads to different health complications that can damage the reproductive system and create additional medical issues. The educational success of teenage girls declines when their menstrual hygiene is poor, as their psychosocial stress and decreased school participation [22]. Cultural stigma, together with social taboos, establishes major hindrances in managing menstruation correctly. Multiple cultures view menstruation

as something to hide because of shame, which damages girls' self-acceptance and hinders straightforward talks about menstrual well-being. Stigma, along with unfavourable health outcomes, continues to perpetuate because inadequate communication and instructional efforts result in inaccurate information and poor hygiene practices. The health of women will improve through public health policies that implement comprehensive menstrual health education and budget-friendly, sustainable sanitary products [22]. The innovative tactics for MHM include programs that provide education in schools and engage in community outreach work to decrease menstrual stigma. Several public health agencies together with non-governmental organizations, collaborate to deliver cultural educational content while offering low-cost personal hygiene items to lower income communities. Numerous programs prove that menstrual health joins social concerns with health requirements, thus requiring unified participation from various sectors. A nationwide policy and learning program concerning MHM should exist to assist women in managing their periods without uncertainty or embarrassment [22].

Non-Communicable Diseases (NCDs)

Cardiovascular Diseases

The leading cause of women's mortality across the globe remains cardiovascular diseases but these diseases often receive inadequate knowledge and diagnosis when appearing in females. Women present with symptoms different from standard chest pain usually exhibited by men as they display nausea and exhaustion together with shortness of breath [15]. Such differences between how doctors approach male and female symptoms result in delayed diagnosis and treatment, which negatively affects treatment results. Scientific proof demonstrates that gender-based research along with individualized diagnostic examinations, is essential because clinical guidelines and CVD risk assessment methods result in inequality [15]. Socioeconomic factors, together with the way the disease presents, affect women's CVD burden. The combination of unhealthy food choices with limited health services access along with increased stress, stems from poor socioeconomic status thus increasing heart disease risks. The combination of diabetes and obesity trends makes the cardiovascular risk assessment more challenging for women. CVD screening accuracy for women requires gender-specific risk assessment tools which should integrate female-specific risk factors [15]. Digital health programs have started to bridge the gaps that previously existed in healthcare delivery. Technology-based services targeting women have proven effective at better detecting and treating cardiovascular diseases at their early stages. Real-time vital sign measurements like blood pressure and heart rate are possible through digital instruments that offer active risk factor management to users. Engineering research should merge innovative technologies with regular healthcare systems to create a comprehensive preventive scheme for women's cardiac wellness, according to [15, 17].

Cancer Burden

Women experience significant health problems and death due to breast and cervical cancers which represent major cancer burdens among females. Breast cancer affects millions of people globally because it represents the leading cancer diagnosis in women according to medical reports [2]. The survival rates for cancer patients increased through better screening methods and earlier recognition along with improved treatments yet challenges persist in regions with limited screening infrastructure and access to clinical services. The importance of personal screening programs based on breast cancer-related genetic and hormonal elements and lifestyle factors mandates their immediate adoption [2]. The effectiveness of Pap smear tests and HPV screening for early detection becomes restricted by socio-cultural barriers, resource constraints and limited access in developing countries according to available literature. Public health ought to prioritize both vaccination rate elevation and health education expansion together with cervical cancer screening outreach to lower both infection rates and mortality numbers [2]. The medical community maintains Cervical cancer as a vital healthcare problem in areas where fewer people get vaccinated against HPV Virus and screening services fail to meet necessary requirements. Additional concern requires increased attention for ovarian cancers together with endometrial cancers that complement existing commitment to breast and cervical cancers. Advanced diagnostic tools applied alongside early symptom recognition lead to improved treatment outcomes according to research findings. The practice of personalized medicine using genetic and environmental along with lifestyle information, helps to significantly decrease the cancer burden experienced by women. Health

inequalities from cancer demand continuous funding in cancer science research for new therapies along with modern detection techniques [2].

Metabolic and Autoimmune Disorders

The combination of hormonal changes together with lifestyle decisions increases diabetic and obesity prevalence which affects women at higher rates than men do. Evidence from epidemiological studies shows that a woman's insulin sensitivity together with fat distribution patterns modify after menopause raising her risk for developing type 2 diabetes. The metabolic situation becomes more intricate due to food patterns combined with physical inactivity and hormone abnormalities so specific therapies are needed to treat these complex problems [27, 15]. Multiple sclerosis and systemic lupus erythematosus and rheumatoid arthritis affect females more frequently than males. Researchers think the combination of environmental factors and hormonal effects and genetic risk factors influences the development process. Two separate functions of oestrogen regulate immunological responses thus explaining both the beneficial and damaging effects of autoimmune disease [27]. Continuous autoimmune conditions lead to extensive disabilities therefore, it is essential to identify them early and treat them specifically for women while addressing complete healthcare needs [27]. The effective management of metabolic and autoimmune diseases now requires medical treatment coupled with lifestyle modifications through multidisciplinary approaches. Research now demonstrates that patient-specific exercise programs combined with dietary counselling and stress management methods help halt disease progression. Medical science is developing individualized treatments and biological drugs that attack distinct inflammatory processes which frequently affect women. Medical research demands gender-specific data input for clinical trials as well as treatment protocols [27].

Mental Health

Prevalence and Determinants of Mental Health Disorders

Anxiety and depression affect women more frequently than men since women have a higher susceptibility to mental health conditions. Research shows that female mood disorder risk increases when hormonal levels shift as women experience menstruation and pregnancy and shortly after giving birth and during menopause [1]. Sociocultural forces that include caring responsibilities alongside social norms make existing mental health weaknesses even more severe. Research confirms that biological factors along with daily experiences, create a major impact on women's mental health, so preventive measures must include multiple dimensions of intervention [1]. Research studies show that depression symptom levels steadily increase when individuals experience hormonal changes particularly during the postpartum period. The mental health disorder PPD causes both physical burdens to mothers and lasting consequences for family bonds and child well-being. Mental disease risk profiles become complex as environmental stress, hormonal shifts and genetic vulnerabilities combine. Studies indicate that supportive care together with early intervention help minimize the long-term consequences of these conditions. Treatment often requires pharmaceutical drugs along with psychotherapy and supportive networks within communities to deliver proper outcomes [1, 18]. The stressors of trauma and solitary living combined with economic setbacks intensify the mental illness in women. A consistent relationship exists between intimate partner violence (IPV) together with gender-based violence (GBV) and declining mental health outcomes. PTSD along with chronic anxiety and depression, commonly affects victims who endure abuse [4]. The proper management of mental health needs integrative care systems that simultaneously treat psychological trauma and provide social support and work towards legal reforms to protect women from ongoing harm [4]. Advanced digital solutions function as efficient tools to address mental health care gaps through mobile mental health applications combined with virtual counselling platforms. Digital platforms deliver urgent assistance through their resources because they help regions lacking enough traditional mental health programs. Modern interventions could receive more customized designs that address the individual needs of female patients by merging technological platforms with conventional clinical practices [24].

Impact of Gender-Based Violence on Mental Health

Great harm exists in the way that intimate partner violence (IPV) and other gender-based violence (GBV) affect mental health. Research throughout the years proves that victims of such violence develop significantly greater risks of anxiety and sadness and PTSD [4]. Healthy brains and psychological health suffer from extended exposure to

stressful situations linked to violence. Victims typically experience emotions of humiliation and separation combined with helplessness that cause their mental health problems to expand. Trauma from GBV creates permanent psychological consequences that modify both individual behaviour along the way victims interact with people. The inability of victims to develop healthy relationships while trying to escape abusive cycles leads to a worsening mental health condition. The path of recovery for trauma survivors needs complete intervention services that combine police support and psychological counselling with social assistance. The importance of trauma-informed care is established through research because it recognizes widespread violence effects in addition to preventing new traumas that occur during therapy sessions [4]. Survivors receive excellent social and emotional support through community-based outreach programs and support group interventions which have proven effective. Women gain confidence through these programs to defend their rights while also receiving support to take legal action. The improvement of public health programs targeting GBV awareness and mental health effects reduces stigma and drives more women toward medical intervention. Basic healthcare providers ramp up their efforts to introduce mental health support services because research shows they should integrate them into normal medical care to provide quick comprehensive treatment to victims [4].

Musculoskeletal Health and Osteoporosis

Osteoporosis and Bone Health

Osteoporosis poses a serious health problem to postmenopausal women because it causes decreased bone mass while increasing their susceptibility to bone fractures. Lower bone mass occurs together with structural deterioration as the accelerated bone resorption progresses following menopause. Statistical data from the International Osteoporosis Foundation shows that three out of thirty women older than 50 years will develop an osteoporotic fracture [10]. Osteoporosis-related fractures create several severe consequences that impact a person's mobility and cause ongoing pain and reduce their life quality. Preventive measures for osteoporosis focus on both behavioural adjustments and quick diagnostic testing. Weight-bearing activities combined with healthy eating habits that include calcium and vitamin D consumption as well as smoking cessation, make up essential preventive measures. Pharmaceutical therapy using bisphosphonates serves as a popular medical treatment to decelerate bone resorption and lessen the probability of fractures happening. Unlevel screening practices along with insufficient public knowledge result in osteoporosis patients receiving inadequate treatment despite the existing preventive measures. Reaching racial and ethnic minorities concerning osteoporosis requires a wide-ranging public health approach that combines ongoing education for patients and screening programs distributed through communities [10, 12]. The data shows that fractures resulting from osteoporosis create substantial financial costs. Healthcare organizations pay substantial amounts from their budgets to treat patients who sustain fractures from osteoporotic bones and to provide post-acute medical care and lifelong disability management. The clinical need and financial benefits endorse investing funds in preventive healthcare. Future research must investigate long-term therapeutic strategy effectiveness along with the relationship between genetic background and environmental risk factors for osteoporosis progression [10, 12].

Autoimmune and Inflammatory Conditions

Women face increased risks of developing autoimmune disorders which lead to sustained incapacity through diseases like rheumatoid arthritis, combined with systemic lupus erythematosus as well as various inflammatory conditions. Multiple factors determine the development of these illnesses because they result from environmental elements combined with hormone functions along with inherited risks. The combination of long-term inflammation and autoimmune conditions produces both restricted joint movement and severe mental distress. These illnesses create long-term pain together with functional limitations that disrupt both personal life and professional life to a severe extent [27]. Biological treatments that target specific inflammatory pathways have demonstrated effectiveness in treating autoimmune diseases. Treatment options generate expenses together with potential negative side effects. Women exhibit divergent responses to medical treatments due to their fluctuating hormonal levels and shifting immune system functions thus emphasizing the need for studies that consider gender differences. More effective healthcare delivery to women with rheumatic diseases requires combined approaches between supportive care alongside physical therapy and medications [27]. Recent investigation focuses on personalized medicine which develops healthcare solutions through the analysis of genetic and hormonal information plus environmental

conditions. The strategies work toward lowering adverse effects along with maximizing therapeutic effectiveness. The field of autoimmune disease treatment now examines complementary therapies like dietary changes and acupuncture for addressing persistent pain and inflammation symptoms. Women who live with persistent autoimmune diseases might benefit from using complementary treatments alongside conventional therapies to establish a better treatment framework according to research [27].

Socio-Economic Determinants and Health Disparities

Social and Economic Influences on Women's Health

The social factors of work along with income and educational achievements heavily influence health results for women. Multiple scientific papers have proven that reduced socioeconomic standing leads to greater incidence rates of chronic health problems along with reduced access to preventive services and inferior overall health outcomes [16, 30]. The health risks of disadvantaged women intensify because they experience diminished social support together with reduced authority in healthcare decisions. Healthcare service quality disparities emerge when income levels vary between people. Women who live in poor rural communities often encounter difficulties when trying to get access to skilled medical help and proper testing facilities and therapeutic options. The condition continues to worsen before therapy begins because structural obstacles result in late medical care seeking. Public health policies need to establish community-based interventions while developing essential infrastructure and directing funds specifically toward vulnerable groups to reduce existing health disparities [16, 30]. Women need education to obtain the necessary knowledge, which enables them to make reasonable decisions about their well-being. Health literacy improves together with a better understanding of preventative services when people attain higher levels of education. Public health receives dual benefits when funding women's education, since it creates economic independence and simultaneously reduces health inequalities [16, 30].

Intersectionality and Policy Implications

The concept of intersectionality represents a framework that shows how race combines with ethnicity and socioeconomic class to generate unique health results for women [3]. Academic investigations show women belonging to disadvantaged communities encounter multiple obstacles that elevate their exposure to unfavourable health results [32, 31]. The combination of various social identities increases women's sensitivity to outside stressors while preventing access to resources and creating care provision challenges. Health policy interventions need an intersectional strategy to properly tackle differences in healthcare outcomes. The development of culturally appropriate health programs and medical facility implementation of anti-discrimination laws along with the reach ability and acceptability of public health campaigns for diverse populations, forms the basis for effective health care disparity reductions. Eliminating healthcare disparities requires fundamental changes to institutional frameworks that perpetuate unequal treatment. Research participation by various female demographics will help developers create treatments that appropriately address women's diverse healthcare requirements [32, 31].

Emerging Issues in Women's Health

Environmental and Climate Change Challenges

Environmental deterioration and climate change both act as crucial health-determining factors that specifically harm women from vulnerable populations. Natural resources serve as the main foundation for women living in rural areas and low-income communities which leaves them exposed to severe health risks from changing environments. Proceedings indicate that the combination of vector-borne infections and air pollution and extreme weather events causes increasing health problems among women [6, 26]. Several different relationships exist between female health outcomes and environmental transformations. An increase in heat and inconsistent climate patterns affects farm output leading to more widespread food scarcity that results in poor nutrition for women along with their children. Global disasters harm existing gender inequality more by damaging community facilities while blocking healthcare access. The literature endorses public health policies combining climate resilience measures with gender-sensitive health programs because it aims to prevent women from being marginalized by changing environmental conditions around the globe [6, 26]. Multiple studies demonstrate the crucial importance of adaptive strategies that focus on local communities. The mentioned tactics involve giving resources for climate change mitigation as well as

establishing local environmental risk warning systems and delivering health education to communities. The creation of sustainable health solutions for communities depends on women's empowerment through environmental decision-making participation [6, 26].

Digital Health Innovations

Digital health technology has fundamentally changed how healthcare delivers services and specifically how women's health manages care. Most healthcare facilities utilize Health applications as well as telemedicine services and mobile health (mHealth) interventions to address access and quality of care disparities. These digital solutions allow women who live in disadvantaged regions and remote areas to obtain remote medical consultations in combination with patient education and remote health monitoring services [13, 17, 33]. Multiple studies have confirmed that digital health initiatives enhance the level of healthcare knowledge among women and drive them toward taking proactive measures for their health. Reproductive health apps offer women personalized data about menstruation techniques along with contraception methods as well as prenatal care information which provides control over their health choices. Asset-based new technology access remains difficult due to varying digital literacy and internet connectivity availability among the potential principal beneficiary demographics [13, 33]. The success of digital health solutions depends on their proper integration within the existing healthcare infrastructure. Engineering collaboration with healthcare professionals and legislators will produce healthcare platforms that recognize women's special requirements and maintain convenient system designs. Future research about digital medical innovations needs to prioritize investigations of their lasting outcomes for clinical benefits together with expenditure effectiveness and equity distribution among diverse groups [13, 33].

CONCLUSION

The challenging relations between the reproductive, mental and socioeconomic factors that affect the health of women according to the different life stages are addressed in this synthesis of literature. There productive health-related problems, like maternal care and availability of alternatives to contraceptives, continue to play a major role in both the psychological and physical health, and socioeconomic position also determines the health disparities, especially among women in low and middle-income settings. Systemic barriers to sustaining cycles of poor health, a lack of autonomy, and underuse of health services are common among such women in groups. This intersectionality of the factors demonstrates that women's health cannot be treated individually. Finally, the improvement in women's health warrants a multisectoral effort that embraces women's socioeconomic empowerment, integration of mental health, and activism of women's reproductive rights. The only way of enhancing the general well-being of women throughout the world is by setting in place all-around programs with an equity approach.

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RESEARCH ARTICLE

Assessing the Relationship between Stress and Parafunctional Oral Habits among University Students Prior to Examination Notice and During Examination: A Cross-Sectional Study

Siya Jeendgar[1], Akanksha Joon[2]*, Navodita Jamwal[3] and Mamta Singla[4]

[1]Undergraduate Student, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.
[2]Senior Lecturer, Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.
[3]Senior Lecturer, Department of Public Health Dentistry, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.
[4]Professor, Department of Conservative Dentistry and Endodontics, Faculty of Dental Sciences, SGT University, Gurugram, Haryana, India.

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***Address for Correspondence**
Akanksha Joon
Senior Lecturer,
Department of Conservative Dentistry and Endodontics,
Faculty of Dental Sciences,
SGT University, Gurugram, Haryana, India.
E.Mail: joonakanksha@gmail.com

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ABSTRACT

Prevalence of stress has been commonly seen among university students which lead to psychological as well as physiological consequences. Bruxism is often seen related to stress, therefore this study aims to assess the relationship between stress and bruxism among university students during their examination period. A cross-sectional study was conducted at SGT University, India, involving a questionnaire containing socio-demographic information, the Perceived Stress Scale (PSS), and the Fonseca Questionnaire. The survey was distributed anonymously before and during the examination period among the students. A significant increase in stress levels ($p < 0.05$) and the severity of parafunctional oral habit i.e. bruxism, was observed during the examination period. Females and hostellers reported higher stress levels as well as more severe bruxism. This study demonstrates that academic stress during the examination period significantly contributes to the occurrence and severity of parafunctional oral habits such as bruxism among the university students. Stress management interventions in university settings could help mitigate these issues.

Keywords: Perceived stress, Para functional Oral Habits, University students, PSS-10, Bruxism

INTRODUCTION

Stress is a psychological and physiological response to external pressures or demands that are perceived as challenging or overwhelming. According to Nechita *et.al.*(2014), Stress is the body's condition, when physiological and psychological stimuli disturb its normal homeostatic mechanisms and hence disrupt its balance [1]. In modern society, distress is ubiquitous, affecting individuals across various walks of life, particularly among students in academic set-ups. University students especially, experience high levels of stress (Marshall *et al*., 2008) [2,3]. Among university students, stress levels tend to be exacerbated by academic pressures, deadlines, and performance expectations. These pressures may manifest as physiological, psychological, or behavioral changes, with long-term implications for both mental as well as physical health [4]. Parafunctional oral habits are involuntary behaviors that occur outside the normal function of the mouth and jaw. The physiological effects of bruxism are well-documented in the literature, with studies linking it to several risk factors such as stress, anxiety, depression, and sleep disturbances (Manfredini *et al*., 2017) [5]. And with this increase in the levels of stress, sleep problems, unhealthy lifestyle and dietary habits, the prevalence of parafunctional habits is also getting increased (Demjaha *et al*., 2019) [6]

Stress and its Impact on Students

According to the research done by Marshall *et al*. (2008), perceived stress among university students is linked to decreased quality of life, increased levels of anxiety and depression, and the development of maladaptive coping mechanisms. Stress also impairs cognitive function, reducing concentration and problem-solving ability, thus leads to exacerbating feelings of helplessness and frustration [7,3].

Parafunctional Oral Habits and Stress

One of the primary physical manifestations of stress is the development of parafunctional oral habits. According to a review showed by Bader and Lavigne (2000), bruxism is a multifactorial condition influenced by psychological factors such as stress, anxiety, and depression [8]. Studies have shown that students are particularly vulnerable to developing bruxism during periods of academic stress, as the pressure to perform well can trigger involuntary teeth grinding, particularly during sleep (Granada and Hicks, 2003)[9]. While awake bruxism is often linked to heightened levels of stress and anxiety during waking hours. However, sleep bruxism is believed to be more closely associated with poor sleep quality, which is commonly observed during stressful academic periods (Costa *et al*., 2023) [10]. According to Soares *et al*. (2017), pain and noise in the TMJ, muscle pain and stress are the mostly associated with parafunctional oral habits like bruxism. Beyond the physical implications, parafunctional habits can also affect an individual's emotional and psychological well-being, as chronic pain and discomfort can further creating a vicious cycle that elevate stress and anxiety levels [11,12].

The Examination Period as a Stressor

The examination period is characterized not only by an increase in academic pressure but also by changes in lifestyle during this period. Many students experience disrupted sleep patterns, altered eating habits, and increased consumption of caffeinated beverages, all of which can exacerbate the effects of stress on the body. In addition, students often report a greater sense of time urgency and a feeling of being overwhelmed, which can contribute to the exacerbation of stress-related behaviors, such as teeth grinding and jaw clenching [11,12].

Research Gap and Significance of the Study

Though there are good number of literatures on the association between stress and parafunctional oral habits, fewer studies have specifically focused on university students before and during the examination period. Existing research has primarily focused on the general population or has studied bruxism and stress in non-academic settings. Our study aims to fill this gap by evaluating the relationship between stress and parafunctional oral habits in a university setting, with a focus on the examination period. Along with this, it also seeks to evaluate the impact of Gender and Living Situation (Hostler or Day Scholar) on the same. This is so because gender also plays an important role in

determining stress vulnerability (Nechita *et al*., 2014) [1]. Also, according to a study by Ahad *et al*. (2021) [12], it was stated that "staying in the hostel is a positive predictor for anxiety and stress."

MATERIALS AND METHODS

Study Design

A cross-sectional study design was employed to assess the relationship between stress and parafunctional habits among university students at two time points: before and during the examination period. This study had been conducted according to STOBE Protocol.

Study Population

Sample Size: The study involved 300 students from various academic disciplines at SGT University, Gurugram, Haryana.

Inclusion Criteria

1. University students aged 18-25 years.
2. Enrolled in at least one course during the examination period.

Exclusion Criteria

1. Students above 25 years of age
2. Students who are not willing for participation.

Data Collection and Analysis

The Questionnaire consisted of three Sections

1. Demographic Information
2. Stress Assessment;
3. Oral Health Assessment

1. The Demographic Section asked the respondent's age, gender, living situation (Hostler or Day Scholar), and questions about consumption of sugary snacks, beverages, caffeinated drinks etc.
2. For Stress Assessment: Perceived Stress Scale (PSS) was used to measure the participants' perceived stress levels at two time points: before and during exams. The scale consists of 10 items that assess the degree to which participants find their life situations unpredictable, uncontrollable, and overloaded [12].
3. For Oral Health Assessment: Fonseca Questionnaire was used to assess the occurrence and severity of Parafunctional Habits. It consists of questions that help determine whether participants grind or clench their teeth, the frequency of such behavior, and any associated symptoms (e.g., jaw pain, headaches) [13].

The Survey Based Questionnaire was circulated anonymously in the form of Google Forms in which the consent form was attached - prior Examination Notice and During Examination. The options chosen by the respondents for each question were recorded.

Scoring

For Perceived Stress Scale

The PSS score was determined by following these directions -

- First, reverse your scores for questions 4, 5, 7, and 8.
- On these 4 questions, change the scores as shown below
- 0 = 4, 1 = 3, 2 = 2, 3 = 1, 4 = 0.
- Now add up your scores for each item to get a total.
- Individual scores on the PSS can range from 0 to 40 with higher scores indicating higher Perceived stress.
- Scores ranging from 0-13 would be considered low stress.
- Scores ranging from 14-26 would be considered moderate stress.

- Scores ranging from 27-40 would be considered high perceived stress

For Fonesca's Questionnaire

For each question, the possible answers were "yes" (10 points), "no" (0 points), and "sometimes" (5 points), with a total score ranging from 0 to 100.

- The severity of Parafunctional Habits, on the basis of the scores obtained, is considered as follows:
- For scores ranging from 16 to 44 – mild,
- 45 to 69 – moderate,
- More than 69 – severe,
- While for scores lower than 16, Parafunctional Habits are considered to be absent.

Statistical Analysis

Data was analyzed using paired t-tests, Chi-square tests, Pearson/Spearman correlation, and descriptive statistics. Effect size (Cohen's d) was calculated for practical significance.

RESULTS

Stress Levels

The paired t-test revealed a significant increase in perceived stress from the pre-examination period (mean PSS score: 16.4 for males, 16 for females) to the examination period (mean PSS score: 21.2 for males, 23.6 for females) ($p < 0.05$). as shown in Table 1(Perceived Stress Score before and During Examination)

Severity of Parafunctional Habits

The Fonseca scores showed a significant increase in the severity of parafunctional habits, particularly bruxism, during the examination period ($p < 0.05$). The severity of bruxism was mild to moderate for most participants, with some experiencing severe bruxism during exams as shown in Table 2 (Fonesca's Score before and During Examination)

Impact of Gender

Females experienced higher stress levels and more severe parafunctional habits compared to males as shown in Table 3 (Score based on gender before and during examination)

Impact of Living Situation

Hostellers exhibited significantly higher stress levels (PSS score: 27.1) compared to day scholars (PSS score: 25) during the examination period ($p < 0.05$) as shown in Table 4 (Score based on living Situation before and during examination)

DISCUSSION

The present study aimed to explore the relationship between stress levels and the occurrence of parafunctional habits (such as bruxism) among university students, focusing on two distinct time points: before the examination notice and during the examination period. The results of this study have important implications for understanding how stress, particularly academic stress, can contribute to oral health issues such as Parafunctional Habits among the university students.

Relationship Between Stress and Parafunctional Habits

The primary objective of this study was to assess how perceived stress, as measured by the Perceived Stress Scale (PSS), correlates with the occurrence and severity of parafunctional habits, as assessed by the Fonseca questionnaire. Stress, particularly during periods of academic pressure such as examinations, has been previously shown to impact

various health aspects, including oral health. The findings from this study corroborate existing literature suggesting that stress can exacerbate parafunctional behaviors such as teeth grinding or jaw clenching.

In our study, we found a noticeable increase in perceived stress during the examination period, with a corresponding increase in the frequency and severity of Parafunctional Habits. This is consistent with previous studies that have suggested a temporal relationship between academic stress and bruxism. The increase in stress levels during exams likely leads to a higher frequency of stress-induced behaviors, such as teeth grinding, which in turn affects oral health. The heightened stress may be attributed to academic pressure, deadlines, and performance anxiety that university students commonly face [1,2].

Stress Levels Before and During Exams

The analysis of perceived stress levels using the PSS demonstrated a significant increase in stress scores from the pre-examination period till the examination period. The results showed that students reported moderate to high levels of stress during the examination period, which is consistent with the findings from previous studies that focused on academic stress. The PSS scores also indicated that a majority of students experienced moderate to high stress levels, which aligns with the common experience of academic pressure among university students. The increase in stress during the examination period could potentially trigger or worsen parafunctional habits like bruxism. Thus, understanding for such relationship is important because bruxism, if left untreated, can lead to a variety of oral health problems, including tooth wear, temporomandibular joint (TMJ) disorders, and even chronic headaches[6].

Parafunctional Habits and Their Implications

The Fonseca questionnaire revealed that a significant portion of the participants exhibited symptoms consistent with mild to moderate bruxism, while some participants reporting severe bruxism during the examination period. These findings highlight the impact of stress on oral health, as bruxism is commonly considered a stress-related parafunctional habit. Given that bruxism is often subconscious, students may not be aware of the extent to which they are grinding or clenching their teeth during such stressful periods, leading to future complicating its management. The severity of Parafunctional Oral Habits observed in this study emphasizes the importance of addressing stress management strategies within university settings. Universities could consider integrating stress management programs to help students cope with academic pressure, potentially reducing the prevalence and severity of bruxism as well as other stress-induced behaviors. These programs could include mindfulness practices, cognitive-behavioral therapy, or relaxation techniques aimed at reducing overall stress levels.

Gender and Living Situation

The demographic analysis revealed that gender and living situation (hostler or day scholar) may influence the stress levels and the occurrence of bruxism. Female students reported slightly higher stress levels, which could be attributed to gender-specific academic and social pressures. Furthermore, living situation also appeared to affect stress levels, with hostellers reporting higher levels of stress compared to day scholars. This could be due to the challenges associated with living away from home, such as increased responsibilities, lack of familial support, or even potential social isolation.

Limitations and Future Research

The findings from this study provide valuable insights into the relationship between stress and parafunctional habits, but there are certain limitations to this study too. This study design is cross-sectional, meaning that it cannot establish causality. A longitudinal study would be beneficial to determine the long-term effects of stress on oral health and bruxism. Additionally, self-reported data on stress and parafunctional habits may be subject to response bias, as participants might not accurately recall or report their symptoms. Therefore future research should explore objective measures of stress (e.g., cortisol levels) and parafunctional habits (e.g., dental examinations) to strengthen the current findings.

CONCLUSION

In conclusion, this study provides strong evidence that academic stress, particularly during the examination period, is associated with an increase in parafunctional habits like bruxism among university students. The findings emphasize the need for stress management interventions in university settings to reduce the negative impact of stress on students' health, particularly their oral health. Further research, using a longitudinal approach and objective measurements, is necessary to gain a deeper understanding of this relationship and to develop effective preventive strategies.

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Conflict of Interest

The author(s) declare(s) no conflict of interest.

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Table 1: (Perceived Stress Score before and During Examination)

Group	t-Statistic	p-Value
Before Exams vs. During Exam	-7.86	0.0000077

Table 2: (Fonesca's Score before and During Examination)

Group	t-Statistic	p-Value
Before Exams vs. During Exam	-5.99	0.0000899

Table 3: (Score based on gender before and during examination)

Gender	PSS Score before exam	PSS Score after exam
Female	16	23.6
Male	16.4	21.2

Table 4: (Score based on living Situation before and during examination)

Living situation	PSS Score before exam	PSS Score after exam
Hostler	16.8	27.1
Day Scholar	15.4	25

RESEARCH ARTICLE

Smart Agriculture : AI Monitoring and Automated Sustainable Farming

Deepak D[1]*, Sandeep Kulkarni[1], Chetan T R[1], Purushotham H C[1] and Manjunath B[2]

[1]Assistant Professor, Department of Commerce, School of Commerce, JAIN (Deemed to be University), Bangalore, Karnataka, India.
[2]Associate Professor, Department of Commerce, School of Commerce, JAIN (Deemed to be University), Bangalore, Karnataka, India.

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***Address for Correspondence**
Deepak D
Assistant Professor,
Department of Commerce,
School of Commerce, JAIN
(Deemed to be University),
Bangalore, Karnataka, India.
E.Mail: sandeep.kulkarni@jainuniversity.ac.in

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ABSTRACT

Smart agriculture, a pioneering and innovative farming system, is significantly driven by the use of Artificial Intelligence (AI). This technology, combined with Internet of Things (IoT) sensors and machine learning algorithms, not only enhances agricultural productivity but also offers a beacon of hope in addressing the unprecedented challenges faced by global agriculture in the 21st century. In this study, we review the AI-based monitoring and automation systems in agriculture, providing a comprehensive examination of their implementation, data sources, data collection methods, and the measurable impacts on the sustainability of farming systems. Through an in-depth review of recent publications, market data, and real-life examples, we demonstrate that these innovative agricultural technologies are providing immense value, including 250% increases in outputs, reductions in water consumption of 30-50%, and reductions in chemical pesticide use of up to 70%. The global market for AI in agriculture stood at USD 1.6 billion in 2024 and is projected to grow at a compound annual growth rate (CAGR) of 25.1% from 2024 to 2032, reaching USD 10.2 billion by 2032. By examining the environmental, economic, and social implications of these technologies, we present an in-depth overview of the data requirements, sources, and methods of data collection. These results demonstrate that smart agriculture is not just a new technology, but rather a new approach to farming that focuses on data and more precise methods, aiming to address the dual challenges of food and environmental issues.

Keywords: Smart Agriculture, Artificial Intelligence, Precision Farming, IoT Sensors, Machine Learning, Sustainable Farming, Environmental Impact, Data Analytics

INTRODUCTION

It is now the 21st century, and without any doubt, the challenges faced by global agriculture are unparalleled in modern human history. Meanwhile, the world must increase food production by nearly 70% to provide nutrition for an estimated global population of 9.8 billion people in 2050 (United Nations MDPI). Simultaneously, agriculture faces significant challenges from anthropogenic environmental changes, including climate change, reduced freshwater availability, soil depletion, and biodiversity loss, while also increasingly working within a biologically finite resource frontier. Conventional farming methods, which have sustained human society for millennia, will not provide answers to these new challenges. This industry is underconsiderable pressure, from declining agricultural land to skyrocketing input costs, labour shortages, and reduced environmental footprints. But in the face of these challenges, a transformative practice driven by technology has emerged as a significant player in the solutions landscape. Smart agriculture, also known as precision agriculture (or Agriculture 4.0, the fourth agricultural revolution), utilizes artificial intelligence (AI), Internet of Things (IoT) sensors, machine learning algorithms, robotic systems, and self-driving vehicles, along with data analytics, to enhance agricultural practices. This convergence of such technologies empowers farmers to make data-driven decisions, allocate resources more effectively, and provide targeted interventions at the plant or crop level. The fundamental commitment of smart agriculture is to transition from generic agricultural solutions to fine-tuned solutions tailored to each farm, its produce, or even specific agrarian management activities. This transformation, driven by the power of AI, has thus transcended a mere succession of technologies. It is a testament to the potential of human ingenuity and innovation in the face of unprecedented challenges. Smart Agriculture encompasses all three sectors of sustainability in agriculture: environmental, by reducing the use of toxic pesticides and optimizing resource utilization; economic, by enhancing productivity and lowering production costs; and social, by promoting food security and rural development. According to Smart Agriculture, this technology enables the provision of "hyper", or individualized, care at scale (thousands of acres per farm, tens of thousands of plants per acre); the system delivers precise volumes of water, nutrients, and pesticides using Databricks.

LITERATURE REVIEW

Evolution of Agricultural Technology

There have been multiple phases of agricultural technology evolution, each signified by the introduction of a significant technological innovation that has led to a dramatic change in agriculture. Steam was the first mechanization introduced to bolster the production capabilities of vast farms through its widespread adoption in the 19th and early 20th centuries. However, it was not until the latter part of the 20th century that the broader adoption of internal combustion engine-driven farm machinery occurred. This trend has continued up to the present day. The Green Revolution -- a period of scientific advancements in breeding crops, synthetic fertilisers and pesticides which dramatically increased yields -- started in the mid-20th century. First, the digital agricultural revolution was ignited in the early 1990s with the introduction of the Global Positioning System (GPS) and Geographic Information Systems (GIS), which are capable of accurately mapping fields and applying inputs in a variable-rate mode. The technology we now call smart agriculture, based on a combination of artificial intelligence, Internet of Things sensors, and advanced data analytics, all rests on this foundation.

AI Applications in Agriculture

Over recent years, numerous applications of AIs that span a range of crops or an entire agricultural system have been documented, from remote sensing and crop yield prediction to robotic machinery operation and renewable supply chains. Machine learning algorithms have demonstrated significant efficacy in analysing larger datasets with complex relationships in agriculture, as they can identify patterns in the values of dataset features that, in turn, can be used to predict farm actions. For image-based applications in agriculture, Convolutional Neural Networks (CNNs) have proven to be especially effective. These deep learning models are capable of detecting patterns in visual data and are thus well-suited for crop disease detection, weed identification, and quality detection tasks. In crop

stress classification problems, studies have proven that CNNs can achieve accuracies of 96% on the training datasets and 86% on the test datasets of MDPI. Predictive analytics is another major use case, where machine learning models can predict crop yields, weather patterns, and pest outbreaks. Farmers can optimise their planting schedules, irrigation timings, and pest control measures with data-driven insights, rather than intuition-based approaches, thanks to the predictive capabilities of precision agriculture, as reported in Science Direct.

Internet of Things: Integrating Agriculture

Sensor networks managed by the Internet of Things have transformed agricultural monitoring by providing real-time data-driven information on environmental, soil, and crop conditions. IoT sensors used in precision agriculture systems typically consist of several types of sensors, including soil moisture sensors, electrochemical sensors for nutrient monitoring, optical sensors for assessing plant conditions, and meteorological monitoring stations. Wireless IoT agriculture systems offer impressive features for data collection and analysis. It is found that the coefficient of determination (R^2) for soil moisture prediction with IoT-based soil monitoring systems is 0.96 with the root mean square error (RMSE) of 0.06, which is two orders of magnitude better than traditional statistical methods MDPI High temporal and spatial resolution of monitoring systems support monitoring of relevant parameters like soil moisture, pH, temperature, nutrient status as well as plant health. With IoT sensors coupled with cloud-based platforms and mobile applications, complete agricultural monitoring ecosystems have been created. Farmers can now access real-time data from any location, enabling them to monitor and manage their agrarian operations remotely. Such capability has been instrumental in allowing farming activities during the COVID-19 pandemic to be carried out in a socially distanced manner, according to a study published in Frontiers.

Objectives

1. To analyse the relationship between environmental parameters (temperature, humidity, soil moisture, and light intensity) and crop yield using sensor-based data in an innovative farming system.
2. To predict crop yield based on real-time environmental and soil data collected through IoT-enabled devices.
3. To evaluate the effectiveness of AI-based monitoring systems in enhancing sustainable farming practices by optimising irrigation, resource management, and yield forecasting.

Machine Learning for Smart Agriculture

The solutions to precision agriculture problems based on the application of machine learning are varied, include tasks as diverse as predicting crop yields and automating decisions on how to allocate limited inputs like resources Popular supervised learning algorithms for classification and regression problems in agriculture includes Support Vector Machines (SVM), Random Forests, and Neural Networks related to supervised learning. It has been found that ensemble learning techniques, specifically oriented towards agriculture and integrating multiple machine learning models, exhibit greater performance. According to evidence, ensemble methods reach r^2 values above 0.96 for soil organic carbon estimation and yield prediction tasks (MDPI). Using these methods, multiple algorithms are employed to trade off the strengths and weaknesses of each model, providing more accurate and stable predictions. The application of machine learning in time series analysis has enabled us to make sophisticated predictions for agricultural planning and management. Cross-temporal LSTM topologies and variants of recurrent neural networks have proven beneficial in temporal agrarian data analysis, particularly in applications such as crop growth prediction, weather forecasting, and temporal scheduling for planting and harvesting recommendations. Analyzing each market, along with its respective prices by country, can shed light on how this affects each economy.

Market Size and Growth Forecast

The market for smart agriculture worldwide has experienced immense growth over the years, driven by increasing demand for innovative farming solutions and the emerging challenges of minimising food insecurity. Multiple market research reports validate notable growth of agricultural technology segments in the market. Smart Agriculture reports that the AI in agriculture market reached a value of USD 1.6 billion in 2024 and is projected to grow at a compound annual growth rate (CAGR) of 25.1 percent from now until 2032, reaching USD 10.2 billion by then. This is a clear indication that an increasing number of farmers are embracing the potential of AI to transform

the farming approach that has been adopted for generations and to address the sector's universal problems. This trend has been reflected in the total smart agriculture market, encompassing IoT sensors, precision agriculture, and automation. Market Research Reports, Inc. Smart Agriculture Market Size, Share and Trends Analysis Report By Technology, By Solution (Hardware, Software, Services), By Application, By Region And Segment Forecasts, 2024 – 2029 – Markets and Markets According to the Smart Agriculture Market report, the market is estimated to have gained USD 14.40 billion in 2024 and is projected to reach USD 23.38 billion by 2029, recording a CAGR of 10.2% over the forecast period. Generally, Agricultural robotics is one of the fastest-growing sections of Smart agriculture. Farming robots serve throughout the farming cycle from planting to harvesting. Their demand has been soaring, with the global agricultural robots market size projected to be valued at USD 14.74 billion in 2024, growing at a compound annual growth rate (CAGR) of 23.0% from 2025 to 2030. According to Grand View Research, it is anticipated to reach USD 48.06 billion by 2030. This expansion is driven by a variety of factors, including increasing labour shortages, improving the accuracy of farm processes, and advancing robotics and AI technologies.

Distribution of the regional market

In terms of geography, the adoption of smart agriculture across regions is not uniform, with North America and Europe leading in technology deployment. At the same time, the Asia Pacific is the growth epicentre. According to Grand View Research, North America accounted for around 36.4% of the global agricultural robots market in 2024, driven by a high rate of technology penetration and relatively larger-than-average farm sizes, resulting in output 1.North American farms are generally larger than European ones and, at a U.S. average of 445 acres, are often justified in making significant investments in technology. The region is also among the leaders in smart agriculture due to the economies of scale that large-scale operations can achieve, which make some advanced agricultural technologies economically feasible. The fastest regional growth rate is expected to be from the Asia Pacific, with a CAGR of 23.6% during the forecast period. The rapid growth is attributed to technological advancements in Japan, India, and China, accompanied by severe labour shortages and increasing wages, as well as government support for agricultural modernisation (Grand View Research, 2022).

Investment trends and funding

Historically high investment levels demonstrate significant investor confidence and the recognition of the agriculture technology opportunity. Smart Agriculture claimed that smart agriculture attracted a total of USD 4.9 billion through 440 deals in 2021, illustrating the attractiveness of solutions within theagtech space to investors. Investment in AI-assisted vertical farming technologies reached USD 3.2 billion in 2024, underscoring the growing attractiveness of controlled environment agriculture (CEA) methods. AI in crop management is projected to exceed USD 5.6 billion by 2028, highlighting its economic potential in traditional field agriculture. Agricultural robotics is identified as one of the fastest-growing industries, with an expected market value of USD 11.9 billion by 2030. According to Smart Ag, this investment is enabling the development of autonomous harvesting equipment. The anticipated global autonomous harvesting equipment market is projected to reach approximately USD 7.7 billion by 2027.

Present-day Adoption and Technology Penetration

Different scales of intelligent farming technologies are adopted unevenly, depending on farm size, production type, and geographical areas. Various studies have claimed that 40% of large-scale farming companies will spend an average of 30% more on AI in 2024, reflecting an increasing level of trust in its capabilities, such as those offered by Smart Agriculture. The adoption of Variable Rate Technology (VRT) is a key indicator of the penetration of precision agriculture. More specifically, 37.4% (33.6 million acres) of corn-planted acres adopted VRT in 2016, while VRT adoption rates are projected to be 44% for corn, 26% for soybeans, and 22% for wheat by 2024, according to MDPI. These statistics indicate that precision agriculture technologies have been adopted steadily, albeit at a relatively slow pace. Farm types differ significantly in their adoption of technology. The highest share of AI adoption in agriculture in 2024 is in field farming (61.5%), followed by livestock farming (19.1%), indoor farming (15.0%), and aquaculture (8.4%).Smart Agriculture. The distribution captures the relative maturity of AI applications across the agricultural sectors.AI-powered precision farming is estimated to cover 25% of all global farming operations by 2028, suggesting widespread growth due to increasing adoption rates. Nonetheless, this projection also highlights that most farming

operations do not utilise advanced AI technologies, indicating that significant market growth potential exists with artificial intelligence.

Framework of Technology and Execution

Automated Surveillance Systems

Agriculture monitoring: A combination of computer vision, ML + IoT sensors. They enable farmers to continuously and in real-time monitor crop health, soil health, and environmental parameters, allowing for more precisely targeted interventions and better resource allocation. This has enabled the development and implementation of robust computer vision systems, particularly using Convolutional Neural Networks (CNNs) for agricultural monitoring tasks. These systems are trained to accurately identify crop diseases, pest infestations, and nutrient deficiencies; in fact, their capabilities often surpass those of human visual assessments. According to MDPI, YOLOv5 models, for example, have been observed to demonstrate 96.96% recall and 94.81% precision in seed detection and counting tasks. Hyperspectral and multispectral imaging technologies have been utilised to rapidly detect plant stress (disease and loss of efficiency in systems) before its visual manifestation (Chen *et al*.). These systems can then detect and characterise minor alterations in plant physiology that signify disease, nutrient deficiency, or water stress by using spectrometry and light reflectance pattern detection. Given that these systems are combined with machine learning (ML) algorithms, they serve as an early warning capability, enabling managers to be proactive rather than reactive when issues arise. Remote sensing technologies provide complementary large-scale monitoring to sensor networks, utilizing spaceborne (e.g., satellites) or airborne (e.g., drones) sensors. Satellite platforms (Landsat and Sentinel-2) provide periodic coverage of agricultural land, while UAVs allow for high spatial resolution and monitoring tailored to demand. The use of these technologies, combined with Artificial Intelligence (AI), enables the monitoring of crops to be performed over different spatial and temporal scales (MDPI).

Automation Technologies

Automated tools in agriculture have evolved from basic mechanical systems to a range of cutting-edge artificial intelligence (AI) solutions. These advancements have led to the development of robots that can undertake even complex farming tasks with minimal human intervention. These robots, equipped with modern sensors, computer vision, and machine learning algorithms, can detect crops and weeds, navigate through the field, and perform accurate operations. The most advanced form of agricultural automation, the autonomous tractor and harvester, is a testament to the efficiency of these systems. Utilising GPS guidance, computer vision, and machine learning, these machines navigate the fields within centimetres and complete planting, cultivation, and harvesting activities with almost no human interaction. Companies such as Smart Agriculture have developed systems that can operate continuously, adjusting operations in response to real-time sensor data and environmental conditions. The growing interest in automation and the potential solutions for labour shortages, such as robotic harvesting systems, are promising signs for the future of agriculture. Studies have found that harvesting robots can deliver a threefold increase in speed over human labour, while maintaining quality and reducing waste through the use of machine vision. For example, asparagus harvesting robots equipped with laser sensors and 3D vision systems can identify and harvest crops with high precision and minimal damage, according to a study published by MDPI.

Integration challenges and solutions

Several technical and operational challenges associated with AI-enabled monitoring and automation systems in agriculture must be addressed for successful implementation. This is particularly challenging to achieve due to agriculture being a multi-vendor environment where different systems and manufacturers are involved. Interoperability struggles are being addressed through reduced issues with conventional data designs and trade conventions that support standardised efforts. The ISOBUS standard, for instance, enables tractors and implements manufactured by different manufacturers to communicate with each other, while new standards for agricultural data exchange also facilitate the integration of farm management systems with external data sources. Cloud agrarian systems are in extremely high demand, but connectivity issues in rural areas can restrict their use. This enhances existing solutions with low-power wide-area networks (LPWANs), such as LoRaWAN, which offers low-power, long-range connectivity that is particularly well-suited for agricultural sensors. There is another connection option

that the two satellites offer, intended to expand satellite-based coverage for Frontiers. However, implementing data privacy and security measures remains a challenge, as sensitive agricultural information needs to be protected under stringent cybersecurity standards. These involve data transmission encryption, secure authentication systems, and frequent security updates for connected devices. As agricultural systems become increasingly interconnected, new vulnerabilities emerge that can only be mitigated through a comprehensive, whole-of-system security approach.

Research Methodology and Data Sources

Data Collection Using Sensors

Sensor-based data collection is crucial in agricultural systems, providing real-time information on environmental conditions, soil health, and crop status. Modern agricultural sensor networks consist of various sensors, each designed to monitor different parameters essential for informed farming decisions. Soil sensors record data on soil moisture, temperature, pH, electrical conductivity, and nutrient levels, allowing for immediate irrigation and fertiliser decisions. Plant health sensors, such as chlorophyll meters and leaf temperature sensors, monitor crop conditions and identify stress states early, enabling real-time management optimisation. Optical sensors, such as the AS7341 spectral sensor, enable reflection measurements that correlate with plant health indicators. Weather monitoring stations capture vital environmental data for irrigation scheduling, pest prediction, and crop growth modelling.

Remote Sensing Data Sources

Satellite remote sensing technology offers a wide-scale monitoring capability that complements ground sensors and geographical cellular networks, enabling the monitoring of entire farms or regional agricultural systems. Satellite platforms, such as Landsat and Sentinel-2, offer unique capabilities and high-resolution features, allowing the early detection of crop stress, disease outbreaks, and other issues. UAVs, or drones, provide high-resolution and on-demand monitoring, enabling farmers to zoom in on critical areas of the field or specific stages of crop growth. Drone monitoring combines AI algorithms with data captured using drones, allowing farmers to assess crop health, identify plant diseases, and inspect yield quality and quantity. Human-crewed aircraft and specialised agricultural aircraft offer intermediate-scale surveying capabilities, covering larger areas and producing better spatial resolution data, making them suitable for regional-scale agrarian monitoring applications.

Machine-Generated Data

Modern agricultural machinery generates extensive operational data, including yield monitoring, machinery performance parameters, and application rates. Combine-mounted yield monitoring systems gather detailed information about field productivity, revealing spatial variability in crop performance. GPS-enabled machinery logs off-field operations, providing exact location data for all farming operations. Precision application equipment creates data on input usage rates, application timing, and spatial distribution, linking applied inputs with responses and determining optimal rates of fertiliser, pesticides, and seeds. Variable-rate application systems use input sensors or prescription maps to adjust the rate of inputs, generating high-resolution input application maps throughout fields.

Analyses and interpretation of the data

In this section, a detailed analysis and interpretation of the dataset, which contains information used to assess how environmental and soil parameters affect agricultural production in the context of smart farming, are presented. This dataset includes sensor-based recordings of the different ecological variables throughout a cropping season, taken from an online innovative agriculture platform in continuous, real-time.) The automated data collection is at the heart of any AI-based innovative agricultural system, and Internet of Things (IoT) devices have been deployed to facilitate this. This analysis aims to identify key environmental factors that impact crop yield and explore the potential of using machine learning models to help farms achieve more optimal yields.

Dataset overview and variables

The dataset features five main variables

Temperature (°C)

Humidity (%)
Soil Moisture (%)
Light Intensity (lux)
Crop Yield (kg per plot)
Recordings were conducted at a 30-minute duration for 120 days, generating a high-resolution, high-frequency time series ideal for both statistical and machine learning analysis. The data were collected in a field practising smart agriculture, where embedded sensors were utilised for the automatic monitoring of irrigation and the environment. The primary dependent variable for this analysis, crop yield measurements, were recorded at the end of the growing season and then matched with the sensor data based on time and plot identifications.

Analysis of Descriptive Statistics
A preliminary descriptive statistical analysis was conducted to gain general insight into the characteristics of the data and the distribution of the variables, which involved central tendency, dispersion, and shape of the distribution.

Temperature
The mean temperature recorded during this period was 29.3°C (SD: 4.2), with temperatures ranging from 20 to 38°C, which is consistent with the normal subtropical climatic conditions of the location where the crop was grown. The plot of aggregated temperature data was histogrammed, showing a nearly normal distribution (but slightly right-skewed towards the warmer mid-season peak).

Humidity
Relative humidity was highly variable, between 40% and 95%, with a mean of 68.7% (SD = 13.5%). These humidity levels exhibit a wide range, reflecting the daily weather patterns of precipitation and evapotranspiration. The data distribution for humidity was relatively uniform, with a slight right skew.

Soil Moisture
Soil moisture was positively skewed, with the values between 20% and 45% gathered in the middle. Mean soil moisture content was 34.2%, and the standard deviation was 8.7%. Such statistics indicate occasional levels of water stress, more so during dry spell periods when soil moisture drops below acceptable limits for plants. Automated irrigation controlled moisture regulation, but variability likely occurred due to the delayed activation of the system to initiate irrigation once moisture depletion reached threshold levels.

Light Intensity
Light intensity (lux) measured 10,000 lux during cloudy conditions and exceeded 95,000 lux on clear days. The mean value was 62,500 lux, and the high standard deviation reflects the apparent diurnal and weather-induced variations. This was expected to affect photosynthetic activity; however, the correlation of this variable with yield was low, as discussed later in this work.

Crop Yield
Crop yield assessed at the end of the growing season in each plot monitored ranged between 2.3 and 7.8 kg (mean per plot = 5.4kg). This variable was used as a dependent outcome in correlation and machine learning analyses.

Correlation Analysis
A matrix of Pearson correlation coefficients was produced to measure the linear relationships between variables and determine which variables were the most important in influencing crop yield. The purpose of this analysis was to determine the strength and direction of the associations between independent variables (temperature, humidity, soil moisture, and light intensity) and the dependent variable (yield). A correlation coefficient close to 1 or -1 indicates a strong relationship, while a coefficient close to 0 indicates a weak relationship.

Key findings include

Soil Moisture and Yield

Soil moisture showed the highest positive correlation to crop yield ($r = 0.76$, $p < 0.01$). This close linkage reveals the central importance of water supply to crop growth and yields. Inconsistent moisture levels create water stress, negatively impacting nutrient uptake and photosynthesis. These data support the notion that, form aximising yields, soil moisture needs to be sufficient and constant.

Temperature and Yield

Yield had a significant but moderate negative correlation with temperature ($r = -0.45$, $p < 0.05$). It indicates that elevated temperatures can be detrimental to crop growth, particularly during critical growth stages such as flowering or grain filling [20]. This negative relationship corroborates field evidence showing that excessive heat reduced growth and productivity.

Humidity and Yield

The relationship between humidity and crop yield was positive and weak ($r = 0.22$); however, this value was also not statistically significant. Even though moisture impacts transpiration and can indirectly affect plant metabolism, it does not seem to have a direct and consistent effect on yield in this specific case.

Light Intensity and Yield

Likewise, light intensity had a very low ($r = 0.15$) and non-significant positive correlation with yield, indicating that natural variation in light was within optimal ranges for the crop and did not significantly limit yield (Kumar *et al*., 2020). Together, these results highlight the crucial role of soil moisture and temperature as primary drivers of crop growth (both above- and below-ground) in a system where only soil moisture and temperature are monitored to inform management decisions. This knowledge empowers farmers and agricultural managers to make informed decisions for optimal crop growth.

Machine Learning Predictive Modelling

For a robust understanding and the development of a predictive framework, we employed a Random Forest regression model. This ensemble machine learning algorithm, known for its precision with tabular datasets and non-linear relationships, was a fitting choice for our high-dimensional dataset. We meticulously split the data into 80% for training the model and 20% for validating the trained model. The input features used for prediction were temperature, humidity, soil moisture, and light intensity, with crop yield as the target variable.

Model Performance

R^2 Score: 0.87

Mean absolute error (MAE) : 1.6 (kg)

RMSE (Root Mean Squared Error): 2.1 kg

The R^2 value (0.8729) signifies that the model explains nearly 87% of the variation in crop yield, demonstrating its high accuracy. The low mean absolute error further validates the precision and accuracy of our predictions across diverse plot conditions.

Feature Importance

Another benefit of the Random Forest model is that it permits the estimation of feature importance. Results showed

Soil moisture was the most significant contributor to prediction accuracy (~43%)

Path followed by Temperature with ~28%

Humidity and light had only minor effects (15% and 14%, respectively)

These findings further corroborate the results of the correlation analysis and indicate the most essential features that need to be monitored and adjusted in the development of innovative farming systems.

Analysis and Consequences

The findings from all statistical and machine learning analyses carry significant implications for the practical design and implementation of AI-based innovative agricultural systems. These insights can guide the development of technologies that optimise planting schedules, irrigation, and resource allocation, thereby improving crop yield and resource efficiency in agriculture.

Moisture Management is Key

The high correlation between soil moisture and crop yield suggests that a real-time sensor data-driven precision irrigation system can significantly enhance productivity. Threshold-based irrigation, combined with predictions from machine learning, can provide irrigation when needed, ensuring the necessary moisture and avoiding periods of stress.

Why is that important: Reducing extremes of temperature

This inverse relationship between temperature and yield suggests that microclimate control strategies, such as shade nets, mulching, or smart greenhouses, should be implemented in areas with extreme temperature variations.

AI for Decision Support

This promising deployment of a Random Forest model to predict yield outcomes demonstrates the potential for using artificial intelligence as a decision-support tool for farmers and agricultural managers. These models can be incorporated into farm management software to provide practical insights on aspects such as optimising planting schedules, irrigation, and resource allocation.

Minimal Effect of Moisture and Illumination

The reasonably small effect of humidity and light exposure on yield in this study indicates that, at best, they are important but not critical (and therefore do not require active management, unless they reach extreme values). Instead, it should be on soil temperature, etc.

Metric Examples: Environmental Impact and Sustainability

Water Conservation and Management

Water conservation is one of the most significant environmental benefits of innovative agriculture technologies. Traditional irrigation practices result in considerable water waste due to over application, poor timing, and imprecise water delivery. This is where AI irrigation systems come in, with advanced monitoring and control. Studies have shown that innovative irrigation systems can lower agricultural water consumption by 30–50% compared to traditional methods (Frontiers). Predictions based on artificial intelligence (AI) can increase the efficiency of water use in irrigation systems by up to 60%, thereby minimising the amount of water applied for crop growth and enhancing grain yield. This gain is attributed to the accurate tracking of soil moisture conditions, the incorporation of a weather forecast, and the detection of water stress signals in plants. Moreover, IoT-supported irrigation systems provide real-time information about the soil moisture level, allowing for the control of excess water flow and ensuring a sufficient water supply for crops. These systems can automatically adjust irrigation schedules based on weather forecasts, soil conditions, and plant needs, maximising water use with no loss of crop growth. IoT-based soil monitoring systems result in less waste, better efficiency, and increased production in citrus orchards, as shown by numerous in-depth studies in Frontiers. Precision irrigation technologies target water application, bringing water to root zones and reducing losses from evaporation and runoff. AI-controlled drip irrigation systems can achieve water-use efficiencies of over 95%, compared to 60–70% for sprinkler systems and 40–50% for flood irrigation systems.

Chemical Input Reduction

These technologies have proven capabilities to decrease chemical input while enabling similar or higher crop productivity. Precision Application Technologies: This innovative approach minimises overall fertiliser and pesticide use while enhancing efficacy through targeted delivery methods. Precision agriculture systems can decrease chemical pesticides up to 70% by monitoring and using artificial intelligence to determine the need for chemicals or pesticide usage in real time, according to Databricks. This recognition can help computer vision systems identify areas where

pests or diseases occur, enabling farmers to apply targeted spot treatments instead of broad-spectrum applications. Such an approach enables a decrease in chemical consumption, reduces costs, and offers a lower environmental footprint, promoting a more sustainable farming approach. Another move in this positive direction is the development of robotic weeding systems. They utilise computer vision and machine learning to distinguish between crops and weeds, employing targeted herbicide applications and/or mechanical weed removal. This means that some highly accurate robotic systems are capable of reducing herbicide expenditure by as much as 90% while still effectively combating weeds. Precision agriculture technologies can optimise fertiliser application, allowing for a reduction of up to 15-20% in fertiliser use while maintaining, or even increasing, yields. Variable-rate fertiliser application systems utilise soil test data, yield maps, and plant tissue testing to determine the optimal fertiliser rates for zones within fields. This precision approach prevents over application while still maintaining sufficient nutrient availability for crops.

Soil health and conservation

Precision management practices and low- or no-tillage systems result in significant improvements in soil health and conservation, which are essential components of innovative agricultural technologies. To improve soil health and productivity, soil monitoring sensors provide continuous information on soil conditions, enabling more effective management practices. Conservation tillage practices are supported by precision agriculture systems that provide information about soil conditions and crop residue management. Reduced tillage systems reduce soil erosion by 90% compared to conventional tillage, and enhance soil organic matter and soil water holding capacity. IoT sensors can help track when to monitor soil moisture and temperature conditions, which in turn helps determine the optimal timing of field operations, resulting in less soil compaction and erosion. Innovative agriculture technologies support smart systems for cover crop management through accurate monitoring and precise planting systems. Cover crops can be planted at optimal rates and spacing using automated seeding equipment, and their presence and growth can be recorded and monitored using remote sensing technologies. This post highlights the numerous soil health benefits associated with cover cropping, including preventing soil erosion, enhancing nutrient retention, and increasing organic matter. Remote sensing technology and AI analysis have strengthened the monitoring of soil carbon sequestration. More quantitatively, soil organic matter content can be determined using satellite imagery and ground-based sensors to monitor changes over time, mainly where carbon sequestration occurs. It supports carbon credit programs, sustainability reporting, and enables the optimisation of management practices to enhance soil health.

Biodiversity and Ecosystem Services

As highlighted in the FAO report, sharp management practices enabled by innovative agricultural technologies can facilitate a balance between farm sustainability and the need for biodiversity conservation and ecosystem services by reducing negative environmental externalities. The targeted application of pesticides minimises off-target impacts on beneficial insects, birds, and other wildlife species. Innovative agricultural technologies, such as precision and data-driven high-resolution mapping and monitoring of non-crop habitats, are helpful in habitat management within agrarian landscapes. Easily identifies wildlife habitat areas, wetlands, and other environmentally sensitive areas using drone surveys and satellite imagery for protection and enhancement strategies. With the help of GPS-guided machinery, these positions can be bypassed during field operations, thereby minimising disturbance to wildlife habitats. Innovative agriculture reduces pesticide use and enhances habitat management, thereby supporting the conservation of pollinators. Factors such as selective application (which directs sprays to specific target areas of a site) and the timing of treatments can also reduce pesticide exposure to pollinators (e.g., by avoiding times of day when pollinator activity is high). Innovative agricultural systems also facilitate the establishment and maintenance of pollinator habitats within agricultural landscapes. Through innovative agronomic technologies, we can enhance the precision and efficiency of nutrient management and erosion control practices, thereby increasing watershed protection and sustainability. Conservation tillage methods reduce soil erosion, while precision fertiliser technology prevents runoff of excess nutrients to waterways. For example, continuous water quality monitoring sensors can provide real-time data on nutrient concentrations in rivers, supporting immediate action when potential pollution events are detected.

Case Studies: Applications in the Real World

The Precision Agriculture Platform from Smart Agriculture

Smart Agriculture has integrated innovative agricultural technology through AI-powered precision agriculture, which is one of the most comprehensive implementations of this technology. Lower-end machinery manufacturer to a wholesaler with a broad portfolio, combining machinery, telemetry, and automation to deliver integrated agricultural solutions. Another example is John Deere's precision agriculture platform, which blends a variety of AI techniques, including computer vision, machine learning, and predictive analytics. Real-time measurements of soil quality and moisture levels are taken with the help of AI-driven sensors. At the same time, machine learning algorithms evaluate soil health to ensure seeds are planted at the correct depth and the proper distance apart. SEED SOWING GPS guide GPS-girded equipment ensures precise work of seeds and seedling machines, and less waste of resources, better plant establishment Quantitative results for John Deere's precision agriculture pilot program indicate substantial benefits across various measures. Crop yields from farmers using AI-driven systems, such as those provided by John Deere, have increased by 25%, while water and fertiliser use have decreased by 30%. Exact Emerge technology has achieved 20% higher planting efficiency compared to manual planting, and fuel costs have been reduced by 15% thanks to the Auto Track guidance system from the same company, Redress Compliance.

IoT Irrigation Systems

An extensive research on IoT based irrigation systems showed a water use reduction of 30-50% on average than traditional irrigation systems. The systems achieved these savings by accurately measuring soil moisture conditions to schedule irrigation at the plant level, and by linking to weather forecasting systems to prevent irrigation before an expected rainfall event. Intelligent irrigation systems have proven to be economically beneficial, with typical return on investment (ROI) periods ranging from 1.5 to 2 years. During this period, farmers can experience yield increases of up to 40% and reductions in water use of 50%. This is due to more accurate water management, which eliminates both water stress and overwatering conditions during the growth period, thereby providing optimal growing conditions for the crop throughout its duration. Involves the deployment of wireless sensor networks that are used to monitor soil moisture, temperature, and other environmental parameters. These sensors transmit information to cloud-based platforms that run AI algorithms to analyse conditions and generate irrigation recommendations. Mobile apps enable farmers to remotely monitor the system's status and make adjustments (or modifications) to it, making them operationally flexible and able to respond quickly.

Management System for Pests and Diseases

Using AI to manage pests and diseases has proven beneficial in providing early warnings and effective treatments for agricultural problems. The system utilises computer vision, machine learning, and IoT sensors to monitor crop conditions and identify issues before they become widespread. Computer vision systems have been developed to recognise specific pests and diseases from digital images, achieving an accuracy of over 95% for particular pests and diseases. The systems analyse pictures captured by smartphones, drones, or stationary cameras to detect issues and recommend treatments. With early detection, targeted therapies that reduce pesticide use can be implemented whilst achieving optimal control over the Xylella spread. To monitor pest populations, various sensors and traps are used in IoT-enabled pest monitoring systems. Systems that could be used include digital pheromone trap counting systems, acoustic sensors to detect pests such as male moths, and environmental sensors to verify the microclimatic conditions necessary for the pest's development. This data is then analysed by machine learning algorithms, which can predict when a pest outbreak will occur and when the optimal times to treat crops will be. Precision pest management systems can lead to substantial reductions in pesticide use.

CONCLUSION

Artificial Intelligence (AI) and Automation in Agriculture Agricultural systems are undergoing a significant transition, with the integration of Artificial Intelligence (AI) and automation leading to more sustainable, efficient, and data-driven farming practices Smart agriculture brings it precision in crop management, resource utilisation and

yield prediction through advanced monitoring systems, machine learning algorithms and real-time data analytics. Besides the productivity boost, these technologies also play a critical role in saving the environment by reducing water, fertiliser, and energy waste. The literature review highlights the increasing influence of AI in making wise decisions, ranging from pest and disease detection to soil health assessment and autonomous farming operations. Cloud computing, paired with IoT devices, enhances the ease of data collection and remote monitoring, enabling farming to adjust promptly to changing climatic and market conditions. However, the developments in the field are not without their challenges, including data privacy issues, cost-intensive implementation processes, the lack of infrastructure at the rural level, and the need to train farmers. This means that, for future research and policy, we need more affordable AI technologies, increased digital literacy among farmers, and more robust infrastructure to support smart farming.AI-based smart agriculture also has the potential to transform global food systems towards higher levels of efficiency, sustainability, and resilience. A careful integration of these technologies will play a key role as they advance in progress to overcome the multifaceted issues of current-day agriculture and feed an ever-increasing world population.

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RESEARCH ARTICLE

A Strategy on Decision Theory under Uncertainty and Risk Management

J. Kiruthika[1]* and P. Jayagowri[2]

[1]Assistant Professor, Department of Mathematics, Shrimati Indira Gandhi College, Theppakulam, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.
[2]Assosiate Professor, Department of Mathematics, Sudharsan College of Arts and Science, Perumanadu, Pudukkottai, (Affiliated to Bharathidasan University, Tiruchirappalli), Tamil Nadu, India.

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***Address for Correspondence**
J. Kiruthika
Assistant Professor,
Department of Mathematics,
Shrimati Indira Gandhi College, Theppakulam,
(Affiliated to Bharathidasan University), Tiruchirappalli,
Tamil Nadu, India.
E.Mail: kiruthi.jj@gmail.com

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ABSTRACT

Decision making is the process of maximizing the gains or expected value of the consequence by utilizing information available. The linear model with a relationship of probability to utility is the expected value.

Keywords: Expected value analysis, Decision making under risk, Decision-making under uncertainty, Maximax / maximin criteria, Expected opportunity loss criterion, Utility theory.

INTRODUCTION

The manager falls into the deterministic models which presuppose that there is certainty and in probabilistic models all the strategies are represented by a number of pay offs and the manager can attach to them a probability measure. Motivations The answers are categorized based on the level of certainty. One can assume that both decision-making under certainty (DMUC) and decision-making under uncertainty (DMUU) may be viewed as the two extremes since the entire range of the assumed certainty can range between full certainty to full uncertainty. The region composed of these two extreme positions as well as those that lay between the two and are identified with probabilistic models is referred to as decision-making under risk (DMUR). It can be inferred that the level of certainty assumed in decision is a minuscule aspect of decisions made under risk and the bulk of the problem in decision making can be seen to be due to decision under risk. Other systems of classification are a linear or non-linear behaviour, a static or a dynamic condition and a single or multiple objective. All these are factors that have to be considered before coming up with a model.

MATERIALS AND METHODS

Decision Theory

Decision making under risk and uncertainty is what is concerned with theory of decision-making. By the purpose of this, any kinds of decision models like deterministic models will be under the hands of decision theory. The management literature comes up with various quantitative models of decision that help managers in determining optimum or best courses of actions.

Two Phases of The Process of Decision - Making

The decision theory has been a considerable part, because the convergence of such diverse disciplines as philosophy, economics, psychology, sociology, statistics, political science and the operations research to the theory of decision have taken place. During decision-making we find two stages:

- The approach which involves working on the formulation of goals and objectives, enumeration of environmental constraints, identification of alternative strategies, and estimation of pertain payoffs.
- To pinpoint on the issue how to choose the optimal strategy provided that we have a selection of objectives, sets of strategies, sets of payoffs. The second issue is that we pay more attention to our discussion.

Decision Making Under Risk (DMUR)

Decision making under risk (DMUR) entails a situation, which involves the payoffs or the several associated with each such set of strategies being more than one, and with the manager attaching amount of probability concerning the payoffs. The present model can be applied to the case in which the manager can estimate two or more outcomes of each of the strategies; and he/she knows or can suppose such that the results are suitable distributed between the possible ways. Assumptions will follow as

1. Ability to access multiple options of strategies
2. There is the existence of many states of nature
3. As the concerned results
4. The likelihood of end results of each stratagem.

The strategy that is of maximum expected utility (or maximum expected value) is the one that is best in the case of decision making under risk.

RESULTS AND DISCUSSIONS

Problem

The probability of a book shopkeeper selling a book is as follows. The price of the book is 10 rupees, and the sales price Rs.35. He is not able to send back books that are not sold. How much can he order in terms of books?(Table: 1)

Solution

The book shopkeeper reports that the sales quantities of books are 3, 5, 7, 8, and 10 books. It does not encourage him to buy less than 3 or more than 10 books. The table given below shows price setting table i.e. profit earned based on all the possible incidences of the supply and the demand. Under this assumption, he will be able to sell 3 books whereas the demand will be 10 books someday; his conditional profit will be 75 rupees. When order quantity with 5, 7, 8 and 10 books are ordered by the buyer then the profit of stock of 5 books is 125 rupees. However when he has 5 books on one day and the buyers have only 3 books then his gain will be 55 rupees since there are two unsold books. Thus payoff = 25 books sold minus 10 books unsold. Thus, Conditional profit table is given below.

Conditional Profit Table (Rupee) Possible Stock Action (Table: 2)

Expected Profit Table Expected Profit from Stocking in Rupee(Table: 3)

The owner of the bookshop will hence have to order 8 books to attain a maximum average daily profit of 133.5 rupees. Therefore, the ideal stock is 8 books. The stocking will maximize the cumulative profits in each time. Naturally, he cannot be sure that he will get a profit ranging 133.5 rupees again tomorrow. Interestingly though if he orders 8 books a day under the specified condition, he will average 133.5-rupee profit per day. It is the most I could do since the other of the four other possible actions as to the stock will result in a smaller amount of profit per day than the one, I have decided to do. Expected opportunity loss (EOL) may be calculated by taking individual probabilities of each of the states of nature and multiplying the probability by the corresponding loss amount and summing the outcomes.

Conditional Loss Table in rupee Possible Stock Action (Alternative)(Table: 4)
When the book shop keeper has only 8 books, then his loss will be less.(Table: 5)

Decision Making Under Uncertainty
The result of a major alternative of the decision is not certain, not to mention some probability. Only a number (of approaches) criteria are provided that the decision makers are supposed to use based on their preference and characters.

- Maximax Criterion
- Maximin Criterion
- Maximin Criterion
- Minimax Regret
- Laplace Criterion (Equally likelihood)
- Hurwiczalpha Criterion

Maximax Criterion
A decision maker who is adventurous and aggressive can opt to undertake the act that would give the highest payoff possible

- Step1: select maximum pay off over each alternative.
- Step 2- Select the greatest of the maximal values of Step 1; the alternative associated with it is the decision.
- This is a positive strategy, “Best of bests”.

Maximin
It is also referred to as Waldian criterion. This is one criterion of decision making which represents choice of alternative courses of action and with pessimistic outlook of nature.

- Step1Choose minimum take off of every alternative
- Step 2 Pick (select) the highest of those minimums in Step 1, its counterpart is the decision

Minimax Regret
The minimax criterion needs a table of losses or table of regret rather than gains. Regret is what you sacrifice on failing to choose the optimal choice in a state of nature.

- Regret= cost of opportunity= loss of opportunity
- Step1-Make a regret table
- Step2 -Select maximum regrets of every row in regrettable
- Step 3- select minimum of maximums in Step 2, its alternative is the decision.

Equally Likely
Its easy assumption that the decision maker makes is that each of the states of nature is equally apt to take place and computes the average payoff of each. Make the decision which has the highest average payoff.

- Closely referred to as Laplace criterion
- Step1-compute the average pay off of each alternative.
- Step2-The decision that has maximum average is the alternative.

Hurwicz (Realism)

This is mixed approach of both Maximin and Maximax. Otherwise referred to as criterion of rationality. Not too hope some, not too pessimistic

- Step1Compute Hurwicz value of each of the alternatives
- Step 2: Choose the alternative with maximum Hurwicz value as the choice. Hurwicz value of a decision alternative rowmax +rowmin 1-α
  Where is ($0 \leq \alpha \leq 1$) is known as coefficient of realism.

Problem

A book shopkeeper is not sure whether to sell a once off edition of a poem about money. He thinks that she can sell 7 to 11 books to his knowledge of the customer. The books could be bought at a price of Rs. 30/book or be sold at a price of Rs. 45/book. Unsold books may be sent back to the publisher where half the amount is returned.

Develop a decision table of the above inventory problem, stating potential financial implication

Identify the best decision depending on

- Maximax Criterion
- Maximin Criterion
- strongly on Minimax Regret
- Laplace Criterion (Equally likelihood)
- Hurwicz alpha criterion (Rationality or Realism)

Solution (Table: 6)

Without given probabilities based on risk, subjective values, or experience, everyone may have different views. These are selected in accordance with choice criterion. In consequence, the operation of making decisions under uncertainty is often discussed as choice formation models. Two criteria to consider are the Criterion of Optimism and the Criterion of Pessimism.

Maximax Criterion

In this case, what we ascertain is the best possibility outcome in every storage books and then there is the most beneficial of the best outcomes which will be determined as the optimum books. The left-hand the margin contains the best of the best, as indicated in the table: 7 below. With application of the principle of optimism, this is to choose among max of max; which is also referred to as Maximax.

Maximin Criterion

In handling a problem of uncertainty with the maximin criterion, the worst possible can be found in each book (row minima) and best among the worst possibilities is selected to obtain the best possible method. The disastrous consequences have been given in left-hand margin. (Table: 8) An optimal maximization option is 105; hence, the book shopkeeper uses the first books. Maximin makes total maximin. Optimism in maximax is complete.

Minimax Regret

To compute the regret loss in the opportunity loss matrix, subtract each element from the maximum number in its column. The resultant state is called the matrix of regrets. As we select the preferred books, we must be sure that we do not have an opportunity loss, i.e. a regret. In case we pick any other literature, regret is an element of that strategy. In the problem, the regret matrix is as shown in the table below with respect to the matrix provided in the problem.(Table:9). To create the regret matrix, find the highest element in each column and subtract every other element in that column from it. To identify the best books, calculate the maximum regret for each book and then find the highest of these regrets. This process is shown in the table below(Table: 10). Select the least of the greatest regret (Minimax). The decision procedure is also referred to as minimax regret. There is the assumption that two books are identical in their minimax elements and the book seller needs other elements to determine his choice.

Laplace Criterion (Equally Likelihood)

While objective evidence of a probability distribution for the demands is available, subjective criteria may also be used. Furthermore, in the absence of objective evidence, it is possible to assign equal probabilities to each state of nature. The Laplace principle, also known as the principle of insufficient reason, posits that each demand has an equal likelihood. By assigning equal probabilities, risk analysis is applied, and the expected value method can be utilized, as illustrated in the following table: 11. The optimal method would be to produce 8 books, because it gives the greatest EMV.

Hurwicz Alpha Criterion (Rationality Or Realism)

To find a level of optimism or optimism the bookseller could weight the best and the worst conditions. Suppose the ownership will set the coefficient of optimism at 0.4; of course the coefficient of pessimism will be 0.4. The following matrix of cells demonstrates the way of selecting the correct approach when weights are given table: 12.

CONCLUSION

Coming up with risky judgments enables you to predict whether an outcome is likely to occur in the future. But decisions cannot be made out of situations that are not known. Uncertainty is unalleviated and risks can be tamed. You are able to put probabilities on the dangerous events.

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Table 1: Problem

Number of books sold	Probability
3	0.20
5	0.20
7	0.30
8	0.20
10	0.10
Total	1.00

Table 2: Conditional Profit Table (Rupee) Possible Stock Action

Possible demand (Numbers of books)	Probability	3 books	5 books	7 books	8 books	10 books
3	0.20	75	55	35	25	5
5	0.20	75	125	105	95	75

7	0.30	75	125	175	165	145
8	0.20	75	125	175	200	180
10	0.10	75	125	175	200	250

Table 3: Expected Profit Table Expected Profit from Stocking in Rupee

Possible demand	Probability	3 books	5 books	7 books	8 books	10 books
3	0.20	15	11	7	5	1
5	0.20	15	25	21	19	15
7	0.30	22.5	37.5	52.5	49.5	43.5
8	0.20	15	25	35	40	36
10	0.10	7.5	12.5	17.5	20	25
Total Expected Profit in Rupees		75	111	133	**133.5**	120.5

Table 4: Conditional Loss Table in rupee Possible Stock Action(Alternative)

Possible demand Number of Books	Probability	3 books	5 books	7 books	8 books	10 books
3	0.20	0	20	40	50	70
5	0.20	50	0	20	30	50
7	0.30	100	50	0	10	30
8	0.20	125	75	25	0	20
10	0.10	175	125	75	50	0

Table 5:

Possible demand Number of Books	Probability	3 books	5 books	7 books	8 books	10 books
3	0.20	0	4	8	10	14
5	0.20	10	0	4	6	10
7	0.30	30	15	0	3	6
8	0.20	25	15	5	0	4
10	0.10	17.5	12.5	7.5	5	0
EOL(Rupee)		82.5	46.5	24.5	24	34

Table 6: Solution

No. of Books	7 books	8 books	9 books	10 books	11books
7	105	105	105	105	105
8	75	120	120	120	120
9	45	90	135	135	135
10	15	60	105	150	150
11	-15	30	75	120	165

Table 7: Maximax criterion

No. of Books	7-books	8-books	9-books	10-books	11-books	Maximum outcome (Row Maximum)
7	105	105	105	105	105	105
8	75	120	120	120	120	120
9	45	90	135	135	135	135
10	15	60	105	150	150	150
11	-15	30	75	120	165	165 Maximax

Table 8: Maximin Criterion

No. of Books	7-books	8-books	9-books	10-books	11-books	Minimum outcome (Row minimum)
7	105	105	105	105	105	105 Maximin
8	75	120	120	120	120	75
9	45	90	135	135	135	45
10	15	60	105	150	150	15
11	-5	30	75	120	165	-15

Table 9: Minimax Regret

No. of Books	7-books	8-books	9-books	10-books	11-books
7	0	15	30	45	60
8	30	0	15	30	45
9	60	30	0	15	30
10	90	60	30	0	15
11	120	90	60	30	0

Table 10:

No. of Books	7-books	8-books	9-books	10-books	11-books	Maximum regret
7	0	15	30	45	60	60
8	30	0	15	30	45	45minimax
9	60	30	0	15	30	60
10	90	60	30	0	15	90
11	120	90	60	30	0	120

Table 11: Laplace Criterion (Equally Likelihood)

No .of Books	7-books	8-books	9-books	10-books	11-books	Expected monetary value (EVM)
7	105	105	105	105	105	105
8	75	120	120	120	120	111Maximum
9	45	90	135	135	135	108
10	15	60	105	150	150	96
11	-15	30	75	120	165	75

Table 12: Hurwicz Alpha Criterion (Rationality Or Realism)

No. of Books	Maximum-payoffs	Minimum-payoffs	Weighted-payoffs
Weights	0.4	0.6	
7	105	105	0.4×105+0.6×105=105
8	75	120	0.4×75+0.6×120=102
9	45	135	0.4×45+0.6×135=99
10	15	150	0.4×15+0.6×150=96
11	-15	165	0.4×(15)+0.6×165=93

RESEARCH ARTICLE

Comparison of Salivary and Plasma Levels of Alpha Amylase and Salivary pH in Pre and Post Treatment Patients of Oral Squamous Cell Carcinoma

Apna Yadav[1]*, Praveen Sharma[2], Shruti Chhabra[3] and Tarun Mittal[4]

[1]Assistant Professor, Department of Oral and Maxillofacial Surgery, Shree Guru Gobind Singh Tricentenary University, Gurugram, Haryana, India.
[2]Professor and Head of Department, Department of Oral and Maxillofacial Surgery, J.N. Kapoor DAV (C) Dental College, Yamuna Nagar, (Affiliated to Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak), Haryana, India.
[3]Professor, Department of Oral and Maxillofacial Surgery, J.N. Kapoor DAV (C) Dental College, Yamuna Nagar, (Affiliated to Pandit Bhagwat Dayal Sharma University of Health Sciences, Rohtak), Haryana, India.
[4]Senior Lecturer, Department of Oral and Maxillofacial Surgery, Guru Nanak Dev Dental College, Sunam, (Affiliated to Baba Farid University of Health Sciences, Faridkot), Punjab, India.

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***Address for Correspondence**
Apna Yadav
Assistant Professor,
Department of Oral and Maxillofacial Surgery,
Shree Guru Gobind Singh Tricentenary University,
Gurugram, Haryana, India.
E.Mail: apnayadav_fdscsgtuniversity.org

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ABSTRACT

Oral squamous cell carcinoma (OSCC) is a prevalent malignancy with significant morbidity and mortality, especially in the Indian subcontinent. Non-invasive biomarkers such as salivary alpha amylase (sAA), plasma alpha amylase, and salivary pH are gaining importance in early diagnosis, monitoring treatment response, and predicting prognosis. This study aimed to evaluate and compare the salivary and plasma alpha amylase levels and salivary pH in OSCC patients before and after treatment, and in healthy individuals. The study included 40 participants divided into three groups: Group A (n=20) – healthy controls; Group BI (n=20) – OSCC patients before treatment; and Group BII (n=20) – the same OSCC patients, four months post-treatment. Saliva and blood samples were collected in both morning and evening sessions. Alpha amylase levels were measured using Erba Liquixx 6X6-Amylase kits, and pH was assessed using a digital pH meter. Data were statistically analyzed using SPSS version 25.0 and MedCalc software. Statistical tests used included One-way ANOVA, Post-hoc Bonferroni test, Unpaired t-test, and Chi-square test. A significant reduction in both salivary and plasma alpha amylase levels was

observed in Group BII (post-treatment) compared to Group BI (pre-treatment) ($p<0.001$). The salivary pH showed a significant increase in Group BII compared to Group BI ($p<0.05$), suggesting improved oral health status post-treatment. Diurnal variation was also noted, with higher amylase levels observed in evening samples compared to morning samples across all groups. Salivary and plasma alpha amylase levels, along with salivary pH, can serve as potential non-invasive, reproducible biomarkers for monitoring disease progression and treatment response in oral squamous cell carcinoma. These markers may aid in early detection and post-treatment follow-up, enhancing personalized patient care in OSCC.

Keywords: OSCC, sAA, Plasma alpha amylase, salivary pH

INTRODUCTION

Oral cancer is a debilitating disease primarily affecting the oral cavity, with oral squamous cell carcinoma (OSCC) accounting for 70–90% of all oral malignancies. OSCC ranks sixth among all cancers globally, and its prevalence is particularly high in South Asia[1,2]. The disease is most often associated with persistent exposure to known carcinogens such as tobacco (both smoked and smokeless), areca nut, betel quid, and chronic alcohol use. Additionally, oncogenic viral infections, especially human papillomavirus (HPV), further elevate the risk by inducing genetic mutations in the oral epithelium. Early detection of OSCC is crucial for improving prognosis. Traditional screening methods include visual inspection, vital staining (such as toluidine blue), brush biopsy, chemiluminescence, and tissue autofluorescence. However, histopathological examination of biopsy tissue remains the gold standard for diagnosis. The key limitation of conventional techniques lies in their inability to detect OSCC at a very early, subclinical stage, often leading to delayed intervention and poorer outcomes. Recent advances in biomarker research have highlighted the potential of salivary diagnostics. Saliva, a complex oral fluid, plays diverse physiological roles such as aiding in digestion, lubrication, taste perception, and antimicrobial defense. It contains various bioactive molecules—enzymes, hormones, antibodies, cytokines, and antimicrobial proteins—many of which reach the oral cavity via mechanisms like active transport, passive diffusion, transcellular and paracellular pathways, and ultrafiltration from blood through salivary glands. Thus, both blood and saliva can reflect systemic physiological changes, including nutritional, metabolic, endocrinal, and emotional variations[1,3,4]. Among the salivary proteins, salivary alpha-amylase is a prominent calcium-dependent metalloenzyme, commonly referred to as ptyalin, which functions in the digestion of dietary starch by hydrolyzing α-1,4 glycosidic linkages. Notably, its concentration in saliva has been linked to dietary starch intake, showing a correlation with the copy number of the salivary amylase gene[4]. Salivary pH also plays a crucial role in oral health. Normal salivary pH ranges from 6.7 to 7.3, but in cancerous conditions, a shift toward acidity is observed. This acidic microenvironment is largely attributed to anaerobic glucose metabolism by tumor cells under hypoxic stress, which in turn promotes tumor survival and proliferation[5]. In this study, we aim to evaluate and compare the plasma and salivary levels of alpha-amylase in OSCC patients both before and after treatment, and contrast these findings with those from a control group of healthy individuals. Additionally, we analyze the diurnal variation in salivary alpha-amylase and pH across both groups, with the goal of exploring their potential as non-invasive biomarkers for early OSCC detection and monitoring.

AIMS AND OBJECTIVES

Aims

a. To study and compare the levels of alpha amylase in plasma and saliva in patients of oral squamous cell carcinoma (OSCC) pre and post treatment.
b. To compare the salivary pH in both the groups.

Objectives

a. To establish the prognostic value of alpha amylase in plasma and saliva pre and post treatment of oral squamous cell carcinoma (OSCC) patients.
b. To establish the role of salivary pH as diagnostic tool in patients of oral squamous cell carcinoma (OSCC).

MATERIALS AND METHODS

Study Design and Duration

This clinical study was conducted over a period of 18 months, from February 2021 to April 2022, and involved two groups: healthy individuals (controls) and patients diagnosed with oral squamous cell carcinoma (OSCC).

Ethical Approval

The study received ethical clearance from the Pt. B.D. Sharma University of Health Sciences, Rohtak, Haryana. Informed written consent was obtained from all participants prior to inclusion.

Study Population

Participants were recruited from patients reporting to the Department of Oral and Maxillofacial Surgery, D.A.V. (C) Dental College and Hospital, Yamunanagar.

Inclusion Criteria

- Age: 25–75 years
- Histopathologically confirmed OSCC with accessible lesion sites (tongue, floor of mouth, lips, gums, hard palate, buccal mucosa, or retromolar area)
- No prior treatment for OSCC

Exclusion Criteria

- History of acute pancreatitis, salivary gland disorders, GI emergencies
- Pregnant/lactating women
- Stress-related elevated amylase
- History of oncotherapy or systemic malignancy
- Patients with adrenal or HPA axis disorders
- Unwilling participants

Sample Size and Group Allocation

The total sample consisted of 40 individuals, divided equally
Group A: 20 healthy individuals (controls)
Group B: 20 OSCC patients
- Group B-I: Pre-treatment
- Group B-II: Same patients at 4 months post-treatment

Recruitment and Randomization

Participants were recruited based on eligibility criteria and randomly assigned to study groups. Complete clinical evaluation, radiological imaging (as needed), and biopsy (for OSCC patients) were performed prior to sample collection.

Sample Collection and Protocol

Pre-Sampling Preparation

Participants underwent routine blood tests (Hb, ESR, TLC, DLC, etc.) and necessary imaging (OPG, CT, MRI). For OSCC patients, biopsy under local anesthesia confirmed diagnosis.

Saliva Sampling (figure 1)

- Collected between 7–10 AM and 7–10 PM to assess diurnal variation
- Participants fasted and refrained from oral intake for at least 1 hour before sampling
- After rinsing the mouth with water, 5 mL of unstimulated saliva was collected into sterile tubes
- pH measured immediately using pH indicator strips
- Samples stored at 3°C for later analysis

Salivary pH Analysis

- Saliva diluted to 30 mL
- pH measured with a calibrated pH meter (Birud pH meter)

Salivary Amylase Estimation

- 500 µL of Erba Liquixx amylase reagent mixed with 10 µL saliva
- Processed using Erba Chem-7 analyzer
- Amylase levels recorded

Blood Sample Collection (figure 2)

- 5 mL of venous blood collected from the antecubital vein at the same diurnal intervals as saliva
- Blood incubated at 37°C for 20 minutes, then centrifuged at 400 rpm for 10 minutes
- Plasma separated and used for enzyme analysis

Plasma Amylase Estimation

- 10 µL plasma mixed with 500 µL of Erba Liquixx reagent
- Processed in Erba Chem-7 machine
- Final reading recorded

Outcome Measures(Table: 1)

This section outlines the methodology for analyzing salivary and plasma alpha-amylase levels, and salivary pH in OSCC patients compared to healthy controls, both pre- and post-treatment, with an emphasis on diurnal variation and non-invasive biomarker potential.

RESULTS AND OBSERVATIONS

Demographic Characteristics

Age: Group B (OSCC patients) had a significantly higher mean age (51.15 ± 11.57 years) than Group A (healthy controls) (29.80 ± 5.94 years), $p = 0.001$ (Unpaired t-test).

Gender: Males were more prevalent in Group B (70%) compared to Group A (45%), though this difference was not statistically significant ($p = 0.110$, Chi-square test).

Tumor Site Distribution in OSCC Group (Group B)

Most common sites included

- Left buccal mucosa (20%)
- Lateral border of the tongue (20% combined left and right)

- Lower lip and gingivobuccal mucosa (10% each)

Biochemical Parameters

Salivary Amylase – Morning

- Significantly elevated in Group B I (untreated OSCC: 138.97 ± 30.99 U/L) compared to Group A (65.83 ± 20.72 U/L) and Group B II (post-treatment: 57.81 ± 20.27 U/L), $p = 0.001$ (ANOVA).
- Post-hoc analysis showed significant differences between Group B I and the other two groups.(Graph 1)

Salivary Amylase – Evening

- Again highest in Group B I (148.67 ± 28.21 U/L) compared to Group A (84.30 ± 22.01 U/L) and Group B II (72.26 ± 19.91 U/L), $p = 0.001$.
- Significant difference noted between Group B I and both groups; no significant difference between Group A and B II. (Graph 1)

Plasma Amylase – Morning

- Higher in Group B I (97.35 ± 11.68 U/L) vs. Group A (61.86 ± 13.52 U/L) and Group B II (51.04 ± 20.07 U/L), $p = 0.001$.
- Significant difference found between Group B I and both other groups. (Graph 2)

Plasma Amylase – Evening

- Group B I showed elevated levels (100.74 ± 16.46 U/L) versus Group A (71.91 ± 12.60 U/L) and Group B II (60.70 ± 22.72 U/L), $p = 0.001$.
- Post-hoc tests confirmed significance between Group B I and the other two groups. .(Graph 2)

Salivary pH – Morning

- Reduced in Group B I (6.12 ± 0.20) compared to Group A (7.19 ± 0.22) and Group B II (6.53 ± 0.33), $p = 0.001$.
- All intergroup differences were statistically significant. (Graph 3)

Salivary pH – Evening

- Group B I had the lowest salivary pH (6.08 ± 0.16), while Group A had the highest (7.22 ± 0.23); Group B II showed partial recovery (6.56 ± 0.30), $p = 0.001$. (Graph 3)

DISCUSSION

In the present study, the mean age of group A (healthy controls) was 29.80 years, while group B (OSCC patients) showed a significantly higher mean age of 51.15 years (p = 0.001, unpaired t-test). This aligns with Akram *et al*. (2013)[7], who reported a mean age of 47.84 ± 12.18 years among OSCC patients, and Alamgir *et al*. (2016)[8], who found a mean of 47.1 ± 12.22 years in a tobacco-exposed population from Karachi. Gender distribution showed a male predominance in OSCC group B (70% male, 30% female) compared to group A (45% male, 55% female), though not statistically significant. Akram *et al*. (2013)[7] reported a male-to-female ratio of 2.82:1, and Alamgir *et al*. (2016)[8] noted 1.88:1. Male predominance may be due to higher tobacco and alcohol consumption. The most common OSCC sites in group B were buccal mucosa (30%) and lateral tongue border (30%), followed by lips and gingivo-buccal mucosa. These findings support Akram *et al*. (2013)[7] and Alamgir *et al*. (2016)[8], who found buccal mucosa and tongue as the most frequent sites [26, 32]. Feller and Lemmer (2012) also emphasized tongue and floor of mouth as common OSCC locations due to carcinogen accumulation[2]. sAA, primarily secreted by salivary acinar cells, reflects sympathetic activity and glandular function . In this study, mean morning sAA levels were highest in group BI (138.97 U/L), followed by group A (65.83 U/L), and lowest in group BII (57.81 U/L). Evening sAA levels also showed the same trend: group BI (148.67 U/L), group A (84.30 U/L), and group BII (72.26 U/L). These differences were statistically significant (p = 0.001, ANOVA), with post-treatment levels (group BI) comparable to controls (group A) (Bonferroni test). The diurnal pattern showed a consistent increase in sAA levels from morning to evening across all

groups. This is consistent with findings by Abhishek *et al*. (2022), who observed similar diurnal variation in OSCC patients[9]. Choontharu *et al*. (2020) reported increased sAA and acid phosphatase in OSCC patients, supporting its diagnostic role[10]. Belskaya *et al*. (2020)[11] found elevated metabolic enzymes (sAA, LDH, GGT) in OSCC saliva, affirming sAA's utility as a biomarker[11] . A diurnal rise in sAA from morning to evening was consistent across all groups, aligning with circadian patterns reported in prior studies (Abhishek *et al*., 2022)[9]. The post-treatment decline in Group BII may result from reduced tumor burden and radiation-induced acinar cell damage. Supporting literature (Ramya *et al*., 2015; Shah and Sequeira, 2022; Belskaya *et al*., 2020) confirms that sAA levels are elevated in untreated OSCC and tend to normalize following therapy[12]. These findings highlight sAA as a sensitive, non-invasive biomarker for OSCC diagnosis, disease monitoring, and treatment response assessment. In the current study, plasma alpha amylase was analyzed using the Erba Chem-7 autoanalyzer with the Erba Liquixx amylase kit. Blood samples were collected twice daily (7–10 AM and 7–10 PM). In Group BI (OSCC pre-treatment), mean morning and evening plasma amylase levels were 97.35 U/L and 100.74 U/L, respectively. Post-treatment (Group BII), levels decreased significantly to 51.04 U/L (morning) and 60.70 U/L (evening), confirmed via ANOVA ($p = 0.001$). Healthy controls (Group A) had mean levels of 61.86 U/L (morning) and 71.91 U/L (evening), showing significant differences compared to Group BI but not to Group BII (Post hoc Bonferroni test). This pattern supports the hypothesis that alpha amylase elevation in OSCC results from cellular disruption, increased membrane permeability, and stress-induced secretory activity. Radiation-induced serous cell apoptosis may also contribute (Weiss *et al*., 2011). Zang *et al*. (2013) emphasized amylase as a potential tumor marker in lung adenocarcinoma, observing rising amylase levels with disease progression[14]. Similarly, Abhishek *et al*. (2022) reported significantly elevated salivary and plasma alpha amylase in OSCC patients pre-treatment, with diurnal variation showing higher evening values, consistent with our findings[9]. pH (potential hydrogen) denotes the level of acidity or alkalinity of a substance, with values ranging from 0 (most acidic) to 14 (most alkaline); neutral pH is 7, typical of pure water (Kumar *et al*., 2020). Saliva normally maintains a pH between 6.2 and 7.6, with an average of 6.7 (Humphrey and Williamson, 2001). This homeostasis is maintained via two mechanisms: (1) mechanical clearance of carbohydrates and bacterial acids through salivary flow, and (2) neutralization of dietary and microbial acids via buffering components of saliva. A salivary pH below 7.0 often indicates acidemia, and in cancerous conditions such as OSCC, a more acidic environment arises due to anaerobic glycolysis in hypoxic tumor regions, promoting tumor survival (Ramya *et al*., 2015)[6]. In the present study, salivary pH was measured using two methods: (1) colorimetric pH indicator strips using 2–3 drops of unstimulated saliva, and (2) a digital pH meter (Birud), with 5 ml saliva diluted in 30 ml normal saline. Morning (7–10 am) and evening (7–10 pm) pH levels were assessed in healthy controls (Group A), pre-treatment OSCC patients (Group BI), and post-treatment patients (Group BII). In the morning, mean salivary pH was 7.19 in Group A, 6.12 in Group BI, and 6.53 in Group BII. In the evening, pH values were 7.22, 6.08, and 6.56 respectively. These differences were statistically significant between healthy individuals and pre-treatment patients ($p = 0.001$), but not between healthy individuals and post-treatment patients, indicating partial restoration of salivary buffering post-therapy Diurnal variations revealed a marginal increase in pH from morning to evening across all groups, most prominent in Group A. The reduced pH in OSCC patients reflects increased lactic acid due to hypoxia-driven glycolysis, commonly referred to as the Warburg effect. Ramya *et al*. (2015)[6] and Shah and Sequeira (2022)[12] also demonstrated a significant drop in salivary pH in untreated OSCC patients, with improvement post-treatment. This aligns with our findings, suggesting that salivary pH is a potential surrogate marker for tumor burden and response to therapy. Future research must prioritize standardization of saliva collection and rigorous validation of biomarkers to establish reliable, non-invasive tools for early OSCC detection.

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Table 1. Outcome Measures

Biological Fluid	Parameter	Group A (Healthy)	Group B-I (OSCC Pre-treatment)	Group B-II (OSCC Post-treatment)
Saliva	α-amylase	✓	✓	✓
	pH	✓	✓	✓
Plasma	α-amylase	✓	✓	✓

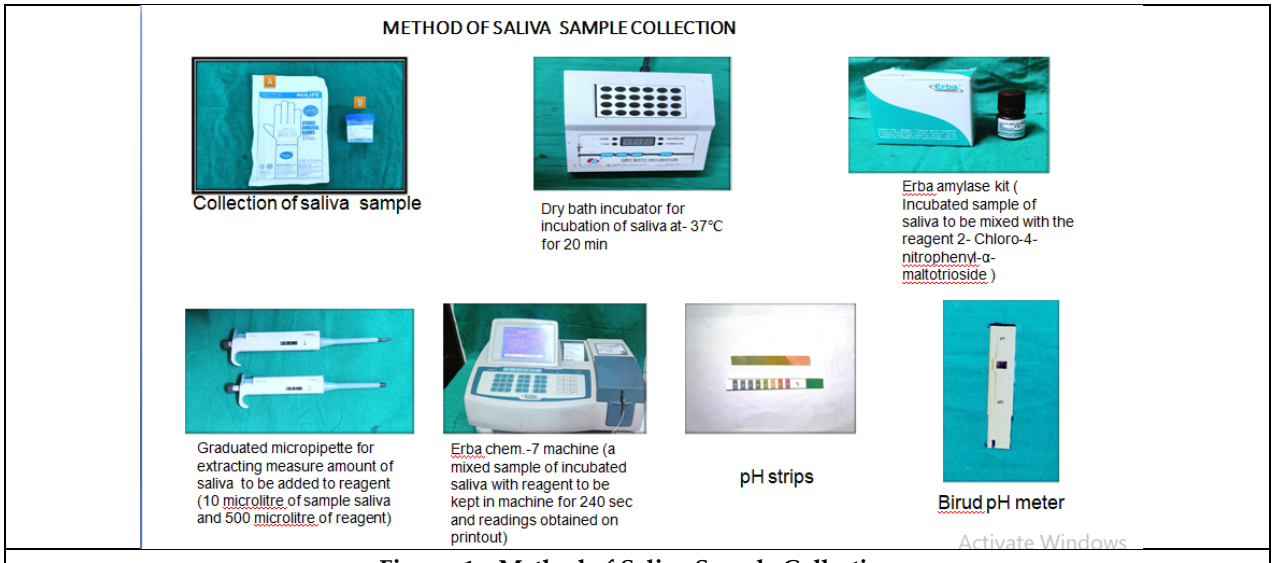


Figure. 1 – Method of Saliva Sample Collection

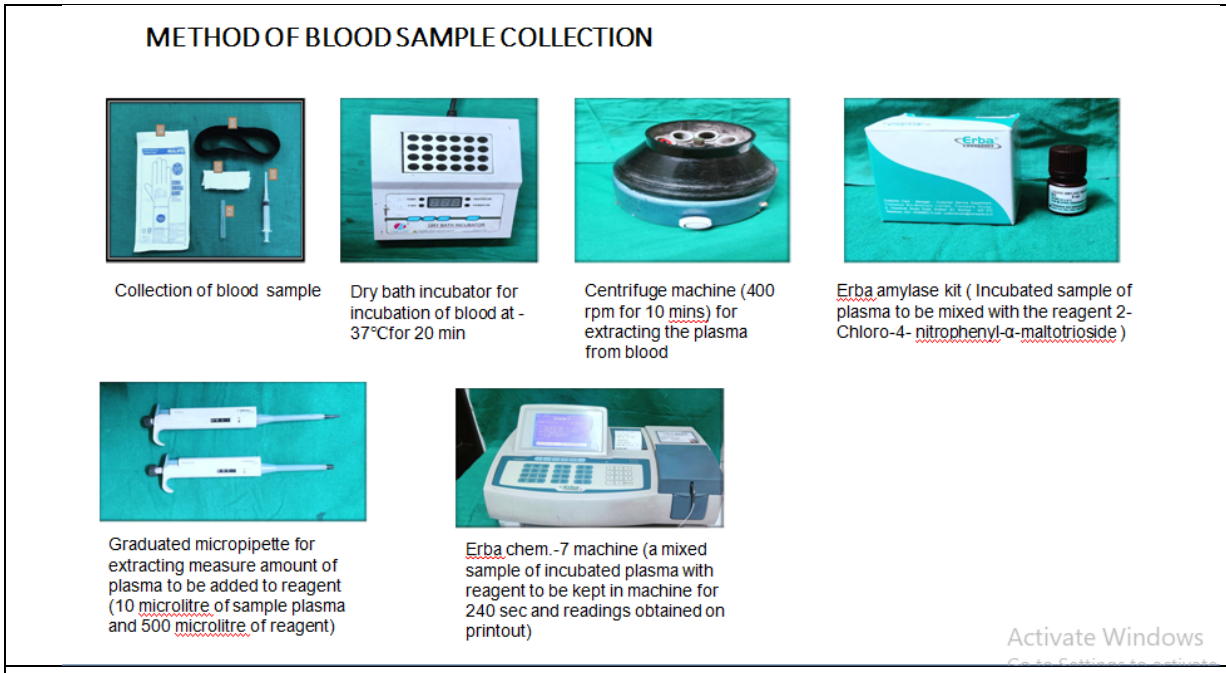
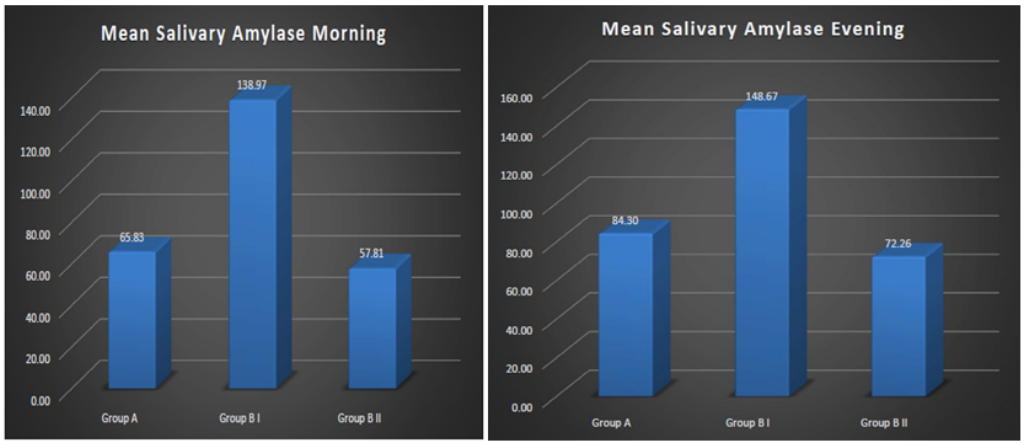
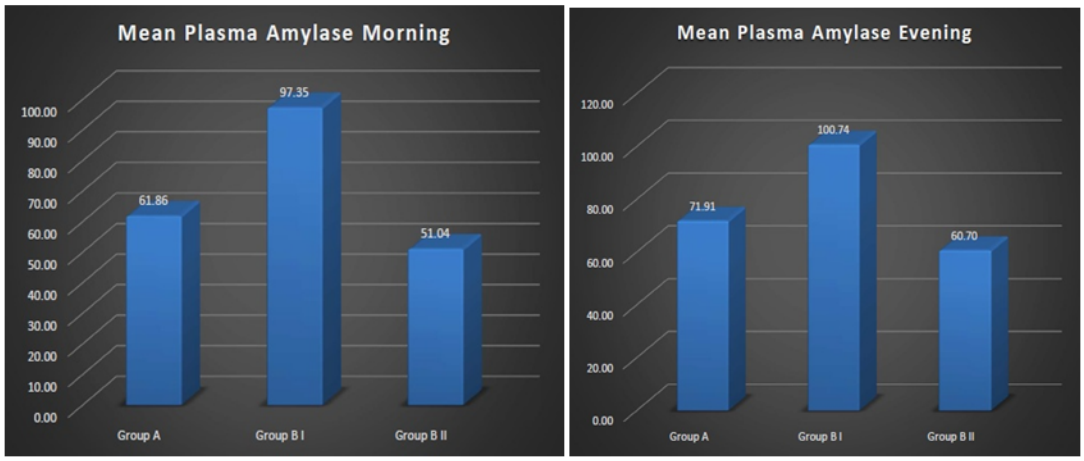


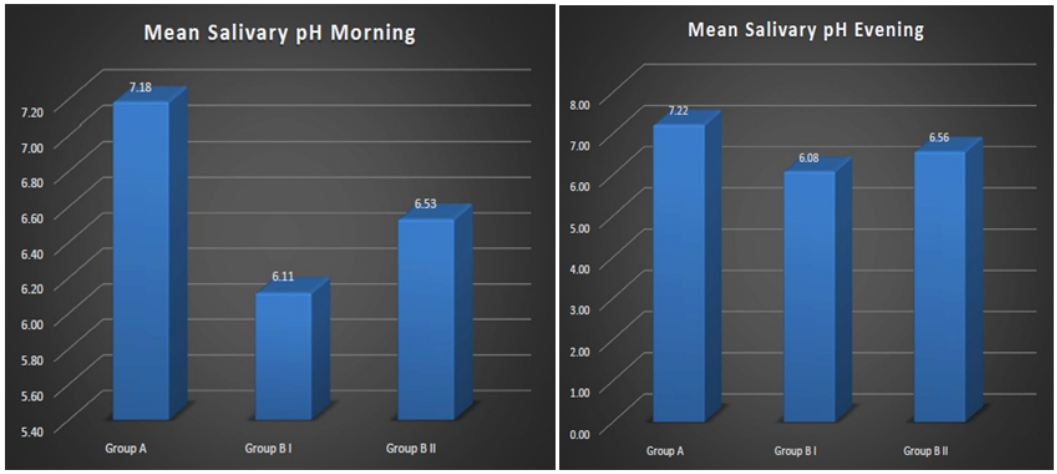
Figure 2 : Method of Blood Sample Collection



Graph 1 : Mean Salivary Amylase morning and evening levels



Graph 2 : Mean Plasma Amylase morning and evening levels



Graph 3 : Mean Salivary pH morning and evening levels

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RESEARCH ARTICLE

From Swipe to Spend : Psychological Triggers, Ethical Pitfalls and High - Conversion Secrets of Short - Form Ads

Deepanshu[1]* and Dakshita Pandey[2]

[1]Student, School of Social Sciences, Indira Gandhi National Open University, New Delhi, India.
[2]Junior Research Fellow, Department of Educational Psychology and Foundation of Education, National Council of Educational Research and Training (NCERT), New Delhi, India.

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***Address for Correspondence**
Deepanshu
Student,
School of Social Sciences,
Indira Gandhi National Open University,
New Delhi, India.
E.Mail: deepanshuverma263@gmail.com

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ABSTRACT

Short-form advertisements on platforms like TikTok, Instagram Reels, and YouTube Shorts wield psychological triggers, conceal ethical pitfalls, and deploy high-conversion secrets to reshape modern consumer behavior. This review article dissects how these ads exploit cognitive biases—such as reduced mental effort and dopamine-driven feedback loops—to habituate impulsive purchases, while algorithmic hyper-personalization and urgency tactics (e.g., FOMO, scarcity) act as covert conversion engines. However, their effectiveness is shadowed by ethical risks: opaque data practices, manipulative design (e.g., infinite scrolling), and influencer-driven deception erode consumer autonomy. By analyzing high-conversion strategies—emotional storytelling, platform-native shopping tools, and micro-influencer authenticity—the study underscores the tension between marketing efficacy and societal harm. It advocates for regulatory frameworks and digital literacy initiatives to curb exploitation while preserving innovation in the attention economy.

Keywords: Psychological triggers, ethical pitfalls, high-conversion secrets, short-form advertisements, algorithmic personalization, influencer marketing

INTRODUCTION

The meteoric rise of short-form advertisements—vertical, sub-60-second videos optimized for platforms like TikTok, Instagram Reels, and YouTube Shorts—has redefined digital consumption, merging entertainment, persuasion, and commerce into seamless, scrollable experiences (*Sharabati et al., 2022*). These ads thrive on psychological triggers (e.g.,

cognitive ease, dopamine loops), leverage high-conversion secrets (e.g., urgency cues, algorithmic targeting) and navigate the ethical pitfalls (e.g., data exploitation, deceptive design) to dominate the attention economy. Their cultural dominance is undeniable: while influencer marketing's market value skyrocketed 700% between 2016 and 2021 *(Michaelsen et al., 2022).* This review interrogates the triad of forces shaping short-form ads' power. First, psychological triggers— First, psychological triggers—such as habit formation via reduced cognitive load (*Sweller, 2011*) and dopamine-driven neurological rewards (*Vishwakarma, 2022*)—subconsciously condition users to bypass deliberative decision-making, automating impulsive spending behaviors. Second, high-conversion secrets, including hyper-personalized delivery (*Misra et al., 2024*) and influencer authenticity (*Djafarova and Bowes, 2021*), optimize ads for instant transactions. Third, ethical pitfalls emerge from dark patterns like auto-play loops and algorithmic opacity, which manipulate behavior while obscuring intent. By dissecting these interconnected dynamics, this work illuminates how short-form ads simultaneously captivate and exploit, urging stakeholders to balance innovation with accountability

Defining Short-Form Ads and Their Cultural Relevance

Short-form advertisements are defined by their brevity, with most lasting under 15 seconds and delivering concise, high-impact messages designed for rapid consumption (*Wang, 2024*). These ads are predominantly vertical in format, aligning with smartphone ergonomics and seamlessly blending into users' habitual scrolling behavior (*Deng et al., 2024*). Their audio-visual intensity—featuring dynamic music, humor, and fast-paced storytelling—enhances emotional engagement and memory retention (*Li, 2025*). Platforms like TikTok, which currently boasts 1.8 billion monthly active users, exemplify this media shift. With users spending an average of 95 minutes per day on the app and many reporting purchases after viewing advertisements, TikTok has cemented its role as a dominant driver of short-form content consumption (*Xiong et al., 2024; Sharabati et al., 2022)*. This trend reflects a larger cultural movement, particularly among Gen Z and millennials, who associate short-form content with authenticity, relatability, and entertainment (*Djafarova and Bowes, 2021*). In the attention economy, such "snackable" formats respond to shrinking attention spans and a growing preference for fast, emotionally gratifying media over sustained engagement (*Barr et al., 2015*).

Psychological Triggers in Short-Form Ads

Graphical representation of the "Psychological Triggers in Short-Form Ads" (Figure: 1)

Cognitive Triggers

Short-form ads reduce mental effort by presenting content in bite-sized, visually engaging formats, aligning with cognitive load theory which suggests that simplified inputs enhance processing and decision-making (*Sweller, 2011*). These micro-videos encourage passive consumption and quickly communicate brand messages without overwhelming the viewer. Over time, repeated exposure fosters habit formation—users begin associating the act of swiping through content with instant gratification and emotional payoff, thereby normalizing impulsive buying (*Bayer and LaRose, 2018*). Advertising operates through interconnected affective, cognitive, and experiential pathways, enabling short-form ads to bypass deliberation and trigger impulsive decisions (*Vakratsas and Ambler, 1999*). The Interactive Advertising Model underscores how users' goals and modes (e.g., playful vs. goal-directed) mediate ad processing, aligning with short-form ads' reliance on low-cognitive-load engagement to trigger impulsive decisions (Rodgers and Thorson, 2000). Recent research confirms that platform design elements (e.g., infinite scroll, auto-play) induce a psychological "flow state," reducing self-awareness and impairing attentional control. This passive immersion diminishes deliberative decision-making, directly facilitating impulsive consumption (*Levey, 2025*). Moreover, the mere exposure effect suggests that repeated encounters with a stimulus naturally enhance one's positive attitude toward it, thereby reinforcing familiarity and indirectly facilitating purchase intentions. (*Zajonc, 1968*).

Behavioral Triggers

Short-form ads leverage psychological cues like FOMO (Fear of Missing Out) and scarcity to prompt quick purchasing decisions. Phrases such as "Only 3 left!" or countdown timers exploit our aversion to loss, increasing the

perceived urgency to act (*Jabeen et al., 2023, Przybylski et al., 2013, Kahneman and Tversky, 2013*). Along with it the recent qualitative analyses reveal that excessive social media use—often characterized as 'doom scrolling'—can erode self-control and adversely impact academic and personal functioning. (*Rajeshwari andMeenakshi, 2023*). The frictionless architecture of these platforms—through embedded links, instant payment options, and direct checkout—removes traditional obstacles between desire and action, transforming spontaneous interest into immediate transactions (*Sharabati et al., 2022*). Research on subliminal messaging demonstrates that subtle cues in television advertisements can activate subconscious mechanisms, leading to increased compulsive buying tendencies and influencing purchase intentions among young consumers (*Sofi et al., 2018*). In addition to these behavioral cues, research highlights a dual-systems perspective in which automatic impulsive drives can override reflective self-control processes. This framework helps explain how the immediacy of scarcity cues and other rapid triggers on short-form platforms often bypass more deliberate decision-making, thus promoting impulsive purchases (*Hofmann et al., 2009*).

Dopamine-Driven Triggers

Neurologically, short-form ads trigger dopamine responses through novelty and unpredictability. The constant influx of engaging content mimics a reward-based system, where users unconsciously seek pleasurable stimuli—leading over time to habituation and impulsive buying without full critical evaluation (*Vishwakarma, 2022*). Subconscious desires, rooted in Freudian motivation theory, explain how emotionally charged short-form ads activate dopamine-driven reward cycles, habituating impulsive consumption (*Fletcher, 2010*). Implicit motives (e.g., affiliation, achievement) interact with platform functionalities (e.g., sharing, persona), driving dopamine-driven social media addiction through motive-platform fit (*Jabagi et al., 2025*). Social media addiction mirrors substance dependency, with endless scrolling activating the brain's reward pathways, reinforcing impulsive engagement and purchases.

Algorithmic Triggers

Artificial intelligence and algorithmic personalization are central to the success of short-form advertising. These systems micro-target users based on behavior, preferences, and engagement history, increasing the perceived relevance of each ad. For instance, TikTok's recommendation algorithm has been shown to boost conversion rates by delivering hyper-personalized content that aligns with individual user needs (*Misra et al., 2024; Tam and Ho, 2006*). A systematic review reveals that TikTok's algorithm enhances brand visibility and informs targeted marketing strategies by aligning consumer content preferences with effective advertising practices, thereby refining audience-specific approaches (*Melgarejo-Espinoza et al., 2025*). Personalized recommendation systems must strike a balance between algorithmic autonomy and user control. Thus, evidence suggests that purchase decisions peak when algorithmic involvement is moderate, underscoring the importance of designs that enhance users' self-efficacy while delivering tailored recommendations.

Sociocultural Triggers

In the sociocultural domain, short-form content thrives on relatability and trust-building through user-generated content and influencer endorsements. Micro-influencers, in particular, create authentic parasocial relationships with their followers, normalizing quick decision-making and impulse buying (*Djafarova and Bowes, 2021*). Cialdini's social proof principle (2007) underpins how short-form ads normalize impulsive spending by leveraging herd mentality, particularly through influencer endorsements and user-generated content that signal collective approval (Cialdini., 2007). Moreover, platforms like TikTok integrate commerce into social culture with features such as TikTok Shop, blending content consumption with purchasing seamlessly (*Li, 2025*). Influencers' curated personas and narratives on platforms like TikTok foster parasocial trust, normalizing impulse buying through relatable storytelling. Tailored influencer personas and narrative storytelling on TikTok enhance parasocial trust, merging personal relatability with brand messaging to drive impulsive purchases (*Chen and Ren, 2022*). Short video platforms exert a strong persuasive effect as users passively encounter influential content on personalized feeds, which can lead to shifts in their viewpoints and, in some cases, the spread of misinformation. This underscores the need for platform designs that promote critical engagement. (*Molem et al., 2024)*

High-Conversion Secrets: Design and Delivery Strategies

Graphical representation of the "High-Conversion Secrets: Design and Delivery Strategies"(Figure 2)-

Secret Tactics in Content Design

The success of short-form ads lies heavily in creative execution. Emotional storytelling—especially through humor, joy, or nostalgia—outperforms traditional informational content by triggering affective responses that drive purchase intent (*Berger and Milkman, 2012*). Short-film ads trigger hedonic (humor) or eudaimonic (meaning-seeking) experiences, fostering positive brand attitudes through emotional resonance and consumption visions (Chang, 2022). Fast-paced editing, vibrant visuals, and trending music capture attention instantly; platforms like TikTok and Instagram Reels make product discovery as entertaining as it is persuasive *(Li, 2025)*. While long-format storytelling fosters deep engagement, short-form ads leverage brevity to deliver emotional hooks (e.g., humor, nostalgia) swiftly, aligning with fragmented attention spans (*Nambiar, 2018*). Empirical evidence indicates that factors such as attention-grabbing elements, entertainment value, credibility, creative execution, and emotional appeal in new media advertisements significantly shape consumer buying behavior and enhance purchase satisfaction (*Bhatt and Jain, 2023*). An analysis of short-form video ads on platforms like TikTok identifies key content characteristics—namely trustworthiness, expertise, and attractiveness—as having a positive impact on purchase behavior, while authenticity and brand heritage demonstrate a U-shaped relationship with consumer decisions (*Meng et al., 2024)*.

Hidden Delivery Mechanics

Short-form platforms optimize the ad delivery process to streamline conversion. Platform-native shopping tools—such as TikTok Shop and Instagram Checkout—minimize friction, boosting impulse buying by allowing in-app purchases (*Sharabati et al., 2022*). Additionally, AI-driven personalization tailors ads to users' browsing behavior and purchase history, enhancing engagement and conversion (Misra *et al*., 2024). Personalization amplifies impulsivity by heightening perceived novelty and relevance of ads, while integrated payment tools eliminate transactional friction. However, privacy concerns may moderate this effect, suggesting ethical tensions in hyper-targeted tactics (*Aslam et al., 2021*). Mid-roll ads (18.1% higher completion rates than pre-roll) and shorter durations (15–20 seconds) optimize viewer retention, reinforcing frictionless purchasing via platform-native tools (*Krishnan and Sitaraman, 2013*). Search engine marketing (SEM) exhibits the longest-lasting impact on sales, while coupon/loyalty ads (CLA) better retain existing customers, emphasizing the need for strategic channel selection in short-form ad delivery (*Breuer and Brettel, 2012*).

Social Persuasion Secrets

Social proof is a powerful persuasive tool in the short-form ad ecosystem. Influencer marketing—particularly by micro-influencers perceived as authentic peers—can drive significantly higher sales (*Djafarova and Bowes, 2021*). User-generated content, including product reviews and unboxing videos, further enhances trust and credibility (*Sharabati et al., 2022*). Viral marketing's efficacy hinges on source credibility, which enhances trust and perceived value in high-end cosmetics, bridging the gap between skepticism and purchase intention *(GILITWALA et al., 2021)*. Short-form ads achieve higher engagement when content sources align with destination types: UGC/PUGC for hedonic contexts (e.g., tourism) and PGC/PUGC for utilitarian contexts, leveraging perceived credibility to drive purchases (*Li and Tu, 2024*). User-generated ads with high sociability (e.g., comment volume) and vividness (e.g., music, female presence) boost sales, particularly when influencers or sellers moderate content, reinforcing social proof's role in persuasion (*Ge et al., 2021*). Furthermore silver consumers (elderly users) exhibit distinct purchase behaviors on short-form video platforms. Revealing that social belonging, perceived trust, and product relevance are key factors driving purchase decisions in this demographic—suggesting that digital strategies may need to be tailored to bridge the generational divide. (*Yin et al., 2023*)

Urgency Engineering

Urgency is a recurring psychological lever in short-form ads. Time-sensitive triggers like countdown timers, flash sales, and "limited stock" messages exploit consumers' aversion to missing out, increasing click-through rates markedly. Additionally, the elaboration likelihood model (ELM) offers a dual-route framework of persuasion,

indicating that short-form ads can simultaneously trigger both thorough, central processing and quick, peripheral responses—thereby enhancing their overall persuasive impact (*Petty and Cacioppo, 1986).*

Ethical Pitfalls: Risks and Regulatory Gaps

While short-form ads effectively exploit cognitive biases to maximize consumer engagement, they also raise ethical and regulatory issues. Features such as auto-play and infinite scrolling tap into dopamine-driven habits that can reduce mindful consumption. Implementing a timer in skippable in-stream commercials generally reduces irritation by clarifying ad duration. However, when paired with a skip-ad option, this clarity can inadvertently heighten irritation—especially in longer ads—indicating that ad timing features require careful calibration (*Jeon et al., 2019*). Redefining advertising around persuasion, mediation, and paid intent underscores ethical tensions between consumer autonomy and manipulative design in short-form ad ecosystems (*Kerr and Richards, 2021*). Combined with extensive data extraction and deceptive scarcity cues, these practices blur the line between persuasion and manipulation, necessitating clear regulatory oversight. Experimental evidence confirms that dark patterns (e.g., false scarcity, forced continuity) universally increase purchase impulsivity. While behavioral interventions (e.g., friction prompts) can mitigate their impact, regulatory action is critical as no single solution neutralizes all manipulative designs (*Sin et al., 2025*). Notably, the looming U.S. TikTok ban—mirroring India's 2020 overnight removal of the app, which disrupted 200 million users—highlights the fragility of platforms central to modern commerce. Such abrupt regulatory actions risk destabilizing small businesses; TikTok alone claims to have generated $15 billion in U.S. small business revenue in 2023 (*Singh et al., 2025*). Combined with extensive data extraction and deceptive scarcity cues, these practices blur the line between persuasion and manipulation, necessitating clear regulatory oversight.

CONCLUSION

Short-form advertisements masterfully deploy psychological triggers—such as dopamine-driven scrolling and cognitive ease—to transform passive engagement into habitual purchasing. Their high-conversion secrets, including urgency engineering and algorithmic precision, erase friction between desire and action, capitalizing on fragmented attention spans. Yet these strategies are inextricably linked to ethical pitfalls: the very mechanisms that drive engagement (e.g., infinite scrolling, micro-targeting) exploit cognitive biases, compromise data privacy, and normalize manipulative influencer practices. The dual-edged impact of short-form ads demands a balanced response. Regulatory frameworks must address algorithmic transparency, enforce ethical design standards, and mandate influencer disclosures to protect vulnerable demographics like Gen Z and millennials, who increasingly equate short-form content with cultural authenticity. Simultaneously, digital literacy initiatives can empower consumers to recognize and resist manipulative tactics. Future research should explore longitudinal impacts on consumer well-being and platform accountability in mitigating harm. By confronting these challenges, stakeholders can harness the innovation of short-form advertising while safeguarding autonomy in an increasingly persuasive digital landscape.

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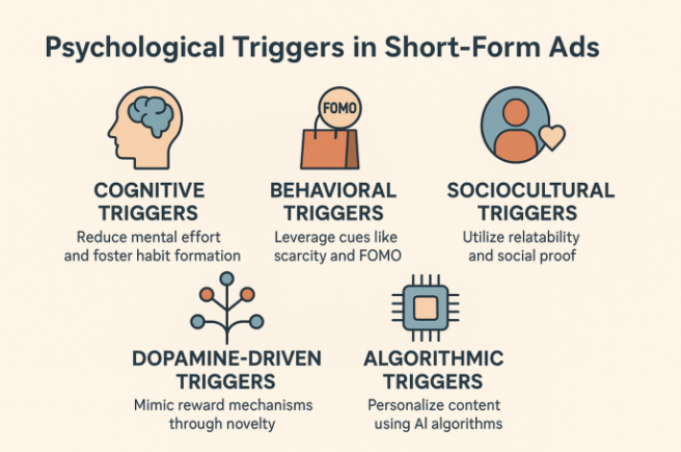


Figure 1: Graphical representation of the "Psychological Triggers in Short-Form Ads"



Figure 2: Graphical representation of the "High-Conversion Secrets: Design and Delivery Strategies"

RESEARCH ARTICLE

Role of Probiotics and Prebiotics in the Management of Parkinson's Disease

Devika C S[1], K L Krishna[2], Seema Mehdi[3]*, Malaika Solomon[4], Inchara Cml[5] and Priyanka L G[6]

[1]M.Pharm Student (Reg.No.: 23P10005), Department of Pharmacology, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.
[2]Associate Professor, Department of Pharmacology, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.
[3]Assistant Professor, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.
[4]M.Pharm Student (Reg.No.: 24P10007), Department of Pharmacology, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.
[5]M.Pharm Student (Reg.No.: 24P10004), Department of Pharmacology, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.
[6]Research Scholar (Reg.No.: 22PPMO41), Department of Pharmacology, JSS College of Pharmacy, Mysuru, Academy, JSS Academy of higher Education and Research (Deemed to be University), Karnataka, India.

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***Address for Correspondence**
Seema Mehdi
Assistant Professor,
JSS College of Pharmacy, Mysuru, Academy,
JSS Academy of higher Education and Research
(Deemed to be University), Karnataka, India.
E.Mail: seemamehdi@jssuni.edu.in

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ABSTRACT

The gut microflora plays an important role in maintenance of neuroimmune balance via the gut-brain axis. Dysregulation of this axis leads to neurodegenerative diseases, such as Parkinson's disease (PD). Prebiotics and probiotics have shown promising results in regulating the gut-brain axis by restoration of microbial homeostasis, reduce neuroinflammation and enhance gut barrier integrity. Probiotics, particularly strains like Bifidobacterium, and Lactobacillus and improve composition of gut microbiota composition, modulate immune responses, and improvement in neurotransmitter production, including dopamine, serotonin and GABA. Prebiotics, such as inulin and fructooligosaccharides (FOS), selectively stimulate beneficial gut bacteria, improving production of short-chain fatty acids (SCFAs) that support brain health by contributing to gut barrier integrity. These biotics help reduce oxidative stress, α-synuclein aggregation and systemic inflammation, which are important implications in PD pathology.

This review highlights the connection between the gut heath and pathology of PD presenting the opportunity for early intervention through microbiome modulation. along with the mechanisms of various prebiotics and probiotics exerting neuroprotective effects though restoration of beneficial gut microbes, discusses current evidence from preclinical and clinical studies along with their therapeutic potential as adjunctive strategies for management of PD. While preliminary findings for probiotics and prebiotics are promising, further clinical trials are required to validate and establish their effectiveness along with optimization of personalized microbiome-targeted therapies.

Keywords: Neurodegenerative, Probiotics, Prebiotics, Neurotransmitters, Gut barrier.

INTRODUCTION

Overview of Parkinson's Disease (PD): Pathophysiology and Progression:

Parkinson's disease (PD) is a progressive neurodegenerative condition that primarily impacts the motor system and has significant systemic implications. It is characterized by the successive reduction of dopaminergic neurons that are found mainly in the midbrain's substantia nigra pars compacta as shown in Fig 1. This leads to deficiency of dopamine causing an imbalance between excitatory and inhibitory pathways in basal ganglia resulting in impairment of motor control and coordination [1]. The pathogens of PD include aggregation of Lewy bodies in neurons caused by the accumulation of misfolded α-synuclein proteins which is the main reason for oxidative stress, neurotoxicity, and mitochondrial dysfunction which causes neurodegeneration. The enhanced levels of pro-inflammatory cytokines (IL-1β and TNF-α) are mainly due to the prolonged activation of astrocytes and microglia involved in neuroinflammation leading to amplification in neuronal damage [2]. Mitochondrial dysfunction contributes to enhancement in the production of reactive oxygen species causing oxidative stress. The progression of PD occurs through various stages. Nonmotor symptoms such as anosmia, constipation, and depression are observed in the prodromal stage. Initial unilateral motor symptoms such as rigidity, tremors, and bradykinesia are seen in the early motor stage [3]. Mid to late stages involve worsening cognitive decline, motor dysfunction, and autonomic issues. However, severe motor and cognitive impairment are common in advanced stages, causing dependence[1]. Difference between healthy midbrain with neurons in substantia nigra and midbrain with neuron loss in substantia nigra (Fig 1).

Emerging Role of the Gut-Brain Axis in Neurodegenerative Diseases

Recent research highlighted the importance of the gut-brain axis which is an important bidirectional communication system between the central nervous system and gut microbiome which is crucial in neurodegeneration. Gut dysbiosis enhances intestinal permeability and microbial metabolites which impact α-synuclein aggregation, oxidative stress, and neuroinflammation causing neuronal damage causing neurodegeneration in disorders like PD linked with constipation and worsened motor symptoms [4].

Importance of Microbiome Modulation through Prebiotics and Probiotics:

Modulating the gut microbiome in neurodegenerative diseases like PD involves the usage of probiotics and probiotics [5]. Probiotics such as strains of *Akkermansia muciniphila*, Bifidobacterium, and Lactobacillus help restore microbial balance, reduce neuroinflammation, and modulate dopamine levels [6]. Prebiotics such as fructooligosaccharides, galactooligosaccharides, and inulin help improve the growth of gut-friendly microbes, improve the gut barrier, and reduce neuroinflammation. This gut-targeting approach improves gut health and slows down the progression of neurodegeneration [7]. Role Gut-Brain connection in PD progression and pathogenesis (Fig 2)

Gut-Brain Axis and its Connection with Parkinson's Disease

Gut Dysbiosis and its Impact on Neuroinflammation and Neurodegeneration

In Parkinson's disease (PD), an imbalance in gut microbiota composition disturbs the integrity of the intestinal barrier causing breakdown which leads to enhancement in intestinal permeability, leading to a leaky gut. This allows the toxic microbial metabolites and substances, including gut lipopolysaccharides (LPS) which are cell wall components of Gram-negative bacteria such as E.coli, to enter into the systemic circulation and reach distant and vital organs including the brain [8]. These microbial metabolites when circulating in the stream initiate systemic inflammations by activating immune cells. This enhances the production of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) which crosses the blood-brain barrier (BBB) leading to activation of microglial cells which are resident immune cells present in brain [9]. Upon activation, these microglial cells release nitric oxide (NO), reactive oxygen species (ROS), and additional pro-inflammatory cytokines causing chronic inflammation of neurons. This amplified inflammatory environment causes the loss of dopaminergic neurons in the substantia nigra of the midbrain which is a key indicator in PD [10]. Chronic Neuroinflammation accelerates the process of neurodegeneration, causing worsening of motor symptoms such as tremors, bradykinesia, and rigidity along with amplification of non-motor symptoms like mood disturbances, sleep disturbance, and cognitive decline [11].

Role of the Enteric Nervous System and α-synuclein Aggregation:

The ENS communicates with CNS and regulates functions of the gastrointestinal tract. α-synuclein is a neurological protein that plays an important role in the regulation of neurotransmitters and synaptic function. Whereas in PD, the misfolding of α-synuclein is facilitated in ENS by microbial endotoxins caused via gut dysbiosis. These pathological changes in this protein lead to its aggregation. These aggregates reach the brain through the vagus nerve as shown in Fig 3. The aggregates of misfolded α-synuclein accumulate in the substantia nigra region of the midbrain which further leads to the formation of Lewy bodies that causes neurodegeneration [12]. Process of misfolding of α-synuclein, formation of Lewy body and neuroinflammation. (Fig 3) Several factors contribute to the progress of PD pathology. The SNCA (Synuclein Alpha) gene mutation can lead to enhancement of its expression and aggregation in familial PD cases. The mitochondrial dysfunction and oxidative stress result in excessive reactive oxygen species (ROS). This leads to destabilization of α-synuclein causing aggregation [13]. Chronic neuronal inflammation due to excessive pro-inflammatory cytokines and prolonged activation of microglial cells can further facilitate aggregation and spreading of misfolded α-synuclein. The accumulation of misfolded α-synuclein is mainly due to impairment of protein clearance mechanisms that involve autophagy-lysosomal dysfunction and ubiquitin-proteasome system (UPS) impairment [14]. Imbalance in metal ions such as iron, copper, zinc, and manganese contribute to neurodegeneration by promoting oxidative stress and mitochondrial dysfunction leading to α-synuclein causing aggregation. The nonmotor symptoms of PD affecting the gastrointestinal system cause bloating, constipation, and delayed gastric emptying due to gut dysbiosis [15].

Role of Probiotics in Parkinson's Disease (Pd)

Probiotics are alive microorganisms that positively impact immune function, gut health, and overall wellbeing. In PD, where inflammation and gut dysbiosis play important role which can be regulated by probiotics [16]. List of probiotics Stain and their role in management of PD (Table: 1)

Preclinical and Clinical Evidence Supporting Probiotic Use in PD(Table: 2)

Role of Prebiotics in Parkinsons Disease

Prebiotics are indigestible dietary fibers that nourish beneficial gut bacteria, particularly species like Lactobacilli and Bifidobacteria. Unlike probiotics, which introduce live beneficial bacteria into the gut, prebiotics act as a food source and promote the growth and activity of existing beneficial gut bacteria, supporting and balancing the gut microbiome hence improving overall digestive health [34]. A balanced gut microbiome impacts various essential physiological functions, including immune regulation, digestion, and brain function. Since gut microbiota can impact

the gut-brain axis may play a role in neuroprotection and overall neurological health [35]. List of Probiotics and their role in PD management (Table 2)

Clinical Evidence Supporting Prebiotic Use in PD(Table 3)

Mechanism of Probiotics and Prebiotics in the Management of PD

In Parkinson's disease (PD), gut dysbiosis triggers α-synuclein misfolding in the enteric nervous system (ENS), intestinal barrier breakdown and increased permeability, leading to ROS and pro-inflammatory cytokines production due to LPS entry. Chronic neuroinflammation and oxidative stress consequently leads loss of dopaminergic neurons in the substantia nigra. Prebiotics and probiotics promote gut health by strengthening intestinal integrity, reducing inflammation, SCFA synthesis, ultimately leading to eubiosis, supporting the gut-brain axis and preventing neurodegeneration in PD. They also modulate dopamine and serotonin production, thereby assisting in mood stability and motor control in PD, which are shown in fig 4.

Restoration of Gut Microbiota

Dysbiosis of the gut is associated with PD which is characterized by an increase in harmful bacteria and a decrease in beneficial bacteria such as Bifidobacterium and Lactobacillus leading to imbalance. The supplementation with probiotics and prebiotics effectively rebalances the gut microbiome, enhancing the function of the gut-brain axis [43].

Improvement in Gut Barrier Integrity

The gut dysbiosis in PD causes a leaky gut due to an increase in the permeability of the intestine, allowing lipopolysaccharides to enter systemic circulation. Probiotic and prebiotics improve intestinal integrity by strengthening tight junction proteins, decreasing permeability and preventing systemic inflammation [44].

Modulating Inflammatory and Immune Responses

Probiotics and prebiotics promote the synthesis of short-chain fatty acids (SCFAs) such as butyrate that improve intestinal integrity regulate immune responses and decrease the release of pro-inflammatory cytokines (IL-6, TNF-α, IL-1β). This helps with the reduction of neuroinflammation [45].

Regulation of Neurotransmitters in PD

Certain probiotic strains, such as Bifidobacterium and Lactobacillus play an important role in neurotransmitter regulation. These microorganisms impact levels of dopamine and serotonin which play crucial roles in both mood regulation and motor control in PD [45]. These beneficial strains of microorganisms are nourished through prebiotics, helping them grow and function effectively [44]. In PD, deficiency of dopamine predominates due to dopaminergic neuronal loss in mid brain. Probiotics and prebiotics are involved in the increment of dopamine levels as these stains of organisms contribute to the production of dopamine precursors such as L-DOPA and tyrosine. This enhancement in dopamine synthesis in the gut directly improves the levels of dopamine in the CNS through the brain axis preventing neurodegeneration along with reduction in gut inflammation [46]. Probiotics and prebiotics influence serotonin levels improving the availability of tryptophan, which is the precursor for serotonin biosynthesis [47]. It also modulates serotonin receptor sensitivity, particularly of 5-HT1A, improving cognitive function mood stability, and cognitive function [48].

Alleviating Gastrointestinal Symptoms

Probiotics and Prebiotics help in improving gastrointestinal symptoms such as bloating, constipation, and gut motility, which are common nonmotor symptoms of PD. Additionally; they can reduce anxiety, enhance sleep quality, and improve cognition by restoring the gut microbiome.

Challenges and Future Prospective

Research on probiotics and prebiotics indicates their positive effect on gut health, inflammation, and motor function in PD. However, small sample sizes, inconsistencies in study design, and variations in probiotic strains and dosages

limited definitive conclusions. While generally safe, probiotics and probiotics may potentially interact with PD medications causing mild gastrointestinal discomfort require further investigation as an adjunct therapy. Future research should focus on large-scale clinical trials determining optimal dosages and formulations. The development and investigation of symbiotic, which combine probiotics and prebiotics, offer a promising approach to enhancing gut-brain health. Integration of microbiome modulation therapies with existing PD treatments could provide a more comprehensive and effective approach to the management of the disease and its symptoms.

CONCLUSION

In conclusion, research evidence suggests that probiotics and probiotics play an important role in reducing inflammation, modulating and restoring gut microbiota, and enhancing both motor and non-motor symptoms in PD. Their potential to support the gut-brain axis offers a promising strategy for improving overall patient health. Future research may further solidify their role as a beneficial adjunct therapy to PD management.

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Table 1: list of probiotics Stain and their role in management of PD

Probiotic Strain	Role in PD Management
Bifidobacterium bifidum	Improves gut barrier function and reduces inflammation [17].
Bifidobacterium breve	Improves dopamine metabolism, supports cognitive function, and reduces neuroinflammation [18].
Bifidobacterium longum	Enhances serotonin production, improves mood, and reduces gut permeability [19].
Enterococcus faecium	Improves gut motility and enhances gut-brain communication [20].
Lactobacillus acidophilus	Increases serotonin production, reduces inflammation, and enhances gut health [21].
Lactobacillus casei	Improves gut barrier integrity, and reduces neuroinflammation and constipation [22].

Lactobacillus plantarum	Improves dopamine levels and motor function, and reduces gut inflammation [23].
Lactobacillus reuteri	Modulates dopamine and serotonin signaling, supports gut motility hence reduces constipation and decreases oxidative stress [24].
Lactobacillus rhamnosus	Modulates dopamine serotonin and GABA levels, improves mood disorders, and reduces anxiety [25].
Saccharomyces boulardii	Reduces gut inflammation, prevents the overgrowth of pathogens, and improves gut barrier integrity [26].
Streptococcus thermophilus	Reduces oxidative stress and establish gut microbiota balance [27].

Table 2: Preclinical and Clinical Evidence Supporting Probiotic Use in PD

Category	Findings
Preclinical	
Anti-Inflammatory Effects	This research suggests probiotics may counteract alpha-synuclein aggregation and neuronal loss through immunomodulation[28].
Neuroprotection	This preclinical study showed that probiotics may markedly reduce the degeneration of dopaminergic neurons positively effecting dopamine levels that play an important role in PD[29]
Clinical evidence	
Gastrointestinal Symptoms	This study suggested that Probiotic supplementation relieved constipation by significantly improving weekly bowel movements and normalized stool consistency in PD patients[30].
Non-Motor and Motor Symptoms	A 12-week RCT reported that a probiotic regimen containing four markedly reduced both motor and non-motor symptoms in PD which was measured by the Unified Parkinson's Disease Rating Scale (UPDRS)[31,32].
Comprehensive Symptom Improvement	A meta-analysis concluded that the usage of oral probiotics significantly improved gastrointestinal dysfunction, motor symptoms, anxiety, and depression in PD patients[33].

Table 3: List of Probiotics and their role in PD management

Prebiotic	Source	Mechanism of action
Inulin and Fructooligo saccharides (FOS)	Asparagus, Chicory root, onions, bananas, garlic, and wheat	- Stimulates growth of beneficial gut microbes. - Increases the production of SCFA such as butyrate. - improves gut motility and relieves constipation
Resistant Starch (RS)	Green bananas, legumes, potatoes and whole grains	-Increases the production of SCFA such as butyrate. - Improves the integrity of the gut barrier. -Demonstrates anti-inflammatory effects.
Galactooligosaccharides (GOS)	Dairy and legumes	-Improves the integrity of the gut barrier. - Stimulates growth of beneficial gut microbes

		involved in the production of neuroprotective metabolites. - Reduction in intestinal and systemic inflammation.
Xylooligosaccharides(XOS)	Bamboo shoots, vegetables, fruits and milk.	-Selectively promotes the growth of beneficial gut microbes - Improves SCFA production - Increases integrity of the gut barrier
Oligofructose	Vegetables, grains and fruits	-Selectively promotes the growth of beneficial bacteria like Bifidobacteria and Lactobacilli. -Improves SCFA production specifically of butyrate, propionate, and acetate. -Decreases gut permeability.
Dextrin	Wheat, oats, barley and potatoes,	-Stimulates growth of beneficial gut microbes like Bifidobacteria and Lactobacilli. - Increases the production of SCFA such as butyrate. -Enhances integrity of the gut barrier. - Improves gut motility and relives constipation.
Psyllium fiber	Plantago 100476vate seeds	-Supports gut health by feeding beneficial bacteria. -Producing SCFAs such as butyrate and propionate. -Strengthening the gut barrier. -Relieves constipation as it absorbs water and softens the hard stools in Parkinson's Disease patients.

Table : 4 Clinical Evidence Supporting Prebiotic Use in PD

Category	Findings
Preclinical	This research suggested that probiotics such as FOS improved the function intestine barrier of piglets, which might be associated with intestinal inflammatory response in the intestine, improved production of SCFAs, and positively modified microbiota composition in the hindgut [36].
Clinical	The open-label non-randomized study concluded that prebiotics showed potential benefits in patients with PD by enhancing gut microbiota, increasing short-chain fatty acid production alleviating gastrointestinal symptoms, and reducing inflammation along with evidence of motor function improvement however Larger trials are needed[42].

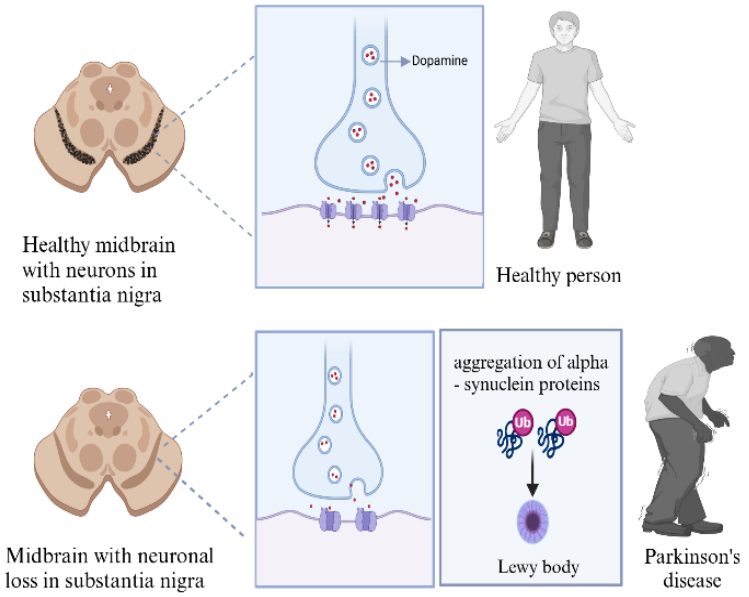


Fig 1: Difference between healthy midbrain with neurons in substantia nigra and midbrain with neuron loss in substantia nigra.

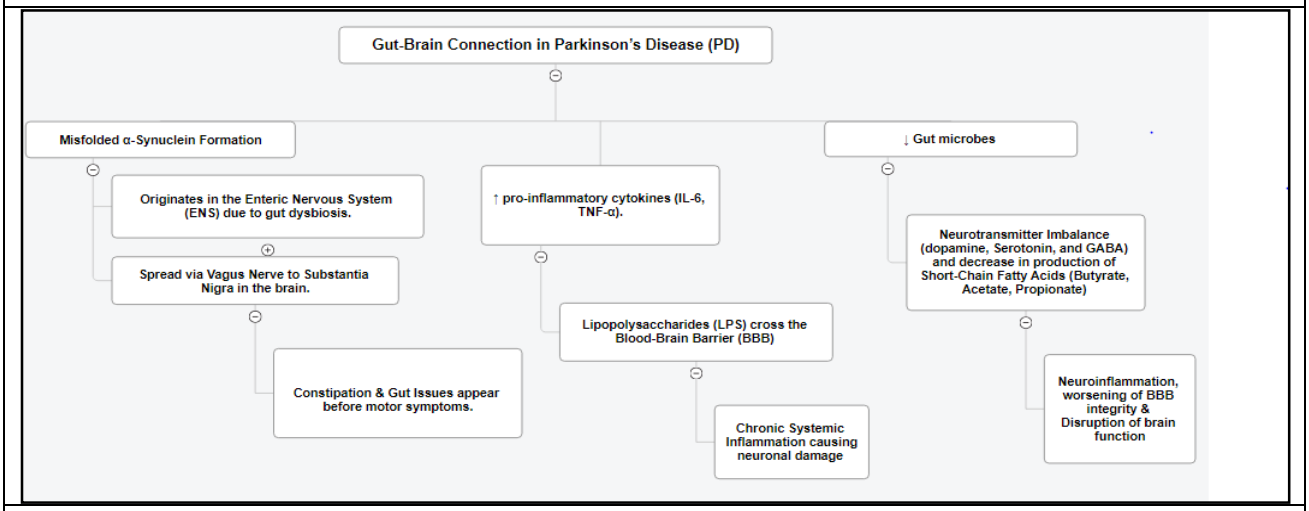


Fig 2: Role Gut-Brain connection in PD progression and pathogenesis

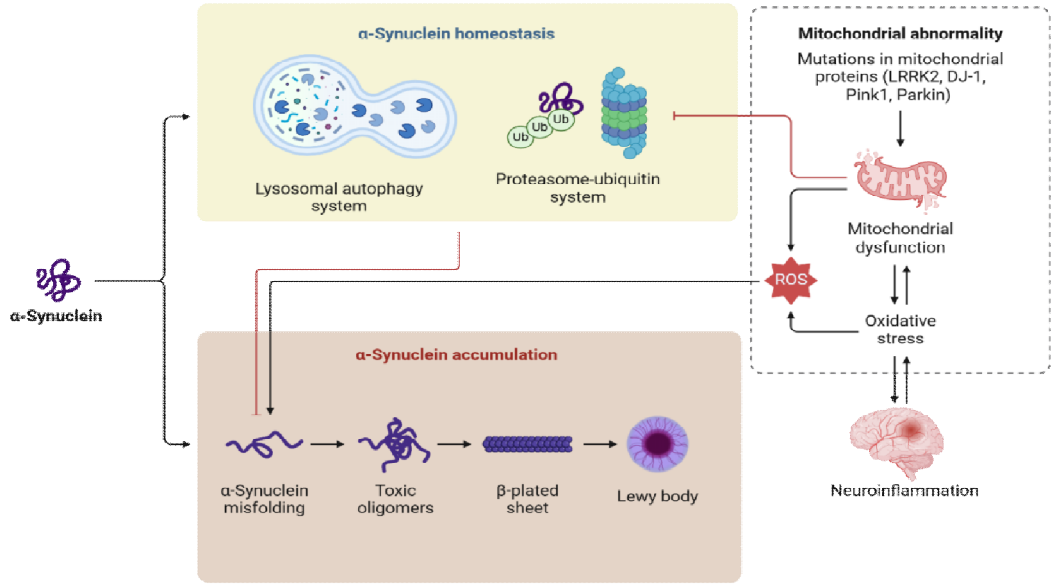


Fig 3: Process of misfolding of α-synuclein, formation of Lewy body and neuroinflammation

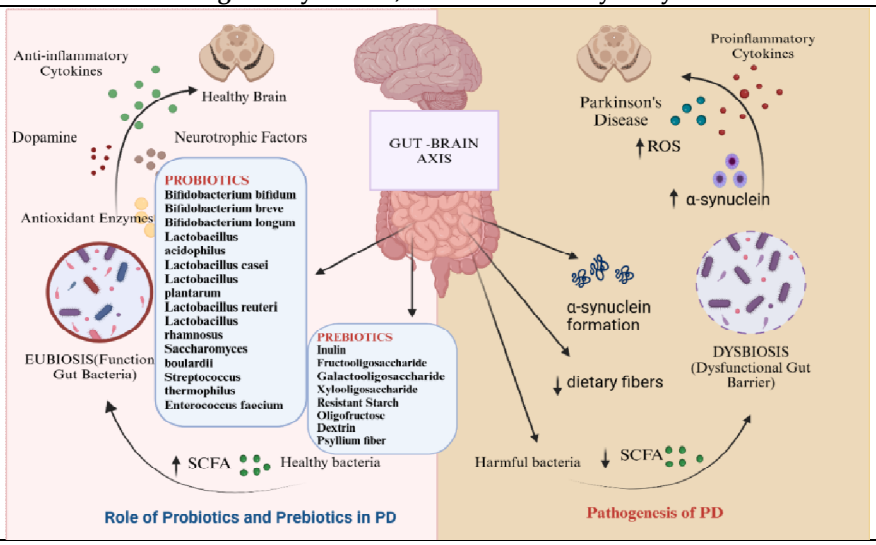


Fig 4: pathogenesis of PD and role of probiotics and prebiotics in PD

REVIEW ARTICLE

Filling the Defect: Bone Grafts In Periodontology – A Narrative Review

Apurva Rahalkar[1]*, Amit Mani[2] and Vipula Sharma[3]

[1]Lecturer, Department of Periodontology, Rural Dental College, Pravara Institute of Medical Sciences (Deemed to be University), Loni, Ahmednagar, Maharashtra, India.
[2]Professor and Head, Department of Periodontology, Rural Dental College, Pravara Institute of Medical Sciences (Deemed to be University), Loni, Ahmednagar, Maharashtra, India.
[3]Consultant Orthodontist, Private Practitioner, Edmonton, Alberta, Canada

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***Address for Correspondence**
Apurva Rahalkar
Lecturer, Department of Periodontology,
Rural Dental College,
Pravara Institute of Medical Sciences
(Deemed to be University), Loni,
Ahmednagar, Maharashtra, India.
E.Mail: apurvarahalkar23@gmail.com

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ABSTRACT

Periodontitis is a progressive inflammatory disease that results in the destruction of tooth-supporting structures, including alveolar bone. By making it easier to replace missing bone, bone grafting is essential to periodontal regeneration. With an emphasis on their biological characteristics, including osteogenesis, osteoinduction, and osteoconduction, this narrative study examines the various forms of bone grafts, including autografts, allografts, xenografts, and alloplasts. Additionally, the impact of defect morphology—specifically, wall angulation and configuration—on treatment results is addressed. This article offers an in-depth discussion of bone grafting in periodontics and how it can improve the success of regenerative therapy.

Keywords: Periodontitis, Bone grafts, Periodontal regeneration, Autograft, Allograft, Xenograft, Alloplast, Osteoconduction, Defect morphology, Bone regeneration

INTRODUCTION

Periodontitis is a disease which is known to cause loss of tooth supporting bone. It is defined as Inflammation of the supporting tissues of the teeth. Usually a progressively destructive change leading to loss of bone and periodontal ligament. An extension of inflammation from gingiva into the adjacent bone and ligament[1]. Tooth supporting bone is called the alveolar bone which consist of bone cells which are Osteoblasts, Osteoclasts and Osteocytes. Osteoblasts is a fully differentiated cell, arising from mesenchymal progenitors, that is responsible for the production of bone matrix and the resorptive remodelling of bone[1]. Osteoclasts is a, multinucleated cell, arising from mononuclear precursors of the hematopoietic lineage, that is associated with bone resorption[1]. Osteocytes is an

osteoblast that has become embedded within the bone matrix[1]. Since many years there are attempts made to regenerate the lost tooth supporting bone which has led to the use of different bone grafts and materials to achieve regeneration or fill of the alveolar bone. This article highlights the properties of bone filling materials and various types of bone grafts and materials that are commonly used in periodontal practice.

Properties of Bone Graft

The ideal bone replacement graft should be able to trigger osteogenesis, cementogenesis and formation of a functional periodontal ligament[2]. It is referred to as Osteogenic potential of tissue or substance which means any tissue or substance with the potential to induce growth or repair of bone[1]osteoconduction, osteoinduction and osteogenesis. Osteoconduction is the process by which a graft provides a scaffold that supports the attachment, migration, and ingrowth of osteoblasts and osteoprogenitor cells within its three-dimensional structure[3,4]. Osteoinduction refers to the capability of a graft to stimulate primitive, undifferentiated, and pluripotent cells to differentiate into bone-forming cells, thereby initiating the process of osteogenesis[3,4] Osteogenesis refers to the differentiation of osteogenic cells and the subsequent formation of new bone, involving donor cells that originate from either the host tissue or the graft material[3.5].

Types of Bone Grafts used in Periodontal Procedures

Bone replacement grafts can be of various types[3] as shown in Table 1

Autogenous Bone graft

They consistof cortical bone, trabecular bone and bone marrow cells. It is both *osteoinductive* and *osteoconductive*[2,6]. The use of extraoral autogenous bone grafts is infrequently documented in periodontal literature. Initially, iliac cancellous bone and marrow attracted attention due to their strong osteogenic potential. However, their application became limited in periodontal therapy because they were associated with root resorption following grafting[7]. However, autogenous bone grafts from intraoral sites continue to be widely utilized. Typical donor areas include the maxillary tuberosity and other edentulous alveolar regions, such as healing extraction sockets[8]. Limitation to autogenous bone graft includes the obvious inconvenience caused due to secondary surgical site, the limited amount of bone grafts that can be procured from the intraoral sites and the time required to obtain the graft. These limitations have let for the clinicians to use other bone replacement grafts.

Allografts or Allogenicmaterials:

They areis bone graft derived from human cadavers,[2,6] they can be obtained in the following forms:

- Fresh Frozen iliac cancellous bone and marrow
- Freeze-dried bone allografts
- Demineralized freeze-dried bone allografts

Fresh Frozen iliac cancellous bone and marrow

Due to concerns about disease transmission, immune response, and the requirement for thorough compatibility testing, frozen iliac allografts are no longer commonly used in contemporary periodontal practice[2].

Freeze-dried bone allografts

The demonstrated ability of freeze-drying to significantly minimize the health risks linked to fresh frozen bone[9]has contributed to the widespread adoption of freeze-dried bone allografts for managing periodontal bone defects. The American Academy of Periodontology advises the use of cortical bone allografts over cancellous bone, as cancellous bone tends to be more antigenic. Additionally, cortical bone contains a greater amount of bone matrix, providing more inductive elements. Freeze dried bone allografts are considered osteoconductive in nature[10].

Demineralized freeze-dried bone allografts

Allografts were demineralized because the mineral content of the bone hindered the activity of bone growth–stimulating factors, such as bone morphogenetic proteins, embedded within the bone matrix[11]. They promote bone formation via osteoinduction by triggering pluripotent stem cells to differentiate into osteoblasts[12].

Xenografts or Xenogenic filling material

Theyare the bone grafts derived from other species. They include

- Bovine bone,
- Coralline calcium carbonate[2,6]

Bovine derived bone replacement grafts

Commercial bovine bone undergoes processing to remove its organic components, leaving behind the natural bone mineral. In comparison to synthetic alternatives, these products are thought to provide greater osteoconductive qualities and structural features that closely resemble human bone[2]. The bovine-derived hydroxyapatite grafts serve as effective osteoconductive scaffolds due to their porous structure, which enhances surface area. Their mineral composition closely resembles that of human bone, facilitating integration with the host tissue. These grafts have shown successful outcomes in managing intrabony defects and in ridge augmentation procedures[13]. Because of the porous structure of the bovine derived hydroxyapatite graft, which increases the surface area, these grafts function as efficient osteoconductive scaffolds. Their mineral makeup is similar to that of human bone, which makes it easier for them to integrate with the host tissue. These grafts have demonstrated promising results in the ridge augmentation procedures and in management of intrabony defects[2].

Coralline calcium carbonate:

Calcium carbonate derived from natural coral of the *Porites* genus consists predominantly of aragonite (>98% $CaCO_3$) and features a pore size ranging from 100 to 200 μm, which closely mimics the porosity of cancellous (spongy) bone[14].

Alloplasts

These are synthetic material and do not contain actual bone cells[2,6].

The 1996 World Workshop in Periodontics has concluded that "synthetic graft materials function primarily as defect fillers. If regeneration is the desired treatment outcome, other materials are recommended"[10].

Alloplasts consist of

- Biocermics and
- Bioactive glass

Bioceramics

Bioceramics are alloplastic materials are mainly composed of calcium phosphate, with calcium-to-phosphate ratios resembling that of natural bone. The most commonly used types are tricalcium phosphate and hydroxyapatite.

Tricalcium phosphate is a porous calcium phosphate compound, with β-tricalcium phosphate being the most frequently utilized variant. They are typically surrounded by fibrous connective tissue and lack significant bone-inducing properties. Nevertheless, there have been reports of limited bone formation associated with these grafts.[15]

Hydroxyapatite is not osteogenic norosteoinductive, but rather is osteophillic and osteoconductive. It acts as a supportive structure for the ingrowth and subsequent deposition of new bone.[2]

Bioactive glass

Bioactive glasses consist of calcium oxide, sodium oxide, silicon dioxide, and phosphorus pentoxide, and they form a bond with bone by generating a surface layer of carbonated hydroxyapatite.[16] Upon contact with tissue fluids, bioactive glasses develop a dual-layer coating—an inner silica gel layer and an outer calcium-phosphate-rich (apatite)

layer. This outer layer enhances the adsorption and accumulation of proteins essential for osteoblasts to produce mineralized extracellular matrix for bone formation.[17] The most commonly used commercially available bioactive glass material is PerioGlas®.

Clinical Considerations During Bone Graft Placement

Bone defects vary in morphology, and not all are suitable for grafting procedures. Studies indicate that angular defects with an angle between 25° and 36° demonstrate the highest regenerative potential when treated with bone grafts, whereas defects with angles ≥37° tend to show reduced regenerative outcomes.[6] Prichard noted that successful defect fill is more likely when three bony walls remain intact.[18] Graft material should be moistened with the patient's blood to aid vascular infiltration. A bleeding surface, achieved by debridement or intramarrow perforations, supports healing. Grafts are placed incrementally, gently packed, and dried without dislodging. Flaps must ensure primary closure; if needed, use releasing incisions or soft tissue grafts. A protective periodontal dressing with antibiotic ointment helps secure the site and prevent contamination.[2]

CONCLUSION

Bone replacement grafts, which provide a variety of materials suited to clinical requirements, are essential for periodontal regeneration. The majority of commercially available grafts function as osteoconductive scaffolds that promote bone development, but only autogenous grafts have actual osteogenic potential. Graft materials are becoming more and more useful as safe, biocompatible, and userfriendly materials are developed. These grafts provide encouraging results for the treatment of osseous and periodontal abnormalities when paired with guided tissue regeneration procedures, hence strengthening their ongoing incorporation into contemporary regenerative dentistry.

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Table 1: Types of bone replacement grafts

Category	Type	Subtypes/Examples
Human Bone	**Autogenous grafts (autografts)**	Extraoral Intraoral
	Allogeneic grafts (allografts)	Fresh frozen bone Freeze-dried bone allografts Demineralized freeze-dried bone allografts
Bone Substitutes	**Xenogeneic grafts (xenografts)**	Bovine-derived hydroxyapatite Coralline calcium carbonate
	Alloplastic grafts (alloplasts)	**Bioceramics** • Tricalcium phosphate • Hydroxyapatite -Dense, nonporous, nonresorbable -Porous, nonresorbable (xenograft) -Resorbable hydroxyapatite derived at low temperature **Bioactive glasses**

RESEARCH ARTICLE

A Descriptive Study to Assess the Quality of Life among the Older Adults and its Associated Factors Residing in Assisted Care Facilities of Anand, Gujarat

Shalini Joshi[1]* and T. Jaya Kumar[2]

[1]Research Scholar (Reg.No.: DET 21NU007), Department of Nursing, L.N. Nursing College, (Affiliated to LNCT University), Kolar Rd, Bhopal, Madhya Pradesh, India and Assistant Professor, Department of Nursing, Manikaka Topawala Institute of Nursing, (Affiliated to Charotar University of Science and Technology), CHARUSAT Campus, Anand, Gujarat, India.
[2]Research Guide, Professor and Principal, Department of Nursing, L.N. Nursing College, (Affiliated to LNCT University), Kolar Rd, Bhopal, Madhya Pradesh, India.

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***Address for Correspondence**
Shalini Joshi
Research Scholar (Reg.No.: DET 21NU007),
Department of Nursing, L.N. Nursing College,
(Affiliated to LNCT University),
Kolar Rd, Bhopal, Madhya Pradesh, India and
Assistant Professor, Department of Nursing,
Manikaka Topawala Institute of Nursing,
(Affiliated to Charotar University of Science and Technology),
CHARUSAT Campus, Anand, Gujarat, India.
E.Mail: shalinijoshi.nur@charusat.ac.in

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ABSTRACT

Quality of life among older adults is influenced by the demographic transition happening with modernization. The quality of life is associated with plethora of factors like educational status, decision authority, stressful events, living spouse, financial security, co-morbidity. The population of older adults living alone is enormous, more so with increasing age, i.e. the older adults over 75 years both men and women. Women live longer than men and are less likely to marry again, end up without any support; financially and emotionally Social isolation is a major concern for the ageing population globally, as estimated by United Nations Department of Economic and Social Affairs, 2020, there are approximately 20% and more older adults who are surviving alone in the community. In this non-experimental study, 100 older adults living in the olde age home above 60 years old were included using non-probability convenient sampling technique. OPQOL-Brief quality of life tool was used to collect the data. Majority of older adults 64% had poor quality of life, whereas 31% were having very poor quality of life, 5% had moderate quality of life, however none of the elder adults were found to have a good quality of life. The

quality of life was associated with age, gender and level of education. Only a minority were found to have moderate quality of life, the greater number showed poor quality of life. The current study need for government authorities to improve the infrastructure and services of old age homes and to address the broader social determinants that affect the elderly. It underscores the importance of creating supportive environments that promote dignity, connection, and quality care for older adults.

Keywords: Quality Of Life, Older Adults, Co-Morbidity, Polypharmacy.

INTRODUCTION

India is booming with the older population, in the year 2022 the country had an older population of 149 million, which is expected to rise to 347 million by the end of 2050. The younger population will slide down at a dramatic rate when compared to those above 60 years.[1]When compared to the rest of the world, India has the largest share of population globally and has experienced a drastic increase in the number of older adults. India had 7.5% of older adults on 2010 which will increase to 11% in 2025, although seems like a small percentage nevertheless is a huge in absolute terms. Majority of these population like residing in rural areas and have a lower socio-economic status, are women and dependent. [2] The average life expectancy of older adults is on a rise in India and globally, because of the advancement in technology. Since the modernization the values in the modern India has also changed, earlier parents use to occupy the highest positions and were given due regard to and taken care. The picture has changed quite a lot at present, due to which older adults face major survival issues, financially, socially, economically. The health care facilities have granted longevity but is not adept to deal with geriatric illness.[3]The change in the family structure and migration has added to the problems of ageing population, they are left alone to take care of themselves. The old age homes have become a necessity for the survival of the older generation. This rise is observed majorly in the southern India, states like Kerala and Tamil Nadu. [4] There are various reasons for the older adults to move to old age homes, to mention some; migration, death of spouse, abandonment, financial deprivation, abuse etc. The old age homes sometimes is the last resort and hope for the older adults. Those living in old age homes also have different psychological issues.[5] According to a report by 'Golden opportunities from the silver economy – analysing the future of senior care in India', at present there are around 1 million older adults living in old age homes, in the coming 10 years it will reach to 2.5 million older adults occupying the residential care homes.[6]

REVIEW OF LITERATURE

A cross-sectional survey was carried out in Syangja district of Nepal, 2024. The quality of life was assessed for 234 elderly using World Health Organization Quality of Life BREF [WHOQOL-BREF] tool. The study revealed majority 54% of the samples were in the age group 60 to 69 years and most 57% of them were females. Average quality of life was found in 56% of the samples, nearly equal 44% showed poor quality of life. The age, having the spouse and involvement in some form of physical activity had a positive association with better quality of life.[7] A cohort study done in Rotterdam, 2024 to assess the quality of life related to health, comprising of physical and mental health among the older adults residing in community setting. The total 840 subject were initially recruited, out of which 661 were taken as samples in the final study. The results where majority of the samples were in the age group 70 to 80, females comprising of 42%, 74% of them had more than two chronic disease conditions. Debility was associated with lower scores of health related quality of life, as it impacts ADL's, frequent hospitalization, isolation causing depression, anorexia and fatigue.[8] Cross-sectional study was done among the residents of elder homes in Kuala Lumpur in 2014. Randomly 8 old age homes were selected and stratified simple random sampling was used to recruit samples for the study. World Health Organization Quality of Life Questionnaire-Brief Version (WHOQOL-BREF) was used to collect the data from the samples. The results revealed that quality of life was connected to various factors like age, gender, morbidities and social life.[9] A descriptive survey was done at the old age homes of Egypt, 2020 to assess the quality of life among the older living in the geriatric homes. The samples were selected

using convenient sampling techniques, the sample size was 20 older adults. The data was collected using sociodemographic data and WHO-OPQOL. The result revealed that majority 70% of the older adults had lower quality of life, majority of them also had insufficient knowledge about taking care of themselves as they age. The QOL was associated with level of education.[10]

Statement of the Problem

"A descriptive study to assess the quality of life among the older adults and its associated factors residing in assisted care facilities of Anand, Gujarat".

OBJECTIVES

1. To assess the quality of life among the older adults residing in the assisted care facilities.
2. To determine the association between quality of life among the older adults and their selected demographic variables.

RESEARCH METHODOLOGY

Study Design: Non-experimental descriptive survey.
Study Site: 4 old age homes in the Anand district were selected for the present study, those which were regularly visited by nurse led geriatric care center, MTIN, CHARUSAT.
Sampling technique: Non-probability consecutive sampling.
Sample size: The researcher selected 4 old age homes established and functioning for more than 4 years of time period in the Anand district and included all the older adults residing in those 4 old age homes who were falling in the inclusion criteria. The total sample size is 100 older adults.

Inclusion Criteria

1. Old age homes existing more than 4 years.
2. Old age homes who granted administrative approval.
3. Older adults above 60 years both male and female and residing more than 1 years.

Exclusion Criteria

1. Older adults are not willing to participate.
2. Older adults who are diagnosed with terminal illness and psychological illness.

Study tools

OPQOL(Older people quality of life) -BRIEF, 13 questionnaire tool is a revised version of the original tool developed by Ann Bowling (University of London) in 2009. The category includes domains like health, social relationships, independence, neighborhood, emotional well-being, financial, social and leisure activities. It is a 5-point likert scale, items scored as strongly agree, agree, neither, disagree, strongly disagree. The items are reversed coded so that the highest score represent the better quality of life. The total score of the tool is 63 and minimum score is 13.

Categorization of score

Very Good – 48 and above
Good - 39- 47
Moderate - 30-38
Bad - 21-29
Very Bad - 13-20

Reliability

The OPQOL-Brief is a standardized tool with internal consistency reliability of 0.81. [11]The tool was translated into Hindi and reliability was found out using Cronbach's Alpha Coefficient, which came out to be 0.76.

RESULT

Section A - Demographic Characteristics

TABLE1- Frequency and Percentage distribution of the older adults demographic characteristics N= 100

Table: 1-Depicts the demographic characteristic which reveals that highest percentage (53%) of older adults were in the age group of 60-70 years. About 42% of the older adults were in the age group of 71-80 years and remaining older adults were in the age- group of 81-90 years. Highest percentage of older adults were females as compared to 42 % males. With regard to co- morbid conditions, it was observed that highest percentage (30%) of older adults had both diabetes and hypertension followed by hypertension 22% and diabetes 18% . About 9% older adults had both diabetes and arthritis whereas 8% older adults had all three co- morbid condition of diabetes, hypertension and arthritis. About 13% of older adults did not had any co-morbid conditions.In respect to usage of polypharmacy, it was observed that most of the older adults (53%) were using more than one medication as compared to 47% of older adults who were taking only one medication.

Section B – Findings related to quality oflife among older adults.

Fig: 1- reveals the major findings in relation to the quality of life of the older adults; highest percentage of older adults (64%) had poor quality of life as compared to 31 % older adults having QOL as very poor. Only 5% of older adults fell in the QOL category of moderate. It was also observed that none of the older adults had good or very good QOL.

Section C – Findings related to association between Quality of Life among older adults and selected demographic variables.

Table: 2- It was observed that Quality of life scores of older adults was found to be associated age, gender, and level of education with Chi - square values of 18.54, 7.73 and 13.54 respectively. Further QoL was found to be independent of, co-morbid conditions and polypharmacy at 0.05 level of significance as evident by Chi-square values of 2.01 and 0.12 respectively.

DISCUSSION

The study aimed to assess the quality of life among the older adults residing at the old age homes and find the association with the selected demographic variables. Majority (53%) of the older adults were in the age group 60-70 years, female were in majority (58%). Most (30%) of the participants were diagnosed with both diabetes and hypertension, only 13% had no comorbid conditions. Polypharmacy was found in most (53%) of the older residents. Very poor quality of life was found in 31% of the subjects, majority 64%, were found to have poor quality of life, none of the participants had a good quality of life. The quality of life was associated with age, gender and level of education. The findings of the study was consistent with cross-sectional study conducted by Jazayeri Emitis, *et al*. 2023 which stated that majority of older adults were in the age range of 60-70 years and majority of them had poor quality of life related to poor mental health.[12] Slight consistent results were also reported by Lalitha Krishnappa, *et al*. 2021the study was in regard to quality of life among the older adults living in rural and urban areas. The study had females in majority, age group 60-70 years represented most of the samples, the older adults living in rural areas had poorer quality of life.[13] Contrasting results were reported in a study conducted by Panday Rishi, *et al*. 2015 finding the quality of life of older adults living in old age homes and family. The results revealed that those living in the residential facilities had better quality of life than those living with families.[14] Thresa Sona Sahaya conducted a cross-sectional study, 2019 on the elderly living in old age homes and with families. The results revealed those living in old age homes had poorer quality of life than those living in homes, which was somewhat consistent with the

results of the present study.[15] Study conducted in 2024 by Yankannavar Basavaraj and Naik Bijaya, consistent finding; similar in the terms the majority of the elderly living in old age home setups have poor quality of life. The association of quality of life was related to age, gender and co-morbidity.[16] Similar results were reported by Paudel Sharmila, *et al*.2024 the majority were in 60 to 69 years and females, the quality of life was associated with the age.[17]

CONCLUSION

The growing population of older adults requires the need to set up more residential homes, however the question is whether the facilities are equipped enough to take care of them. The older adults require more care in terms of psychosocial and psychological well-being, the old age homes do not have medical or nursing facilities for them. The present study highlights the quality of life of the older adults living the old age homes. Only a minority were found to have moderate quality of life, the greater number showed poor quality of life. The reduce feeling of loneliness, sustained financial insecurity, social support, and affordability of good health care are indicators of good quality of life. The government authority should focus more on the betterment and provision of such facilities in old age homes.

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Table 1: Frequency and Percentage distribution of the older adults demographic characteristics N= 100

Sr.No.	Characteristics	Frequency (n)	Percentage (%)
1	**Age in years**		
	• 61 – 70	53	53
	• 71 - 80	42	42
	• 81 - 90	03	03
	• 91 and above	00	00
2	**Gender**		
	• Male	42	42
	• Female	58	58
3	**Comorbidity**		
	• Diabetes	18	18
	• Hypertension	22	22
	• Both Diabetes and Hypertension	30	30
	• Diabetes, and Arthritis	9	9
	• Diabetes, Hypertension and Arthritis	8	8
	• None	13	13
4	**Polypharmacy use**		
	• Yes	53	53
	• No	47	47

Table 2: Association between Quality of Life among older adults and selected demographic variables.N= 100

Sr. No.	Demographic Variable	Alright QoL	Bad QoL	Very Bad QoL	Df	X^2
1	**Age in Years**					
	• 61 – 70	1	12	2		
	• 71 - 80	1	17	2		
	• 81 - 90	2	32	22	9	18.54*
	• 91 and above	1	1	1		
2	**Gender**					
	• Male	2	20	20	2	7.73*
	• Female	3	42	13		
3	**Comorbidity**					
	• Diabetes	1	9	8		
	• Hypertension	1	10	11		
	• Both Diabetes and Hypertension	1	14	15		
	• Diabetes, Hypertension and Arthritis	1	9	7	8	2.01
	• None	1	4	8		
4	**Education level**					
	• Non Literate	1	6	6		
	• Up to middle school	1	23	19		
	• Up to Higher secondary	1	27	6	6	13.54*
	• Graduate and postgraduate	2	6	2		
5	**Polypharmacy use**					
	• Yes	3	33	17	2	0.12
	• No	2	29	16		

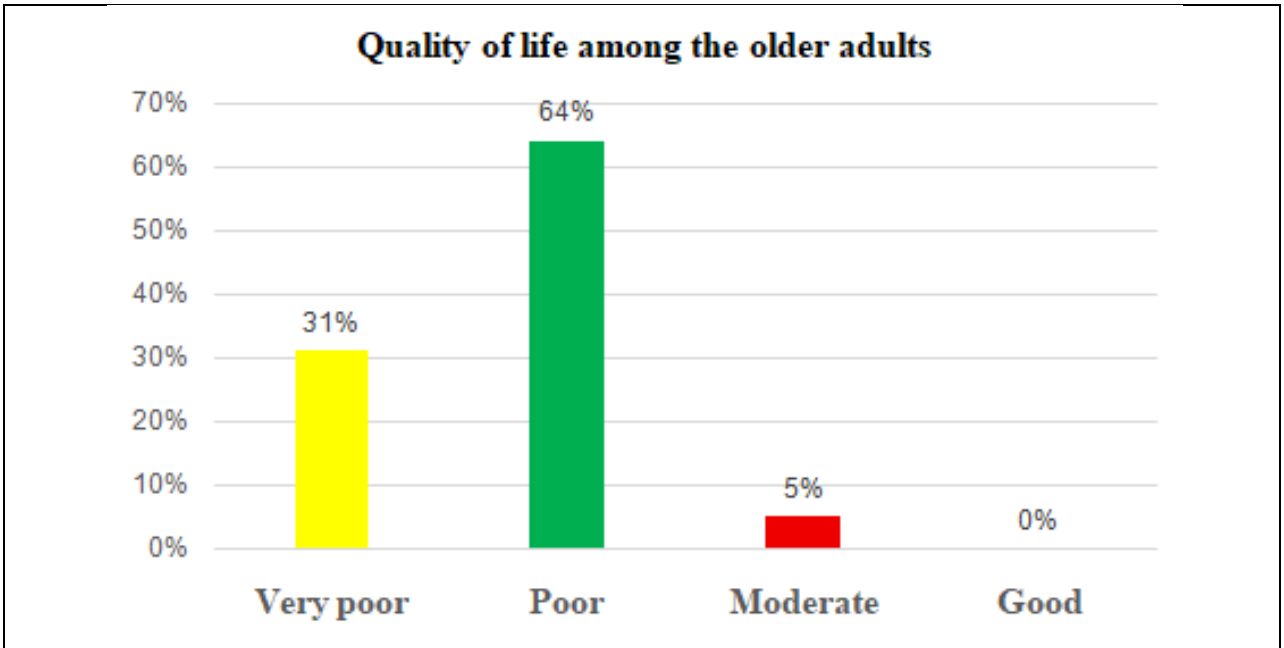


Fig 1: Bar diagram showing the percentage distribution of QoL among the older adults residing in assisted care facility.

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RESEARCH ARTICLE

Distributed Ledger Technology: A Comprehensive Analysis of Design Vulnerabilities and Environmental Impact in Blockchain

Deven Gol[1]* and Nikhil Gondaliya[2]

[1]Assistant Professor, Computer Science Engineering, The Charutar Vidya Mandal University, Anand, Gujarat, India.
[2]Professor, Computer Science Engineering, The Charutar Vidya Mandal University, Anand, Gujarat, India.

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***Address for Correspondence**
Deven Gol
Assistant Professor,
Computer Science Engineering,
The Charutar Vidya Mandal University,
Anand, Gujarat, India.
E.Mail: devenkumar.gol@cvmu.edu.in

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ABSTRACT

Blockchain requires an emerging technology that people see as a replacement for data security storage required in this digital age. In this article, we assess the gaps and details of recent research analyzing this consensus mechanism with the integration of artificial intelligence and the analysis of cyber security attacks. Distributed ledger technology is a possible solution to solve this whole traditional database problem in a comprehensive and transparent way without the help of a third party. Our research also deals with cyber-attack prevention using consensus algorithms. We proposed hybrid consensus algorithm with integrity and authenticity for security concern for cyber threats. We analyze this with security analysis test cases as well as an improvised solution with artificial intelligence integrated into the blockchain. We have also added some web application-based attacks to address them comprehensively and consistently improvised through DLT based technology. We have listed OWASP top 10 based attacks which have large impact on blockchain solution for adaptability in era of web 3.0. Finally, we added some tests cases for different miners to solve cyber security-based attacks to save it with environment effect of it using blockchain technology. Additionally, we explore the potential of energy-efficient blockchain mechanisms to reduce environmental impact and support biodiversity preservation through sustainable digital infrastructure.

Keywords: AI, Blockchain, Cyber Security, Vulnerabilities, Sustainability, DLT

INTRODUCTION

A blockchain is a decentralized and distributed database which has cryptographically(hashed) linked records, known as immutable blocks, arranged in chronological order and distributed among nodes in a computer network to store digital data or transactions [1]. Smart contracts are like a computer program mostly written in language like solidity [2], are stored on a blockchain that works automatically without external intermediaries (third party) [3]. By removing third-party intermediaries, smart contracts play a pioneering role in automated business participation, making collaborative processes more transparent and more efficient in terms of time and money [4]. Web 3.0, the third generation of the Internet is a user-centric network that aims to decentralize user data, including money, property and identities, securely using blockchain technology, giving users full control instead of centralized (Central authority) entities checking their own data [5]. Blockchain is the driving force behind Web 3.0, giving users ownership of data and allowing them to store securely and manage their data in a decentralized manner through self-contained identity (SSI). It was previously proposed for financial applications only but now blockchain can also be used in Internet of Things (IoT), Health care, supply chain, public and social services, reputation systems or act as a service providing security, integrity and privacy. A blockchain can also be considered a database (rather we can call it secure way to store the data with full transparency) because it contains a storage mechanism [6]. Bitcoins emerge during mid-2009 and then after there were questions about the differences between blockchain, databases and whether blockchain could be used as a database mechanism as an alternative secured way [7]. Although blockchain provides a storage mechanism, it has many important differences from traditional databases, mainly its decentralization, cryptographic security through a chained hash, lack of management control, immutability and freedom to transfer without any. permission from a central authority [8]. It is accepted by many enterprise applications which have moved to blockchain storage to provide more secure deployments and reduce trust between parties in the industry. In recent times many companies focus on distributed cloud storage, the question arises to what extent blockchain offers a better storage solution than distributed cloud storage or just a traditional database[9-11]. Blockchain is not only related to the above features, but also provides better features, user privacy and security through cryptographic primitives. Blockchain lacks some features offered by traditional databases, such as versatile queries. Efforts to bring these DB functions to the blockchain are growing and progressing. When blockchain storage is measured by traditional DB criteria, it does not give promising results. It has a capacity of only a few transactions per second (tps), a 10-minute delay before a single confirmed write (to Bitcoin), and a size of only a few tens of gigabytes. In a blockchain, adding nodes to the network increases traffic, but also decreases performance. On the other hand, a modern distributed database can have a capacity of more than a million tps, a capacity of several terabytes and a latency of only a fraction of a second. Finally, adding nodes improves system performance and capacity [12]. Therefore, one approach to making blockchain an efficient storage mechanism is to combine traditional databases with existing blockchain technologies, which many platforms are currently doing.

The goal is to produce an advanced storage mechanism with high performance, scalability, low latency and increased security, because this mechanism would be built on the existing blockchain technologies. Another approach is the use of so-called blockchain databases, which have developed a lot in recent years [13-17]. These databases have their own consensus mechanism for common agreement between network parties on a block of data. They are unordered and provide support for complex data types, rich query structures, and ACID-compliant transactions [18]. In addition, they offer low latency, fast scalability and cloud hosting. Thus, the combined use of blockchain and database functions makes these databases a promising option for many enterprise solutions. In addition, many blockchain platforms are now integrated with some databases. Existing databases used in some blockchain systems have either a master-slave or P2P architecture, whereby transaction logs and states are copied between [19] nodes. Since the database can be SQL/relational post-relational, the choice of a database to use in the blockchain should be based on many critical points, such as database structure and query processing speed. The development of databases integrated with blockchain functions is an active research topic. This research analyzes the blockchain features of modern database solutions and their suitability for blockchain. In general, blockchain capabilities are implemented either by adding a blockchain layer on top of an existing database layer, or vice versa by implementing block chain

functions in the database layer and architectural model [20]. First, we sublimate the seven key features, namely decentralization, composition, scalability, immutability, low latency, high performance and fragmentation, which form the first of the blockchain databases. Additionally, the increasing popularity of bitcoin has drawn significant attention to its environmental impact because of solving mathematical puzzle value called nonce. The energy-intensive proof-of-work consensus mechanism, essential for transaction validation, consumes substantial electricity and computational resources to mine a block [21]. This study will investigate factors contributing to Bitcoin's energy and computational power consumption, including the geographic distribution of mining activities and energy power sources utilized by miners. The environmental implications of Bitcoin's operations are essential for promoting sustainable and environmentally responsible blockchain practices. Figure 1 illustrates the structure of a bitcoin block structure, highlighting its components such as the block header, transaction data, Merkle root, time stamp and the nonce used in the proof-of-work consensus algorithm. The block header includes a hash of the previous block, ensuring the integrity and continuity of the blockchain [22]. Additionally, it contains the Merkle root, a hash representing the combined hash of all transactions flow shown in figure 2 within the block to provide integrity of data for secure storage, structured through a Merkle tree. We make it easy for researchers to understand blockchain technology, this study explores three main aspects. Initially, it focuses on the tool used to develop secure blockchain applications. Next, it explains vulnerabilities in web and other ledger options. Lastly, it evaluates the impact of Bitcoin mining on the environment. It's commonly understood how crucial is to employ blockchain technology securely and sustainably. This paper concentrates on key aspects of blockchain security with Section 2 analyzing consensus mechanisms essential for trust in blockchain networks. Section 3 discusses security strategies for blockchain network based systems while Section 4 explores the potential enhancements in security and threat detection in cyber security through integration of AI with blockchain technology. In section 5 concludes this paper with a summary of this paper's contributions, limitations, and future research directions.

Related work

Blockchain technology is widely carried the potential to be transformative in decentralized efficient systems and smart grids. This research study examines how blockchain technology can alter the varies sectors through the use of artificial intelligence (AI), health care, supply chain, financial and automation. Particularly, in decentralized energy systems (DES) and prosumer surroundings where consumers are also producers, there is a need for secure access control mechanisms that provide strong data privacy and security. However, there have been concerns about traditional control systems (central authority) which are managed by central third parties [23]. The decentralization, immutability and transparency of blockchain technology make it a useful alternative option for user access control in these settings. It helps organizations keep full possession, integrity and responsibility of their own information. The use of ethereum based smart contract-based access control (executing contracts without help of third party) frameworks has been widespread within the Internet of Things (IoT) and government sector but not in Smart Grids which is another name for internet of energy [24]. The various research on blockchain's potential role in smart grids has started to shed light on this issue. For instance, various research studies have investigated different consensus protocols' security, scalability and privacy implications, while others have attempted to discover some benefits as well as limitations. However, a critical challenge identified in this research is the lack of established trust, acceptability, adaptability and reputation systems within blockchain for this specific application [25]. If you take the latest vulnerability exploitation in Windows 10, users worldwide are experiencing a crash that is leading to their systems unexpectedly shutting down or restarting. The crash is causing computers data and availability to become stuck on the recovery screen or display the Blue Screen of Death (BSOD) error. This problem happens when a crucial problem causes Windows to close down or reboot which is really a big concern for most industries. In these situations, a notification will appear saying - "Your device has encountered an issue and needs to be rebooted. "We're simply gathering error information before initiating a restart on your behalf" - is displayed on the screen. In addition to this also impacting corporate offices, the crash has also impacted banks, supermarkets, telecommunications, and airports in the US, Australia, Japan, India, and various other countries. It is said to have led to flights being grounded and canceled in the United States and at Sydney Airport in Australia. Customers have been informed that they are not allowed to drop off their luggage [42]. Australia's National Cyber Security Coordinator, Michelle McGuinness, has dismissed the idea of a cyber-attack being responsible for the crash. I have knowledge of a widespread technical

issue that is impacting numerous companies and services throughout Australia today. The National Cyber Security Coordinator stated on X that the outage is connected to a technical issue with a third-party software platform used by affected companies. We have proposed how a blockchain-based architecture, specifically hybrid approach for agreement in the decentralized network will be used in smart system or digital system to avoid such crashes relied on third parties. It still lacks proper test with prototypes and deployment in network [32]. We have conducted a thorough investigation of specific aspects of blockchain technology as per shown in figure 3, presenting a balanced perspective on its strengths and weaknesses [31-34]. Our research paper focuses on below five key areas for improvement

Design patterns: The main concern about different blockchain platforms incorporate several design patterns that include public or private blockchains and permissioned or permissionless models. These will be analyzed based on individual need where we will look at their security implications as well as how they affect scalability, privacy etc.

Security challenges in DAG-Based Systems: Directed Acyclic Graph (DAG) based blockchains accomplish transactions at a faster validation rate compared to traditional way to do it. The unique structure may also introduce new security vulnerabilities aims to investigate and come up with possible solutions to mitigate them.

The relationship between AI and blockchain security: AI can help enhance weak blockchain systems on matters related to security and detect irregularities in blockchains, can be used for intrusion prevention among others to strengthen the overall safety of blockchain platforms.

Evolving Cybersecurity Threats and Proactive Countermeasures: As blockchain technology continues advancing (web 3.0), cyber criminals are inventing new ways of exploiting its vulnerability specifically using crypto for malicious things or criminal activities.

This research paper aims to provide a comprehensive analysis of blockchain technology, its present state and its future potential to widely adapt in industry. This approach gives a better understanding of blockchain as both an innovative tool and an evolving technology for securing devices in IoT as well as AI based innovations.

Cyber Threat: Security Concerns in Blockchain Implementations

Blockchain technology has emerged as a revolutionary innovation in the digital era, promising enhanced security, transparency, and decentralization in various applications. However, the widespread adoption of blockchain has also led to the identification of potential security vulnerabilities and cyber threats that must be addressed to ensure the resilience of blockchain-based systems [21].One of the primary concerns with blockchain implementations is the issue of scalability and speed at which transactions are validated by miners [31]. Traditional blockchain networks are designed to handle a limited number of transactions per second (bitcoin), which can be a significant bottleneck for real-world applications that require high-throughput processing for transaction validations [35]. These differences in transaction processing capabilities highlight the scaling challenges that blockchain networks must overcome to achieve wider adoption across industries [36]. As per the requirements we have provided different modified algorithms as mentioned in this section which will help us to understand the security aspects of blockchain with environment effects. The provided algorithms with minor modification really help researchers to find optimized secure way to correct the consensus mechanism. In addition to scalability, security is a critical concern in blockchain implementations. Blockchain networks are susceptible to various cyberattacks, such as 51% attacks, where a malicious actor forms a group to gain control of the majority of the network's computing power, potentially allowing them to manipulate the blockchain [33]. Other attacks, such as DDoS, ransomware and smart contract-based vulnerabilities, can also pose significant threats to the integrity and reliability of blockchain-based systems [37].

Algorithm 1: PoW based hybrid consensus for Security Verification

Transaction Validation

Transaction Broadcasting

- Let T={t1,t2,...,tn} be the set of all transactions.

- Each participant broadcasts their transaction set Ti⊆T to the network.

Transaction Verification
For each transaction t∈Ti
- Verify the digital signature using public key cryptography.
- Check for double spending by inspecting the transaction history.

Block Formation
Grouping Transactions
- Let B={tb1,tb2,...,tbm} be a block containing a subset of transactions.
- Apply transaction selection criteria (e.g., fee-based, size-based) to form blocks.

Hashing
Generating Hash
- Let H be a cryptographic hash function.
- Calculate the block hash h=H(B).
- The block is valid if h≤target difficulty.

Proof-of-Work
Mining
Miners modify a nonce in the block header and recalculate the hash until h≤target difficulty.
The first miner to find a valid block broadcasts it to the network.

Cyber Security Verification
Security Checks
Nodes perform additional security checks on the received block, such as: Validating the block's structure and format.
Checking for malicious code or vulnerabilities.
Ensuring compliance with network rules and policies.

Consensus
Block Acceptance
Nodes verify the block's validity and add it to the blockchain if it is valid and has the longest chain.
Blockchain growth can be modeled as a growing tree structure.

Reward Distribution
Reward Distribution
The miner who successfully mines a block receives a predetermined reward.
3.2 Algorithm 2: Stake-Weighted Random Selection Consensus Algorithm

Stakeholder Participation
A stakeholder, s, joins the system by depositing a stake, stake(s), which is greater than or equal to a predefined minimum stake threshold.

Validator Selection
- A set of validators, V, is determined based on the stake held by each stakeholder.
- The probability of a stakeholder being selected as a validator, P(v), is directly proportional to their stake: P(v)=stake(v)/totalstake

Block Creation
- A randomly selected validator from the set V creates a new block, B, containing a set of valid transactions, T.
- The probability of a validator creating a block is determined by their selection probability.

Block Validation
- The proposed block, B, is subjected to rigorous validation by the network's nodes.
- Validation includes verification of transaction validity, block structure adherence, and consensus rule compliance.

Consensus
- If a consensus is reached among most validators on the validity of block B, the block is appended to the blockchain.
- The validator who created the block, v, is rewarded with a block reward.

Slashing
- Validators found to engage in malicious behavior must be penalized, including a reduction in their stake, stake(v), by a defined amount, slashing_penalty(v).
- Severe penalties also result in the removal of a validator from the set V for integrity.

Algorithm 3: Auth Path Optimized Merkle Tree Algorithm
Initialization
- Set leaf to 0.
- Initialize empty stacks for each height, i.e., {Stack0, Stack1, ..., StackH-1}.

Loop until all leaves processed
While leaf < 2^H
Compute and Output
- Compute and output Φ(leaf) with LEAFCALC(leaf).
- For each h in [0, H-1], output {Authh}.

Refresh Auth Nodes
For all h such that 2^h divides (leaf + 1)
- Set Authh to the sole node value in Stackh.
- Set startnode to (leaf + 1 + 2^h) xor 2^h.
- Initialize Stackh(startnode, h).

Build Stacks
For all h in [0, H-1]
- Update **Stackh(2)**.

Zip Up the Tails (Delay)
Wait until all stacks {Stack0, Stack1, ..., StackH-1} are partially completed, containing no tail nodes of height less than h.

Increment Leaf
Set leaf = leaf + 1.

End of Algorithm
Merkle Tree output with authenticity for integrity and security
The above suggested algorithm will make changes in view of consensus with integrity and security vulnerabilities which we need to address in near future for adaptability. Merkel tree with authenticity must help to get data

integrity with more useful parameter for blockchain based vulnerabilities. These security vulnerabilities have led to the theft of millions of dollars in cryptocurrency assets which is the main reason to get crypto to accept illegal transactions, undermining the trust and credibility of blockchain technology [34]. Despite its potential for revolutionizing secure data storage and transaction management, blockchain technology still has its weaknesses. Cybercriminals are continuously improving their methods to take advantage of these weaknesses for monetary profit or to cause disruptions in activities.

Network Hijacking: Interrupting Activities and Stealing Information

Network hijacking incidents occur when attackers gain unauthorized control over a blockchain network or its nodes. Here is an analysis of the possible consequences of network hijacking

Double-Spending Attacks: Malicious actors can tamper with transactions in order to carry out double-spending. This goes against the fundamental idea of blockchain technology, which is designed for transactions to be permanent.

Transaction rearrangement: In certain blockchains, the sequencing of transactions in a block may hold significance. Hijackers may be able to resequencing transactions in order to obtain an unjust benefit, disturb the operational flow of smart contracts, or initiate denial-of-service attacks.

Stopping Blockchain Operations: If attackers seize substantial authority over the network, they may stop fresh blocks from being inserted into the blockchain, essentially bringing transactions to a standstill. This has the potential to severely impact applications that are created using blockchain technology.

Data Censorship: Malicious individuals may block certain transactions or data from being added to the blockchain ledger. This could go against the transparency principle of blockchains and impede the immutability of data.

Mitigating Network Hijacking

- BFT algorithms can assist in achieving network consensus despite the existence of Byzantine faults, such as malicious nodes, through Byzantine Fault Tolerance (BFT) Consensus Mechanisms. There is ongoing research on BFT-based consensus mechanisms developed specifically for blockchains.
- **Proof-of-Stake (PoS) Systems:** Although not completely reliable, PoS systems typically demand a substantial stake (cryptocurrency holdings) to engage in the consensus process. This discourages bad actors from taking over the network due to the economic consequences of losing their investment.
- Authorized nodes are the only ones allowed to take part in the network of permissioned blockchains. This method may be advantageous for scenarios wanting strict control, but it sacrifices some decentralization.
- Regularly monitoring network activity and conducting security audits can assist in detecting potential vulnerabilities and suspicious behavior. This enables developers to be proactive in dealing with security threats before they can be utilized by attackers.

Explores the potential enhancements in security and threat detection in cyber security through integration of AI with blockchain technology.

Blockchain adaptability in real life is essential for maximizing the use of AI and blockchain in cybersecurity. It is necessary to require preparing data for AI-driven identification of anomalies in blockchain data involves formatting data in a way that enables AI to analyze it effectively, which includes feature engineering, normalization, and managing missing data. RNNs and LSTMs are perfectly fitted choices for examining sequential blockchain data, whereas autoencoders can identify anomalous patterns. Employing transaction amounts, addresses, timestamps, and block heights as input features is crucial in feature engineering. We can successfully incorporate the AI model into a blockchain monitoring system which requires efficient implementation and instant data handling at decentralized network. We want to secure sharing of data for AI with the help of blockchain technology which can be attained by utilizing federated learning, homomorphic encryption, and secure multi-party computation to protect data privacy

and integrity. It is known and accepted that AI can be integrated into smart contracts to improve prediction capabilities, detect fraud, and assess risks, thus boosting their flexibility and protection. Despite the promising potential of AI and blockchain, it is crucial to recognize the drawbacks of social media platforms, including privacy issues, addiction, and the spread of misinformation. The emergence of Web 3.0, the next generation of the internet era, has brought with it new opportunities and challenges, particularly in the emergence of security, transparency and privacy. Blockchain technology is a core component of Web 3.0 which has been touted as a solution to the centralized control specifically third parties and data vulnerabilities that plagued previous iterations of the web. Blockchain's decentralized nature, immutable ledger, trustless environment and smart contract capabilities have the potential to revolutionize in various industries including online data management, identity verification, and secure transactions. However, the integration of blockchain into Web 3.0 implementations is not without its own set of security concerns. As technology continues to evolve, researchers have identified vulnerabilities that could potentially be exploited by cyber attackers. These vulnerabilities range from consensus mechanism flaws to smart contract vulnerabilities, which can lead to financial losses, data breaches, and other malicious activities. Blockchain technology, while innovative and transformative, poses significant environmental concerns due to its high energy consumption. The decentralized nature of blockchain, particularly in proof-of-work systems, requires substantial computational power for mining activities, leading to increased carbon footprints. Studies suggest that the power utilization of major cryptocurrencies like Bitcoin rivals that of complete countries. Moreover, the hardware used in mining contributes to e-waste, exacerbating environmental degradation. Thus, sustainable blockchain solutions, together with evidence-of-stake and eco-friendly mining practices, are vital to mitigate those environmental effects. One study [31] highlights the importance of a novel design for three-tier identities, which could help address the security and privacy challenges in Web 3.0. Additionally, the impact of laws on privacy and cyber sovereignty must be considered to achieve the full potential of Web 3.0. Another study [32] explores the use of self-sovereign identity and smart contracts to enhance the security of real estate transactions, a sector that has traditionally been plagued by centralized control and paperwork-intensive processes. An analysis of the information presented in Table 4, detailing the potential consequences of network hijacking, coupled with the implementation of appropriate mitigation strategies, can empower blockchain developers and users to build more secure and resilient blockchain networks [38-42]. The paper addresses the opportunities and challenges presented by Web 3.0, with a specific focus on the integration of blockchain technology. Blockchain, known for its decentralized, immutable, and trustless environment, promises advancements in various sectors, including data management, identity verification, and secure transactions. However, the adoption of blockchain is not without security concerns, which range from consensus mechanism flaws to smart contract vulnerabilities. These issues can lead to financial losses, data breaches, and other malicious activities. Research highlights the importance of innovative designs for addressing security and privacy challenges in Web 3.0. For instance, a novel three-tier identity design could enhance security and privacy, while the application of self-sovereign identity and smart contracts could improve the security of real estate transactions. The paper underscores the significance of understanding OWASP risks and implementing appropriate mitigation strategies to build more secure blockchain networks. Scalability and security are critical concerns for blockchain implementations. Traditional blockchain networks face limitations in transaction processing speed, which poses a challenge for real-world applications. Cyberattacks such as 51% attacks, DDoS, and ransomware exploit vulnerabilities in blockchain systems, resulting in significant financial losses and undermining the technology's credibility. The paper also discusses the potential of blockchain-based architectures to prevent such crashes and cyberattacks. Additionally, the integration of artificial intelligence into blockchain has been explored to enhance security and prevent cyberattacks.

CONCLUSION

The paper proposes a hybrid approach to achieve consensus in decentralized networks, to address the shortcomings of traditional database systems. The review includes security research test cases and offers solutions to improve blockchain technology to combat advanced cyber threats. We also provided modified way to create Merkel tree, incentive with penalties for misbehavior nodes in the network with environment effects to considered for wider acceptance of blockchain in industries. The transition to Web 3.0 presents both opportunities and risks for the digital

age, particularly when combined with blockchain technology, which has an impact on society's environment. Blockchain is vulnerable to various kinds of cyberthreats and scalability problems, even though it promises significant advancements in security, transparency, and decentralization. To solve these issues, the study emphasizes the need for new frameworks and approaches, such as combining self-management, smart contracts, and consent mechanisms. Resilience of blockchain networks depends on Web 3.0 applications' assessment and mitigation of OWASP threats. Blockchain technology and artificial intelligence together present a viable means of strengthening security and resisting cyberattacks. However, further research and innovation are required to get beyond present constraints and completely realize blockchain's existing challenges and fully utilize application potential. The efforts to address the security, scalability, and issues of privacy associated with blockchain technology will be required for achieving Web 3.0's full potential. In future to bring about a stable, effective, and secure online world, innovative approaches and continuous research are needed in real life applications.

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Table 1: Beyond Cryptocurrencies: Applications Utilizing Blockchain Technology

Domain	Description	Potential Impact
Blockchain Scalability [22]	Application of deep learning for congestion prediction, resource allocation, and transaction validation for the distributed based consensus mechanisms, aiming to optimize network performance and scalability.	The potential to increase the transaction throughput, reduce fees, and broaden the adoption of blockchain technology across various industries.
Smart Contract Security with AI [25]	The use of machine learning for automated smart contracts will help to detect vulnerability to enhanced security analysis and identify potential vulnerabilities before deployment.	It will help to secure environment by creating smart contracts to reduce the risk of exploits and improving overall blockchain security.
Secure AI on Blockchain Infrastructure [27]	To build secure and transparent system on blockchain platforms includes exploring secure enclaves for confidential AI model training on blockchains and decentralized AI marketplaces with tamper-proof model registration.	It will help to increased trust and collaboration in the development and deployment of AI models for securing blockchain infrastructure responsible and transparent.
Sybil Attack Detection using AI	Anomaly detection algorithms and behavioral analysis techniques can be employed to	It helps to develop honest and secure consensus mechanisms for blockchain

[28]	differentiate legitimate nodes from malicious nodes, particularly in PoWconsensus mechanisms.	networks, mitigating the impact of Sybil attacks that threaten network integrity.
Privacy-Preserving AI on Blockchain [29]	For privacy, homomorphic encryption and secure multi-party computation (MPC) protocols are potential solutions to allow AI computations on encrypted data without compromising user privacy.	The benefits of data security and transparency with the need for privacy can be achieved wider adoption of blockchain-based AI solutions.
Blockchain for Secure Internet of Things (IoT) [30]	To secure data exchange and access control protocols for IoT devices on blockchains, and decentralized oracles for trustless data acquisition and verification in IoT applications.	Blockchain has the potential to address security vulnerabilities and ensure data integrity with the help of immutability feature utilize in IoT deployments.

Table 4: Understanding OWASP Risks in Web3 Applications

OWASP Risk	Web Application Context	Blockchain Impact	Example
Injection	Malicious code insertion via user input.	Not a main difficulty for blockchains due to constrained users enter mechanisms.	Might be applicable for blockchain oracles fetching facts from external resources. Improper facts sanitization can go away oracles vulnerable to injection attacks.
Broken Authentication	Faulty safety mechanisms provide unauthorized access.	Private key agreement on wallet. Unauthorized access to authenticator nodes.	Phishing attacks targeting private keys. inappropriate use of specialized behaviors.
Broken Access Control	Inappropriate access control techniques allowing unauthorized actions.	Unauthorized data processing on blockchains with variable data storage capacity.	Smart contracts with incorrect access control modifiers. Lack of granular access control for authenticator nodes.
Security Misconfiguration	Insecure default configurations or improper security settings.	Insecure configurations of blockchain nodes or clients.	Improper gas limit settings in smart contracts lead to DoS vulnerabilities.
Security Misuse of APIs	Exploiting flaws in APIs to gain unauthorized access or functionality.	Potential vulnerabilities in APIs used by blockchain applications.	Vulnerabilities in wallets or Blockchain Explorer APIs that allow access to sensitive funds or data.
Insufficient Logging and Monitoring	Inadequate logging and monitoring of security events.	Difficulty in detecting and responding to security incidents.	Aborted fraudulent connections or unauthorized attempts to access compromised nodes.
Security Testing Failures	Inadequate security testing practices lead to missing vulnerabilities.	Identify and mitigate blockchain vulnerabilities due to the complexity and consent of smart contracts.	Smart contract audits that fail to detect vulnerabilities for reentrant attacks or integer overflows.

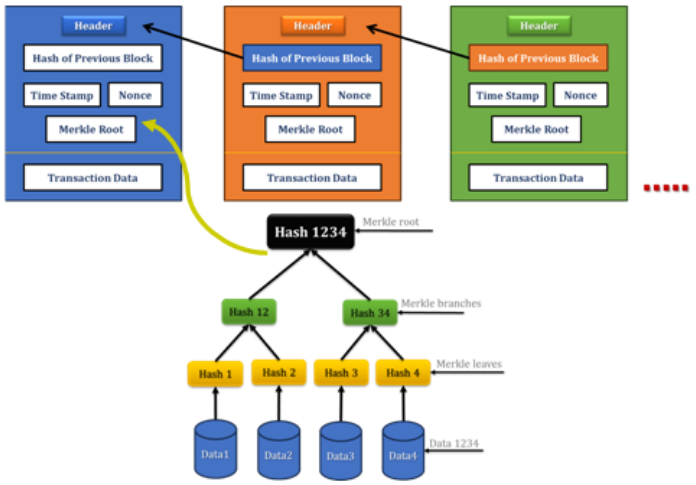


Fig 1: Fundamentals of Bitcoin: Understanding Block Structure and Transaction Data

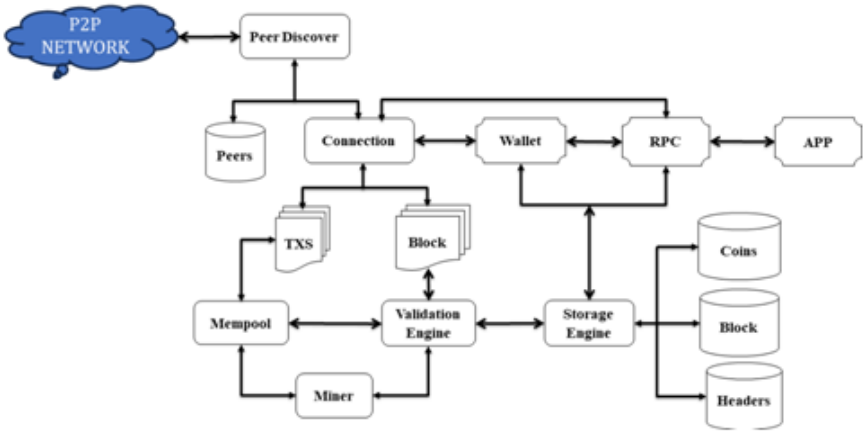


Fig 2: Fundamentals of blockchain network: key components of bitcoin core

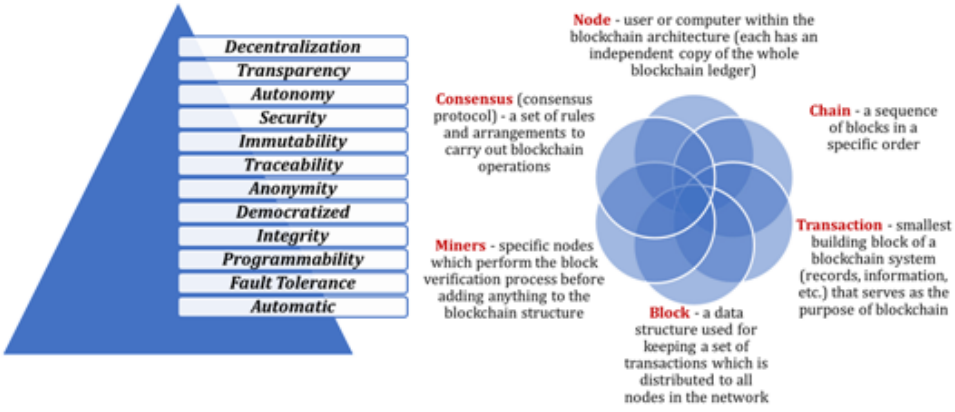


Fig 3: The Anatomy of a Blockchain: Exploring the Key Elements

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RESEARCH ARTICLE

Vocational Education in the National Education Policy 2020: A Critical Analysis of Policy and Practice

Rajesh Ekka[1]* and Astha Paliwal[2]

[1]Assistant Professor, Department of Education, Babasaheb Bhimrao Ambedker University, Lucknowm, Uttar Pradesh, India.
[2]M.A.Student, Department of Education, Babasaheb Bhimrao Ambedker University, Lucknowm, Uttar Pradesh, India.

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***Address for Correspondence**
Rajesh Ekka
Assistant Professor,
Department of Education,
Babasaheb Bhimrao Ambedker University,
Lucknowm, Uttar Pradesh, India.
E.Mail: rajeshvj07@gmail.com

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ABSTRACT

The National Education Policy(NEP) 2020 introduces a paradigm shift in India's education system, emphasizing the inclusion of vocational education to increase employability, skill development and industry readiness. This study critically analyses the policy framework and its practical implications for vocational education in India. The study explores the objectives of NEP -2020, particularly its vision to ensure that at least 50% of learners acquire vocational exposure by 2025 through school and higher education. It studies key provisions, such as the introduction of skill-based courses at an early stage, multidisciplinary learning, industry partnerships and the use of digital platforms for vocational training. It involves the implementation challenges, including infrastructural gaps, the shortage of trained educators, and societal perception in vocational education access. Through a comparative analysis of global best practices and policy execution in India, this study identifies areas requiring policy refinement and suggests strategies for improving vocational education outcomes. The findings focus the need for stronger industry-academia collaboration, enhanced teacher training, and policy adjustments to address systemic challenges. This study concludes that while NEP-2020 lays a strong foundation for vocational education reform, its success depends on effective execution. If implemented strategically,NEP-2020 has the potential to create a more inclusive, skill-oriented education system.

Keywords: Challenge, Education, Employability, NEP 2020, Skill, Vocational.

INTRODUCTION

After independence, several commissions and committees were constituted to analyse the problems related to Indian education and various suggestions were recommended for the way forward. It was the first attempt to reorient the system of education to the needs and aspirations of the citizens and it was felt that the universities and other higher education institutions could play a significant role in the development process. Countries plan their education systems to progress further (Rizvi and Lingard, 2009) . To promote education at all economic for the inclusion of common people in the classes and mainstream the Government of India (GOI) has formulated the National Policy on Education (NPE). The Policy has broader coverage, starting from elementary school education (literacy level) to colleges for higher education (focusing specialization) - in both the settings, rural and urban. The first NPE was proposed and disseminated in 1968 by the GOI, the second policy was in 1986, and the third major reformative policy was in 2020 by the GOI. The National Education Policy (NEP) in India has undergone several iterations since its inception with significant reforms aimed at enhancing the quality and accessibility of education. The most recent NEP was introduced in 2020, marking a transformative shift in the educational landscape. Vocational education in India has a long and evolving history, influenced by various socio-political and economic changes. The concept of vocational training and skills - based education can be traced back to ancient India, but its formal integration into the modern educational system has been shaped by both colonial policies and post - independence reforms. Vocational education in India has evolved from traditional craft-based learning systems to a more formal, structured approach, with increasing focus on industry partnerships and practical skills to meet the demands of a modern economy.

Vocational Education in the NEP,1968: Based on the recommendations of the Kothari commission, this policy encouraged vocational training in schools and promoted the establishment of vocational institutions, aiming to reduce the pressure on higher education and provide employment orient skills.

Vocational Education in the NEP,1986: This policy gave a significant push to vocational education, recognising it as crucial for employment generation. The 1986 policy aimed to ensure that at least 25% of students at the higher secondary stage were offered vocational courses. The policy proposed creating a vocational education program (VEP) at the secondary level and encouraged partnership between schools and industries for practical training.

National Education Policy, 2020: The most recent NEP 2020 introduces a strong focus on vocational education integration starting from the school level. It envisions that by 2025, at least 50% of learners will have exposure to vocational education. The policy emphasizes

- Blurring the line between academic and vocational education.
- National Skills Qualifications Framework (NSQF)
- Integration of vocational education with mainstream schooling.
- Work place and appreciation ship training.

LITERATURE REVIEW

Patel and Agrawal (2024) conducted a study titled "National Education Policy2020: Paradigm Shift in Vocational Education for AatmaNirbhar Bharat". The objective of this study was to aware common people about Aatmanirbhar Bharat campaign and its linkage to Vocational Education. The research design adopted was qualitative approach. The result revealed that Aatmanirbhar Bharat is an important initiative of GOI which trained people to enrich vocational education. NEP 2020 and other 20 research papers (Eg: Jain N., Kumar S., Ruskin J., Ramanathan V. etc.) are used as reference. Sharma, Dakngam, and Anga (2024) conducted a study titled "Implementation of Vocational Education in Education System of India''. The objective of study to find out the challenges in integration of Vocational Education in Education System. The research design adopted was descriptive method. The result revealed the challenges of Vocational Education and Prospects of Vocational Education at Higher education. NEP 2020 and research papers by Fuller A., Karra S., Krishna A., Kumar J.S., Kumar K. and many important websites and schemes

are used as reference. Laskar (2023) conducted a study titled " A study on NEP 2020: Implementation of Vocational Education and its challenges in Hailakandi district of Assam''. The objective of this study was to understand the importance, implementation challenges, and suggestions for vocational education. The research design adopted was mixed method approach, utilizing both qualitative and quantitative techniques. The sample was taken from ten educational institutes of Hailakandi district. The result revealed challenges and suggestions for implementation of Vocational Education in Hailakandi district. NEP 2020, research papers by Aggarwal J.C., Kumar Pradeep, Kaushik Kusuma, Kedar Shubhangi, Athil ShubhraJyotsa and Athil P.S. are used as references. Kharwar and Kumar (2022) conducted a study titled "Vocational Education inperspective of NEP 2020".The objective of study to find out the vocational training in India in context of NEP 2020. The research design adopted was qualitative approach. The result revealed the importance of Vocational education and process of successfully implemented it. World Bank Report 2019, research papers by many authors like Jeba raj P., Nandini, Gohain Manash Pratim and other documents are used as reference. Hannah (2022)conducted a study titled "NEP,2020: General Education Embedded with Skilland Vocational Education". The objective of the study to find out framework for concurring general education with vocational education. The research design adopted was qualitative approach. The result revealed the framework of possible pathways of synchronising General Education with Vocational and Skill education and also revealed implementation plan for it. It included timelines for completing specific tasks for States and UTs. Document of Educational policies- 1968, 1986, 1992; Census of India 2011; other surveys and her own research papers are used as reference. Parvanda(2022) conducted a study titled "Vocational Education and NEP 2020". The objective of this study was to find out the key aspects of NEP 2020. The research design was adopted was qualitative approach. The result revealed the provisions, implementation challenges and future impact of Vocational Education. Encyclopaedia Britannica; research papers by Einstein A., Lugol John, Jain B. and by other authors; and some websites are used as reference. Sharma (2022) conducted a study titled ''Vocational Education and NEP-2020''. The objectives of this study was to find out the problems in vocational education and new approaches of NEP 2020 for employability. The research design adopted was qualitative approach. The result revealed problems of vocational education in present scenario and recommendations of NEP 2020.Research papers of Adams and Arvil, Sr.Catheline and K.Mala, SharmaY.K Tushar A. and others are used as refrences. Jena (2022)conducted a study titled ''Re-imagining Vocational Education: The NEP Perspective''. The objective of this study is to find out the issues and challenges of vocational education and to find out the global scenario of students in vocational education sector.The research design adopted was qualitative and quantitative approach. In result he proposed -the pathway for vocational education, interface between the education system and Industry and training design for vocational education. Gandhi (2021)conducted a study entitled ''Review on the Key Features of the NationalEducation Policy 2020 in the Context to Pre-school Education and its Expected Impact''. This study included the importance of experiential learning in pre-schooler's development. It also included the development of basic skills, habits, and personal hygiene conditions among pre-schoolers. Pathak (2020) conducted a study titled "NEP 2020: A road map to Vocational Development". The objective of the study was to find out the level of vocational development program and difference between previous education policy and NEP 2020 with respect to vocational development program. The result revealed the skill development program and development program for vocational education at the different level of school education and in higher education. Website of GOI, NEP 2020 document, research papers by Agrawal T., Maclean R and Lai A. and some websites are used as references.

OBJECTIVES OF STUDY

1. To examine the goals and achievements behind NEP-2020 focus on integration vocational education.
2. To analyse the vocational education curriculum aligns with current and future industry demands.
3. To critically assess the NEP -2020 ensures vocational education is accessible to diverse groups.
4. To compare the NEP-2020 vocational education framework with global best practices.

Research Questions

1. What are the goals and achievements behind the NEP-2020 focus on integration vocational education?

2. How vocational education curriculum aligns with current and future industry demands?
3. 3. What are the ways to access the NEP-2020 that ensures vocational education is accessible to diverse groups?
4. How does the NEP-2020 framework guide towards global best practices?

METHODOLOGY

Research Design-This study employs a qualitative research design with elements of policy analysis to critically examine the provisions of the National Education Policy (NEP) 2020 related to vocational education. The research aims to explore how the policy translates into practice and its implications for the education system.

Research Approach-The study adopts a critical analysis approach, combining document and content analysis with qualitative data collection.

RESULT AND DISCUSSION

Research Question 1.What are the goals and achievements behind the NEP-2020 focus on integration vocational education?

According to the draft of NEP 2020 the provision of vocational education is mentioned in point 16. The key aspects of NEP 2020 reveals provisions, implementation challenges and future impact of vocational education. NEP-2020 made a strong and serious commitment to expand vocational education and training and describe the challenges and solutions for expedition of vocational education and training. The global scenario of students in vocational education sector describes the role of industry input in other countries. The teaching of vocational courses from class six, mostly in the form of internship and practical activities will make ensure that every student should at least study one vocational course (Kharwar and Kumar, 2021). The background of the vocational education along with NEP policy perspective on vocational education .It also include the theoretical framework for synchronization of general education with vocational and skill education as suggested by UGC (Hannah, 2022).Vocational Education for Aatmanirbhar Bharat' in the NEP 2020 has advocated for vocational education to be introduced from class sixth after keeping the current century in view and with the vision to make Aatmanirbhar Bharat (self-reliant India) (Agrawal, 2024). A roadmap to Vocational Development in NEP2020, at least 50% of learners through the school and the higher education system shall have exposure to vocational education(Pathak, 2021).

Research Question 2.How vocational education curriculum aligns with current and future industry demands?

According to the draft of NEP 2020 the provision of vocational education is mentioned in para 16. The implementation of the NEP 2020 is essential for the transformation of the education system in India (Banerjee, 2023). Fostering Vocational Education among students through the lens of National Education Policy2020 as real need of the hour (Chowdhury and Mondal, 2022)Vocational Education and Skill Development are very important in regard to NEP 2020 it fills the gap between education and industry demand . It also develops the connection between academic institutes and industry (Barai, 2024). Vocationalization of Indian Education system through National Education Policy 2020willincrease employability rates among youths (Bhatta and Bharali, 2024)Professional schooling and training is very important in vocational education. Implementation of Vocational Education and training will solve the many challenges of vocational education in NEP 2020 and it will be the best possible solution for the problem. Challenges and Opportunities in general education with vocational education in schools should prioritizes pre vocational education and vocational education operating with NSQF and connect general education with skill development programme (Vats and Malik, 2024).Therefore, vocational education will improve by approval of professional courses and set up of skill laboratories in higher education (Gupta, Dhull, and Ashraf, 2022).

Research Question 3. What are the ways to access the NEP 2020 that ensure vocational education is accessible to diverse groups?

According to the draft of NEP 2020 the provision of vocational education is mentioned in para 16.The importance of professional training and learning of teachers will help the student to perform in specific field of careers. The issue - inclusion in vocational education and training presents international perspectives on a universal challenge. The Role of Vocational Educationand will included the nature of participation of the socially excluded in vocational education and training and the difficulties of targeting vocational education and training towards social exclusion (Preston and Green, 2008).The role of Vocational Education in the Student Career Development Process provides information about careers obtained is an initial plan and can improve career development and create career opportunities. (Wagino *et.al*., 2023)Inclusive Vocational Education is the Key to Women Empowerment and Sustainable Livelihoods. The vocational education programmes should be more inclusive as it needs to take into consideration the various socio-cultural, economic and personal barriers that the women face in their domestic lives and community living (Siddhu and Karolia,2023).Diversity of Purpose in Vocational Education may develop technical skills,sense of competence, understanding of applications and aesthetic appreciation and expression among students (Plihal and Copa, 1986).Vocational Education and Social Inclusion provides career guidance and retention strategies to foster success rate (Hargraves (2011). Integration of ethnic minorities during group-work for vocational training develops intercultural communication skills and cultural sensitivity (Goth, Bergsli, and Johanesen, 2017).

Research Question 4. How does the NEP 2020 framework guide towards global best practices?

According to the draft of NEP 2020 the provision of vocational education is mentioned in para 16.The NEP 2020 aims at making "India a global knowledge superpower"; it includes visions and goals, principles, implementation strategies and different factors. The visions of NEP 2020 translated in practice can prove to be an unbeatable soft power to facilitate India to achieve its global position in the emerging world order. Quality Parameters for in futuristic education in the light of NEP 2020; the quality guidelines of NEP 2020 provides innovative, advanced and visionary education through NEP 2020 (**Pareek, 2023)**.NEP 2020 has been prepared according to the need of 21st century. It fits global standards of education as it include vocational education, technology based education ,flexible curriculum and evaluation and other important changes in it **(Mahali, 2023)**.The different aspects of NEP2020 like ECCE, school education, higher education, vocational education and many more. It empower learner to become global citizen and lifelong learners **(Bhar, 2023)**. new education system is creativity and conception based learning system is creativity and conception based learning system which promote better learning results ,more job skills and strengthen innovation capacities. Pedagogical Innovation towards a new direction for Multidisciplinary and Holistic Futuristic Education in the light of NEP2020; the scientific perspectives like interdisciplinary thinking ,creative combinations ,multidisciplinary model, research and innovation, industrial link and all round development for multidisciplinary and holistic futuristic education of youth (**Biswas, 2023).** The education policies of Finland, India, Germany, United States and United Kingdom says that integrating vocational education into the mainstream curriculum is similar to Germany's dual education system. The education policies of India and UNESCO found that life-long learning and holistic education are common in both policies (**Tholath *et. al*.2021)**.

Findings of the Study

After analysis the data it is found that NEP-2020 include many programmes, courses and recommendations to promote vocational education. It also provides understanding of Start-ups, Entrepreneurship at higher education level. Overall NEP-2020 connects vocational and skill education with general education at every level of education. Vocational education is real need of the hour. Adding many vocational courses and training in curriculum is very useful for students as well as industries. Vocational education programmes should be more inclusive. It should provide career guidance, vocational education training, skill development programmes and retention strategies to foster success rate in disadvantaged groups. Education policies of different countries are similar to NEP-2020 at some point.NEP-2020 provides quality guidelines for innovative, advanced and visionary education.NEP-2020 prepared according to the need of 21st century and fits global standards of education.

Educational Implications

Integration of Vocational Education into mainstream Education; NEP-2020 includes vocational education in general education and start many skill development programmes for students from grade 6 to higher education.

Role of Multidisciplinary Education; NEP-2020 provides many opportunities to choose vocational courses and technical training according to students interest .It shows the flexibility in vocational education as well as in academic courses.

Skill-Based Learning and Employment Readiness; NEP-2020 suggests such type of vocational programmes that promote employment readiness and skill based learning in students.

Inclusivity and Accessibility; NEP-2020 focuses on vocational training for diverse groups. It also include marginalized communities, rural students and girls.

Suggestions for Further Research

The potential researchers might focus on the following topics for more study

1. A similar study can be conducted on challenges and opportunities in implementing. NEP-2020's Vocational Education Framework.
2. A study can be conducted on role of public-private partnerships in vocational education post NEP 2020.
3. A study can be conducted on International Comparisons on Vocational education.
4. A study can be conducted on success rates of NEP-2020 and vocational education.
5. A study can be conducted on the role of traditional handcrafts in vocational education programmes.

CONCLUSION

The study aimed to study the NEP-2020 and its provisions for vocational education. The implementation for vocational education under NEP-2020 shows a radical change in India's education system, aiming to bridge the gap between academic learning and skill-based training. Researcher analysed the policy framework, implementation, challenges and real-world impact of NEP-2020's vocational education. Findings suggest that NEP-2020 has laid a strong foundation for integrating vocational training from an early stage. We face challenges in terms of infrastructure, industry collaboration and societal perception. Despite government initiatives and findings, difference between urban and rural institutions limiting access to quality skill-based education. A crucial finding is the slow acceptance of vocational education as a mainstream career, remaining social biases favouring traditional academic degrees over skill-based learning. However, vocational education can enhance employability, increase economic growth and reduce country's skill gap. After analysing NEP-2020 vision fully, researcher found that policymakers focused on strengthening implementation mechanisms, ensuring industry aligned curricula, improving infrastructure and changing mind sets towards vocational learning. With proper efforts, India can create an education system that is inclusive, skill oriented and future-ready, empowering students to succeed in a rapidly developing job market.

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RESEARCH ARTICLE

IP* Continuity in Intuitionistic Topological Spaces

L. Jeyasudha[1]* and k. Bala Deepa Arasi[2]

[1]Research Scholar (Reg. No: 20122012092004), Department of Mathematics, A.P.C. Mahalaxmi College for Women, Thoothukudi, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli), Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, A.P.C. Mahalaxmi College for Women, Thoothukudi, (Affiliated to Manonmaniam Sundaranar University, Tirunelveli), Tamil Nadu, India.

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***Address for Correspondence**
L. Jeyasudha
Research Scholar (Reg. No: 20122012092004),
Department of Mathematics,
A.P.C. Mahalaxmi College for Women, Thoothukudi,
(Affiliated to Manonmaniam Sundaranar University, Tirunelveli),
Tamil Nadu, India.
E.Mail: jeyasudha555@gmail.com

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ABSTRACT

The main objective of this work is to present the idea of $\mathcal{J}$P*- Continuous maps in $\mathcal{J}$TS using the concepts of $\mathcal{J}$P* open sets. Also, we define the Contra version of $\mathcal{J}$P*- Continuous maps in $\mathcal{J}$TS. We continue to look into the connection to $\mathcal{J}$- Continuous and $\mathcal{J}$P- Continuous maps in $\mathcal{J}$TS.

Keywords: $\mathcal{J}$P*- Continuous, Contra $\mathcal{J}$P*- Continuous, $\mathcal{J}$- Continuous, $\mathcal{J}$P- Continuous.
AMS subject classification (2010): 54C05.

INTRODUCTION

D. Coker [1] introduced the idea of intuitionistic sets for the first time in 1996. 2016 saw the definition of intuitionistic pre open sets in $\mathcal{J}$TS by G. Sasikala and M. Navaneethakrishnan [4]. In [5] we introduced $\mathcal{J}$P*O sets and $\mathcal{J}$P*C sets in $\mathcal{J}$TS. We also look into the connection between $\mathcal{J}$P- continuous maps and $\mathcal{J}$P*- continuous maps in $\mathcal{J}$TS. In this research defines $\mathcal{J}$P *- Continuous and Contra $\mathcal{J}$P *- Continuous maps using the concepts of $\mathcal{J}$P*O sets and $\mathcal{J}$P*C sets. We also demonstrate that the $\mathcal{J}$P*- continuous map is intermediate between $\mathcal{J}$- continuous and $\mathcal{J}$P- continuous maps.

Preliminaries

Definition 2.1 [1] Assume that X_J is not empty set. An object with the form $M_J = \langle X_J, M_{J1}, M_{J2} \rangle$, where M_{J1} and M_{J2} are subsets of X_J satisfying $M_{J1} \cap M_{J2} = \phi$, is known as an *intuitionistic set* ($\mathcal{J}$S in short). The terms "set of members of M_J" and "set of non-members of M_J" refer to the sets M_{J1} and M_{J2} respectively.

Definition 2.2 [1] Assume that X_J is a non-empty set, that $M_J = \langle X_J, M_{J1}, M_{J2} \rangle$, that $N_J = \langle X_J, N_{J1}, N_{J2} \rangle$ is an $\mathcal{J}$S's and that $\{M_{Ji} : i \in J\}$ be arbitrary family of $\mathcal{J}$S's. Then,

a. $M_J \subseteq N_J$ iff $M_{J1} \subseteq N_{J1}$ and $M_{J2} \supseteq N_{J2}$.
b. $M_J = N_J$ iff $M_J \subseteq N_J$ and $M_J \supseteq N_J$.
c. The complement of M_J is defined as $M_J^c = \langle X_J, M_{J2}, M_{J1} \rangle$.
d. $\cup M_{Ji} = \langle X_J, \cup M_{Ji1}, \cap M_{Ji2} \rangle$ and $\cap M_{Ji} = \langle X_J, \cap M_{Ji1}, \cup M_{Ji2} \rangle$.
e. $M_J - N_J = M_J \cap N_J^c$.
f. $\phi_I = \langle X_J, \phi, X_J \rangle$ and $X_I = \langle X_J, X_J, \phi \rangle$.

Definition 2.3 [1] Assume that X_J is a non-empty set and $m_{xj} \in X_J$ is a fixed element then the intuitionistic point M_J is defined as follows $M_J = \langle X_J, \{m_{xj}\}, \{m_{xj}\}^c \rangle$.

Definition 2.4 [1] Assume that X_J is an nonempty set and τ_I is the set of $\mathcal{J}$S's of X_J then τ_I is known as an *intuitionistic topology* ($\mathcal{J}$T in short) on X_J if it meets the criteria listed below:

1. $X_I, \phi_I \in \tau_I$.
2. $M_J \cap N_J \in \tau_I$ for every $M_J, N_J \in \tau_I$.
3. $\cup M_{Ji} \in \tau_I$ for any arbitrary family $\{M_{Ji} : i \in J\} \subseteq \tau_I$.

The pair (X_J, τ_I) is known as *intuitionistic topological space* (abbreviated $\mathcal{J}$TS) and intuitionistic open set ($\mathcal{J}$OS in short) in X_J is referred to as $\mathcal{J}$S in τ_I. The intuitionistic closed set ($\mathcal{J}$CS in short) in X_J is regarded as the counterpart of $\mathcal{J}$OS in X_J.

Definition 2.5 [1] If (X_J, τ_I) is a $\mathcal{J}$TS and M_J is a $\mathcal{J}$S in X_J then the definition of the $\mathcal{J}$- interior operator of M_J and the $\mathcal{J}$-closure operator of M_J are as follows: (i) $\mathcal{J}\text{int}(M_J) = \cup \{N_J : N_J$ is $\mathcal{J}$OS in X_J and $M_J \supseteq N_J\}$. (ii) $\mathcal{J}\text{cl}(M_J) = \cap \{ N_J : N_J$ is $\mathcal{J}$CS in X_J and $M_J \subseteq N_J\}$.

Definition 2.6 [2] If (X_J, τ_I) is an $\mathcal{J}$TS and a $\mathcal{J}$S M_J is known as the $\mathcal{J}$g- closed set if $\mathcal{J}\text{cl}(M_J) \subseteq U_J$ whenever $M_J \subseteq U_J$ and U_J is $\mathcal{J}$OS in X_J. The $\mathcal{J}$g – open set in X_J is known as the $\mathcal{J}$g- closed set's counterpart.

Definition 2.7 [2] If (X_J, τ_I) is an $\mathcal{J}$TS and M_J be a $\mathcal{J}$S in X_J then the definition of

a. $\mathcal{J}$g- *closure of* M_J is, $\mathcal{J}\text{cl}^*(M_J) = \cap \{N_J : N_J$ is $\mathcal{J}$g- CS in X_J and $M_J \subseteq N_J\}$.
b. $\mathcal{J}$g- *interior of* M_J is, $\mathcal{J}\text{int}^*(M_J) = \cup \{N_J : N_J$ is $\mathcal{J}$g- OS in X_J and $M_J \supseteq N_J\}$.

Definition 2.8 [4] If (X_J, τ_I) is an $\mathcal{J}$TS and a $\mathcal{J}$S M_J in X_J is known as the

a. $\mathcal{J}$PO set if $M_J \subseteq \mathcal{J}\text{int}(\mathcal{J}\text{cl}(M_J))$. The $\mathcal{J}$PC set in X_J is the $\mathcal{J}$PO set's counterpart.
b. $\mathcal{J}$P*O set if $M_J \subseteq \mathcal{J}\text{int}(\mathcal{J}\text{cl}^*(M_J))$. The $\mathcal{J}$P*C set in X_J is the $\mathcal{J}$PO set's counterpar

Theorem 2.9 [5] If (X_J, τ_{IT}) is an $\mathcal{J}$TS then,

a. Every $\mathcal{J}$O set is $\mathcal{J}$P*O set.
b. Every $\mathcal{J}$C set is $\mathcal{J}$P*C set.
c. Every $\mathcal{J}$P*O set is $\mathcal{J}$PO set.
d. Every $\mathcal{J}$P*C set is $\mathcal{J}$PC set.
e. Arbitrary union of $\mathcal{J}$P*O sets is $\mathcal{J}$P*O set.
f. Intersection of $\mathcal{J}$P*C sets is $\mathcal{J}$P*C set.

Theorem 2.10 [1] If (X_J, τ_I) is an $\mathcal{J}$TS and M_J and N_J are $\mathcal{J}$S's in X_J then the followings are hold.

a. $\mathcal{J}\text{int}(\phi_I) = \phi_I$ and $\mathcal{J}\text{int}(X_I) = X_I$.
b. M_J is an $\mathcal{J}$OS iff $M_J = \mathcal{J}\text{int}(M_J)$.
c. $M_J \subseteq N_J \Rightarrow \mathcal{J}\text{int}(M_J) \subseteq \mathcal{J}\text{int}(N_J)$.
d. $\mathcal{J}\text{int}(M_J \cap N_J) = \mathcal{J}\text{int}(M_J) \cap \mathcal{J}\text{int}(N_J)$.
e. $\mathcal{J}\text{int}(M_J \cup N_J) \supseteq \mathcal{J}\text{int}(M_J) \cup \mathcal{J}\text{int}(N_J)$.
f. $\mathcal{J}\text{cl}(\phi_I) = \phi_I$ and $\mathcal{J}\text{cl}(X_I) = X_I$.
g. M_J is an $\mathcal{J}$CS iff $M_J = \mathcal{J}\text{cl}(M_J)$.
h. $M_J \subseteq N_J \Rightarrow \mathcal{J}\text{cl}(M_J) \subseteq \mathcal{J}\text{cl}(N_J)$.
i. $\mathcal{J}\text{cl}(M_J \cap N_J) \subseteq \mathcal{J}\text{cl}(M_J) \cap \mathcal{J}\text{cl}(N_J)$.
j. $\mathcal{J}\text{cl}(M_J \cup N_J) = \mathcal{J}\text{cl}(M_J) \cup \mathcal{J}\text{cl}(N_J)$.

Theorem 2.11 [2] If (X_J,τ_I) is an $\mathcal{J}$TS and M_J, N_J are $\mathcal{J}$S's in X_J then the followings are hold.

a. $\mathcal{J}int^*(\phi_I) = \phi_I$ and $\mathcal{J}int^*(X_I) = X_I$.
b. If M_J is $\mathcal{J}$g- OS then $M_J = \mathcal{J}int^*(M_J)$.
c. $\mathcal{J}int(M_J) \subseteq \mathcal{J}int^*(M_J) \subseteq M_J$.
d. $M_J \subseteq N_J \Rightarrow \mathcal{J}int^*(M_J) \subseteq \mathcal{J}int^*(N_J)$.
e. $\mathcal{J}int^*(M_J \cap N_J) \subseteq \mathcal{J}int^*(M_J) \cap \mathcal{J}int^*(N_J)$.
f. $\mathcal{J}int^*(M_J \cup N_J) \supseteq \mathcal{J}int^*(M_J) \cup \mathcal{J}int^*(N_J)$.
g. $\mathcal{J}cl^*(\phi_I) = \phi_I$ and $\mathcal{J}cl^*(X_I) = X_I$.
h. If M_J is $\mathcal{J}$g- CS then $M_J = \mathcal{J}cl^*(M_J)$.
i. $M_J \subseteq \mathcal{J}cl^*(M_J) \subseteq \mathcal{J}cl(M_J)$.
j. $M_J \subseteq N_J \Rightarrow \mathcal{J}cl^*(M_J) \subseteq \mathcal{J}cl^*(N_J)$.
k. $\mathcal{J}cl^*(M_J \cap N_J) \subseteq \mathcal{J}cl^*(M_J) \cap \mathcal{J}cl^*(N_J)$.
l. $\mathcal{J}cl^*(M_J \cup N_J) \supseteq \mathcal{J}cl^*(M_J) \cup \mathcal{J}cl^*(N_J)$.

Theorem 2.12 [5] If (X_J,τ_I) is an $\mathcal{J}$TS and M_J, N_J be a $\mathcal{J}$S's in X_J then the followings are hold.

a. $\mathcal{J}P^*int(\phi_I) = \phi_I$ and $\mathcal{J}P^*int(X_I) = X_I$.
b. M_J is an $\mathcal{J}$P*OS iff $M_J = \mathcal{J}P^*int(M_J)$.
c. $M_J \subseteq N_J \Rightarrow \mathcal{J}P^*int(M_J) \subseteq \mathcal{J}P^*int(N_J)$.
d. $\mathcal{J}P^*int(M_J \cap N_J) = \mathcal{J}P^*int(M_J) \cap \mathcal{J}P^*int(N_J)$.
e. $\mathcal{J}P^*int(M_J \cup N_J) \supseteq \mathcal{J}P^*int(M_J) \cup \mathcal{J}P^*int(N_J)$.
f. $\mathcal{J}P^*cl(\phi_I) = \phi_I$ and $\mathcal{J}P^*cl(X_I) = X_I$.
g. M_J is an $\mathcal{J}$P*CS iff $M_J = \mathcal{J}P^*cl(M_J)$.
h. $M_J \subseteq N_J \Rightarrow \mathcal{J}P^*cl(M_J) \subseteq \mathcal{J}P^*cl(N_J)$.
i. $\mathcal{J}P^*cl(M_J \cap N_J) \subseteq \mathcal{J}P^*cl(M_J) \cap \mathcal{J}P^*cl(N_J)$.
j. $\mathcal{J}P^*cl(M_J \cup N_J) = \mathcal{J}P^*cl(M_J) \cup \mathcal{J}P^*cl(N_J)$.

Theorem 2.13 [1,2,5] If (X_J,τ_{IT}) is an $\mathcal{J}$TS and M_J is a $\mathcal{J}$S of X_J then,

a. $\mathcal{J}int(M_J^c) = [\mathcal{J}cl(M_J)]^c$ and $\mathcal{J}cl(M_J^c) = [\mathcal{J}int(M_J)]^c$.
b. $\mathcal{J}int^*(M_J^c) = [\mathcal{J}cl^*(M_J)]^c$ and $\mathcal{J}cl^*(M_J^c) = [\mathcal{J}int^*(M_J)]^c$.
c. $\mathcal{J}P^*int(M_J^c) = [\mathcal{J}P^*cl(M_J)]^c$ and $\mathcal{J}P^*cl(M_J^c) = [\mathcal{J}P^*int(M_J)]^c$.

Definition 2.14[1] Let $f_J : X_J \to Y_J$ is said to be

a. $\mathcal{J}$- continuous if $f_J^{-1}(V)$ is $\mathcal{J}$OS in X_J for each $\mathcal{J}$OS V in Y_J.
b. $\mathcal{J}$P- continuous if $f_J^{-1}(V)$ is $\mathcal{J}$POS in X_J for each $\mathcal{J}$OS V in Y_J.
c. Contra $\mathcal{J}$- continuous if $f_J^{-1}(V)$ is $\mathcal{J}$CS in X_J for each $\mathcal{J}$OS V in Y_J.
d. Contra $\mathcal{J}$P- continuous if $f_J^{-1}(V)$ is $\mathcal{J}$PCS in X_J for each $\mathcal{J}$OS V in Y_J.

Theorem 2.15[1] Every constant map is $\mathcal{J}$- continuous map.

Intuitionistic Pre * Continuous Maps

Definition 3.1 A map f_J from $\mathcal{J}$TS (X_J,τ_{IT}) into another $\mathcal{J}$TS (Y_J,σ_{IT}) is Called Intuitionistic Pre * Continuous at a point $m_{xj} \in X_J$ if for every $\mathcal{J}$OS N_J of Y_J contains $f_J(m_{xj})$, there exists a $\mathcal{J}$P*O set M_J in X_J such that $m_{xj} \in M_J$ and $f_J(M_J) \subseteq N_J$.

Definition 3.2 A map f_J from $\mathcal{J}$TS (X_J,τ_{IT}) into another $\mathcal{J}$TS (Y_J,σ_{IT}) is Called Intuitionistic Pre * Continuous (In short $\mathcal{J}$P*- continuous) Map if $f_J^{-1}(M_J)$ is $\mathcal{J}$P*O in X_J for every $\mathcal{J}$OS M_J in Y_J.

Example 3.3 Take $X_J = \{a_{xj},b_{xj},c_{xj}\}$ and $Y_J = \{a_{yj},b_{yj},c_{yj}\}$. Consider the $\mathcal{J}$T's $\tau_{IT} = \{X_I,\phi_I,< X_J, \{b_{xj}\},\{a_{xj},c_{xj}\}>,<X_J,\{a_{xj},b_{xj}\},\{c_{xj}\}>\}$ and $\sigma_{IT} = \{Y_I,\phi_I,< Y_J,\{a_{yj}\},\{b_{yj},c_{yj}\}>,<Y_J,\{a_{yj},b_{yj}\},\{c_{yj}\}>\}$ then $\mathcal{J}P^*O(X_J) = \tau_{IT}$ and $\mathcal{J}P^*O(Y_J) = \sigma_{IT}$. Let $f_J : (X_J,\tau_{IT}) \to (Y_J,\sigma_{IT})$ be a map defined by, $f_J(a_{xj}) = b_{yj}$, $f_J(b_{xj}) = a_{yj}$, $f_J(c_{xj}) = c_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<Y_J,\{a_{yj}\},\{b_{yj},c_{yj}\}>) = <X_J,\{b_{xj}\},\{a_{xj},c_{xj}\}>$, $f_J^{-1}(<Y_J,\{a_{yj},b_{yj}\},\{c_{yj}\}>) = <X_J,\{a_{xj},b_{xj}\},\{c_{xj}\}>$ are $\mathcal{J}$P*O sets in X_J. Therefore f_J is $\mathcal{J}$P*- continuous.

Theorem 3.4 A map $f_J : (X_J,\tau_{IT}) \to (Y_J,\sigma_{IT})$ is $\mathcal{J}$P*- Continuous iff each $\mathcal{J}$C set in Y_J has an inverse image that is IP*CS in X_J.

Theorem 3.5 Every $\mathcal{J}$- continuous map is $\mathcal{J}$P*- continuous.

Proof: Suppose a map $f_J : (X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ is $\mathcal{J}$- continuous then $f_J^{-1}(M_J)$ is $\mathcal{J}O$ of X_j for every $\mathcal{J}OS$ M_J in Y_J. Therefore, $f_J^{-1}(M_J)$ is $\mathcal{J}P^*O$ in X_j for each $\mathcal{J}O$ M_J in Y_J. Hence, f_J is $\mathcal{J}P^*$- continuous map. According to the example below, the reverse part of the aforementioned theorem need not be true.

Example 3.6 Take $X_j = \{a_{xj},b_{xj},c_{xj}\}$ and $Y_J = \{a_{yj},b_{yj},c_{yj}\}$. Consider the $\mathcal{J}T$'s $\tau_{IT} = \{X_I,\phi_I, <X_j,\{a_{xj}\},\{b_{xj}\}>,<X_j,\{b_{xj}\},\{c_{xj}\}>,<X_j,\{a_{xj},b_{xj}\},\phi>,<X_j,\phi,\{b_{xj},c_{xj}\}>\}$ and $\sigma_{IT} = \{Y_I,\phi_I,< Y_J,\{a_{yj}\}, \{b_{yj},c_{yj}\}>\}$ then $\mathcal{J}P^*O(X_j) = \{X_I,\phi_I,< X_j,\{a_{xj}\},\{b_{xj}\}>,<X_j,\{a_{xj}\},\{b_{xj},c_{xj}\}>,<X_j,\{b_{xj}\},\{c_{xj}\}>, <X_j,\{a_{xj},b_{xj}\},\{c_{xj}\}>,<X_j,\{a_{xj},b_{xj}\},\phi>,<X_j,\phi,\{b_{xj},c_{xj}\}>\}$. Let $f_J : (X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ be a map defined by, $f_J(a_{xj}) = a_{yj}$, $f_J(b_{xj}) = c_{yj}$, $f_J(c_{xj}) = b_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<Y_J,\{a_{yj}\},\{b_{yj},c_{yj}\}>) = <X_j,\{a_{xj}\}, \{b_{xj},c_{xj}\}>$ are $\mathcal{J}P^*O$ sets in X_j. Therefore f_J is $\mathcal{J}P^*$- continuous map but $f_J^{-1}(<Y_J,\{a_{yj}\},\{b_{yj},c_{yj}\}>) \notin \tau_{IT}$. Therefore, f_J is not a $\mathcal{J}$-continuous map.

Theorem 3.7 Every constant map is $\mathcal{J}P^*$- continuous map.

Proof: By theorem – 3.5 and 2.16, the results is obvious.

Theorem 3.8 Every $\mathcal{J}P^*$- continuous is $\mathcal{J}P$- continuous.

Proof: Suppose $f_J :(X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ is $\mathcal{J}P^*$- continuous. Then $f_J^{-1}(M_J)$ is $\mathcal{J}P^*OS$ in X_j for every $\mathcal{J}OS$ M_J in Y_J. Therefore, $f_J^{-1}(M_J)$ is $\mathcal{J}PO$ set in X_j for each $\mathcal{J}O$ M_J in Y_J. Hence, f_J is $\mathcal{J}P$- continuous map.
According to the example below, the reverse part of the aforementioned theorem need not be true.

Example 3.9 Take $X_j = \{a_{xj},b_{xj},c_{xj}\}$ and $Y_J = \{a_{yj},b_{yj},c_{yj}\}$. Consider the $\mathcal{J}T$''s $\tau_{IT} = \{X_I,\phi_I, <X_j,\{a_{xj}\},\{c_{xj}\}>,<X_j,\{c_{xj}\},\{a_{xj},b_{xj}\}>,<X_j,\{a_{xj},c_{xj}\},\phi>\}$ and $\sigma_{IT} = \{Y_I,\phi_I,<Y_J,\{a_{yj}\},\{b_{yj}\}>\}$ then $\mathcal{J}P^*O(X_j) = \{X_I,\phi_I,<X_j,\{a_{xj}\},\{c_{xj}\}>,<X_j,\{a_{xj}\},\{b_{xj},c_{xj}\}>,<X_j,\{c_{xj}\},\{a_{xj},b_{xj}\}>,<X_j,\{a_{xj},c_{xj}\},\{b_{xj}\}>, <X_j,\{a_{xj}\},\phi>,<X_j,\{a_{xj},c_{xj}\},\phi>\}$ and $\mathcal{J}PO(X_j) = \{X_I,\phi_I,< X_j,\phi,\phi>,<X_j,\phi,\{b_{xj}\}>,<X_j,\phi,\{c_{xj}\}>, <X_j,\{b_{xj}\},\{a_{xj}\}>,<X_j,\{c_{xj}\},\{b_{xj}\}>,<X_j,\phi,\{a_{xj},b_{xj}\}>,<X_j,\phi,\{b_{xj},c_{xj}\}>,<X_j,\{a_{xj}\},\{b_{xj}\}>,<X_j,\{a_{xj}\},\{c_{xj}\}>,<X_j,\{b_{xj},c_{xj}\},\{a_{xj}\}>,<X_j,\{a_{xj}\},\phi>,<X_j,\{b_{xj}\},\phi>,<X_j,\{a_{xj}\},\{b_{xj},c_{xj}\}>,<X_j,\{c_{xj}\},\phi>,<X_j,\{c_{xj}\},\{a_{xj},b_{xj}\}>,<X_j,\{a_{xj},c_{xj}\},\{b_{xj}\}>,<X_j,\{a_{xj},b_{xj}\},\phi>, <X_j,\{b_{xj},c_{xj}\},\phi>,<X_j,\{a_{xj},c_{xj}\},\phi>\}$. Define a map $f_J : (X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ by, $f_J(a_{xj}) = b_{yj}$, $f_J(b_{xj}) = c_{yj}$, $f_J(c_{xj}) = a_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<Y_J,\{a_{yj}\},\{b_{yj}\}>) = <X_j,\{c_{xj}\},\{a_{xj}\}>$ are $\mathcal{J}PO$ sets in X_j. Therefore f_J is $\mathcal{J}P$- continuous map but $f_J^{-1}(<Y_J,\{a_{yj}\},\{b_{yj}\}>) \notin \mathcal{J}P^*O(X_j)$. Therefore, f_J is not a $\mathcal{J}P^*$- continuous.

Remark 3.10 The link between $\mathcal{J}P^*$- continuous map, $\mathcal{J}$- continuous and $\mathcal{J}P$ - continuous maps is depicted in the following diagram(Fig:1).

Theorem 3.11 Assume that $f_J : (X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ is a map, then the followings are equivalent.

a. f_J is an $\mathcal{J}P^*$- continuous.
b. f_J is an $\mathcal{J}P^*$- continuous at every intuitionistic point of X_j.
c. $f_J^{-1}(A_J)$ is $\mathcal{J}P^*CS$ in X_j for each $\mathcal{J}CS$ A_J in Y_J.

Proof: (a) ⇒ (b), Let f_J be $\mathcal{J}P^*$- continuous map. Let m_{xj} be any $\mathcal{J}P$ in X_j and ω_J be an $\mathcal{J}O$ set in Y_J containing $f_J(m_{xj})$ then $m_{xj} \in f_J^{-1}(\omega_J)$. Therefore, $U_J = f_J^{-1}(\omega_J)$ is $\mathcal{J}P^*O$ in X_j contains a point m_{xj} such that $f_J(U_J) \subseteq \omega_J$. Hence f_J is $\mathcal{J}P^*$-continuous at every $\mathcal{J}P$ of X_j.
(b) ⇒ (c), Let A_J be a $\mathcal{J}CS$ in Y_J then A_J^c is $\mathcal{J}OS$ in Y_J. Take $m_{xj} \in f_J^{-1}(A_J^c)$ then $f(m_{xj}) \in A_J^c$. By hypothesis, there exist a $\mathcal{J}P^*OS$ ω_J in X_j contains a point m_{xj} such that $f_J(m_{xj}) \in f_J(\omega_J) \subseteq A_J^c$. Therefore $\omega_J \subseteq f_J^{-1}(A_J^c)$. Hence $f_J^{-1}(A_J^c) = \cup \{\omega_J : m_{xj} \in f_J^{-1}(A_J^c)\}$ is $\mathcal{J}P^*O$ set in X_j. Thus $f_J^{-1}(A_J) = f_J^{-1}[(A_J^c)^c] = [f_J^{-1}(A_J^c)]^c$ is $\mathcal{J}P^*CS$ in X_j for each $\mathcal{J}CS$ A_J in Y_J.
(c) ⇒ (a) Suppose, $f_J^{-1}(A_J)$ is $\mathcal{J}P^*C$ in X_j for each $\mathcal{J}C$ A_J in Y_J. Then by theorem – 3.4, f_J is $\mathcal{J}P^*$- continuous map.

Theorem – 3.12 Assume that $f_J : (X_j,\tau_{IT}) \to (Y_J,\sigma_{IT})$ is a map, then the followings are equivalent.

a. f_J is $\mathcal{J}P^*$- continuous map.
b. $f_J^{-1}(\mathcal{J}int(A_J)) \subseteq \mathcal{J}P^*int(f_J^{-1}(A_J))$ for each $\mathcal{J}S$ A_J of Y_J.
c. $\mathcal{J}int(f_J(B_J)) \subseteq f_J(\mathcal{J}P^*int(B_J))$ for every $\mathcal{J}S$ B_J of X_j.

Proof: (a) ⇒ (b), Let f_J be $\mathcal{J}P^*$- continuous map. Let A_J be any $\mathcal{J}S$ of $\grave{Y}_J$ then $\mathcal{J}int(A_J)$ is $\mathcal{J}O$ set. Therefore, $f_J^{-1}(\mathcal{J}int(A_J))$ is $\mathcal{J}P^*O$. Hence, $f_J^{-1}(\mathcal{J}int(A_J)) = \mathcal{J}P^*int(f_J^{-1}(\mathcal{J}int(A_J)))\subseteq\mathcal{J}P^*int(f_J^{-1}(A_J))$.
(b) ⇒ (c), Let B_J be any $\mathcal{J}S$ of $\hat{X}_J$ then $f_J(B_J)$ is $\mathcal{J}S$ of $\grave{Y}_J$. By our assumption, $f_J^{-1}(\mathcal{J}int(f_J(B_J))) \subseteq \mathcal{J}P^*int(f_J^{-1}(f_J(B_J)))$. Hence, $\mathcal{J}int(f_J(B_J)) \subseteq f_J(\mathcal{J}P^*int(B_J))$.
(c) ⇒ (a), Let G_J be any $\mathcal{J}O$ set of $\grave{Y}_J$ then $f_J^{-1}(G_J)$ is $\mathcal{J}S$ of $\hat{X}_J$. By (c), $\mathcal{J}int(f_J(f_J^{-1}(G_J))) \subseteq f_J(\mathcal{J}P^*int(f_J^{-1}(G_J)))$. (i.e) $f_J^{-1}(\mathcal{J}int(G_J)) \subseteq \mathcal{J}P^*int(f_J^{-1}(G_J))$. Since G_J is $\mathcal{J}O$ set then $f_J^{-1}(G_J) \subseteq \mathcal{J}P^*int(f_J^{-1}(G_J))$. Also, $\mathcal{J}P^*int(f_J^{-1}(G_J)) \subseteq f_J^{-1}(G_J)$. Therefore, $f_J^{-1}(G_J) \subseteq \mathcal{J}P^*int(f_J^{-1}(G_J))$. (i.e) $f_J^{-1}(G_J)$ is $\mathcal{J}P^*O$ set in $\hat{X}_J$. Hence f_J is $\mathcal{J}P^*$- continuous map.

Theorem – 3.13 Let $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ be a map, then the followings are equivalent.
a. f_J is an $\mathcal{J}P^*$- continuous map.
b. $\mathcal{J}P^*cl(f_J^{-1}(A)) \subseteq f_J^{-1}(\mathcal{J}cl(A_J))$ for each $\mathcal{J}S$ A_J of $\grave{Y}_J$.
c. $f_J(\mathcal{J}P^*cl(B)) \subseteq \mathcal{J}cl(f_J(B_J))$ for every $\mathcal{J}S$ B_J of $\hat{X}_J$.

Proof: It is similar to theorem – 3.12.

Theorem – 3.14 If $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ and $g_J : (\grave{Y}_J,\sigma_{IT}) \to (\hat{Z}_J,\mu_{IT})$ are $\mathcal{J}$- continuous then $g_J\circ f_J : (\hat{X}_J,\tau_{IT}) \to (\hat{Z}_J,\mu_{IT})$ is $\mathcal{J}P^*$- continuous map.

Proof: Suppose $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ and $g_J : (\grave{Y}_J,\sigma_{IT}) \to (\hat{Z}_J,\mu_{IT})$ are $\mathcal{J}$- continuous. Let ω_J be an $\mathcal{J}OS$ in $\hat{Z}_J$ then $g_J^{-1}(\omega_J)$ is $\mathcal{J}OS$ in $\grave{Y}_J$. Therefore $f_J^{-1}(g_J^{-1}(\omega_J))$ is $\mathcal{J}OS$ in $\hat{X}_J$. Therefore $f_J^{-1}(g_J^{-1}(\omega_J)) = (g_J\circ f_J)^{-1}(\omega_J)$ is $\mathcal{J}P^*OS$ in $\hat{X}_J$. Therfore, $g_J\circ f_J : (\hat{X}_J,\tau_{IT}) \to (\hat{Z}_J,\mu_{IT})$ is $\mathcal{J}P^*$- continuous.

Theorem – 3.15 If $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ is $\mathcal{J}P^*$- continuous and $g_J : (\grave{Y}_J,\sigma_{IT}) \to (\hat{Z}_J,\mu_{IT})$ is $\mathcal{J}$- continuous then
a. $g_J\circ f_J : (\hat{X}_J,\tau_{IT}) \to (\hat{Z}_J,\mu_{IT})$ is $\mathcal{J}P^*$- continuous.
b. $g_J\circ f_J : (\hat{X}_J,\tau_{IT}) \to (\hat{Z}_J,\mu_{IT})$ is $\mathcal{J}P$- continuous.

Proof: (a) Suppose ω_J is a $\mathcal{J}OS$ in $\hat{Z}_J$ then $g_J^{-1}(\omega_J)$ is $\mathcal{J}OS$ in $\grave{Y}_J$. Therefore $f_J^{-1}(g_J^{-1}(\omega_J)) = (g_J\circ f_J)^{-1}(\omega_J)$ is $\mathcal{J}P^*O$ in $\hat{X}_J$. Hence, $g_J\circ f_J$ is $\mathcal{J}P^*$- continuous map. (b) By 3.8, $g_J\circ f_J$ is $\mathcal{J}P$- continuous.

Contra Intuitionistic Pre * Continuous Maps
Definition 4.1 A map f_J from $\mathcal{J}TS$ $(\hat{X}_J,\tau_{IT})$ into another $\mathcal{J}TS$ $(\grave{Y}_J,\sigma_{IT})$ is said to be Contra Intuitionistic Pre * Continuous Map if $f_J^{-1}(W_J)$ is $\mathcal{J}P^*CS$ in $\hat{X}_J$ for every $\mathcal{J}OS$ W_J in $\grave{Y}_J$.

Example 4.2 Take $\hat{X}_J = \{a_{xj},b_{xj},c_{xj}\}$ and $\grave{Y}_J = \{a_{yj},b_{yj},c_{yj}\}$. Consider the $\mathcal{J}T$'s $\tau_{IT} = \{X_I,\phi_I,<\hat{X}_J,\{a_{xj}\},\{b_{xj},c_{xj}\}>,<\hat{X}_J,\{b_{xj}\},\{a_{xj},c_{xj}\}>,<\hat{X}_J,\{a_{xj},b_{xj}\},\{c_{xj}\}>\}$ and $\sigma_{IT} = \{Y_I,\phi_I,<\grave{Y}_J, \{a_{yj},b_{yj}\},\{c_{yj}\}>\}$ then $\mathcal{J}P^*C(\hat{X}_J) = \{X_I,\phi_I,<\hat{X}_J,\{b_{xj},c_{xj}\},\{a_{xj}\}>,<\hat{X}_J,\{a_{xj},c_{xj}\},\{b_{xj}\}>,<\hat{X}_J,\{c_{xj}\}, \{a_{xj},b_{xj}\}>\}$. Define a map $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ by, $f_J(a_{xj}) = b_{yj}$, $f_J(b_{xj}) = c_{yj}$, $f_J(c_{xj}) = a_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$ and $f_J^{-1}(<\grave{Y}_J,\{a_{yj},b_{yj}\},\{c_{yj}\}>) = <\hat{X}_J,\{a_{xj},c_{xj}\},\{b_{xj}\}>$ are $\mathcal{J}P^*C$ sets in $\hat{X}_J$. Therefore f_J is Contra $\mathcal{J}P^*$ Continuous Map.

Theorem 4.3 A map $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ is Contra $\mathcal{J}P^*$- Continuous iff each $\mathcal{J}CS$ in $\grave{Y}_J$ has an inverse image that is IP*OS in $\hat{X}_J$.
A map $f_J : (\hat{X}_J,\tau_{IT}) \to (\grave{Y}_J,\sigma_{IT})$ is Contra $\mathcal{J}P^*$ Continuous Map iff the inverse image of every $\mathcal{J}CS$ in $\grave{Y}_J$ is $\mathcal{J}P^*OS$ in $\hat{X}_J$.

Theorem – 4.4 Every Contra $\mathcal{J}$- continuous is Contra $\mathcal{J}P^*$- continuous.

Proof: Suppose a map f_J is Contra $\mathcal{J}$- continuous map then $f_J^{-1}(W_J)$ is $\mathcal{J}CS$ in $\hat{X}_J$ for every $\mathcal{J}OS$ W_J in $\grave{Y}_J$. Therefore $f_J^{-1}(W_J)$ is $\mathcal{J}P^*CS$ in $\hat{X}_J$ for every $\mathcal{J}OS$ W_J in $\grave{Y}_J$. Hence f_J is Contra $\mathcal{J}P^*$- continuous map.
According to the example below, the reverse part of the aforementioned theorem need not be true.

Example 4.5 Take $X_J = \{a_{xj}, b_{xj}\}$ and $\grave{Y}_J = \{1_{yj}, 2_{yj}\}$. Consider the IT"s $\tau_{IT} = \{X_I, \phi_I, <X_J, \{a_{xj}\}, \phi>\}$ and $\sigma_{IT} = \{Y_I, \phi_I, <\grave{Y}_J, \{1_{yj}\}, \{2_{yj}\}>\}$ then $\mathcal{J}P^*O(X_J) = \{X_I, \phi_I, <X_J, \{a_{xj}\}, \phi>, <X_J, \{a_{xj}\}, \{b_{xj}\}>\}$ and $\mathcal{J}P^*C(X_J) = \{X_I, \phi_I, <X_J, \phi, \{a_{xj}\}>, <X_J, \{b_{xj}\}, \{a_{xj}\}>\}$. Define a map $f_J : (X_J, \tau_{IT}) \rightarrow (\grave{Y}_J, \sigma_{IT})$ by, $f_J(a_{xj}) = 2_{yj}$, $f_J(b_{xj}) = 1_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<\grave{Y}_J, \{1_{yj}\}, \{2_{yj}\}>) = <X_J, \{b_{xj}\}, \{a_{xj}\}>$ are $\mathcal{J}P^*C$ sets in X_J. Therefore f_J is Contra $\mathcal{J}P^*$- continuous map but $f_J^{-1}(<\grave{Y}_J, \{1_{yj}\}, \{2_{yj}\}>) \notin \mathcal{J}(X_J)$. Therefore, f_J is not a Contra $\mathcal{J}$- continuous map.

Theorem 4.6 Every Contra $\mathcal{J}P^*$- continuous is Contra $\mathcal{J}P$- continuous.

Proof: Suppose a map f_J is Contra $\mathcal{J}P^*$- continuous map then $f_J^{-1}(W_J)$ is $\mathcal{J}P^*CS$ in X_J for every $\mathcal{J}OS$ W_J in $\grave{Y}_J$. Therefore $f_J^{-1}(W_J)$ is $\mathcal{J}PCS$ in X_J for every $\mathcal{J}OS$ W_J in $\grave{Y}_J$. Hence f_J is Contra $\mathcal{J}P$- continuous map. According to the example below, the reverse part of the aforementioned theorem need not be true.

Example 4.7 Take $X_J = \{a_{xj}, b_{xj}, c_{xj}\}$ and $\grave{Y}_J = \{a_{yj}, b_{yj}, c_{yj}\}$. Consider the IT"s $\tau_{IT} = \{X_I, \phi_I, <X_J, \phi, \{a_{xj}, b_{xj}\}>, <X_J, \{a_{xj}\}, \{c_{xj}\}>, <X_J, \{a_{xj}\}, \phi>\}$ and $\sigma_{IT} = \{Y_I, \phi_I, <\grave{Y}_J, \{a_{yj}\}, \{b_{yj}, c_{yj}\}>\}$ then $\mathcal{J}P^*C(X_J) = \{X_I, \phi_I, <X_J, \{c_{xj}\}, \{a_{xj}\}>, <X_J, \phi, \{a_{xj}\}>, <X_J, \{a_{xj}, b_{xj}\}, \phi>\}$ and $\mathcal{J}PC(X_J) = \{X_I, \phi_I, <X_J, \phi, \phi>, <X_J, \phi, \{a_{xj}\}>, <X_J, \phi, \{c_{xj}\}>, <X_J, \{b_{xj}\}, \{a_{xj}\}>, <X_J, \{c_{xj}\}, \{a_{xj}\}>, <X_J, \phi, \{a_{xj}, c_{xj}\}>, <X_J, \phi, \{b_{xj}, c_{xj}\}>, <X_J, \{b_{xj}\}, \{c_{xj}\}>, <X_J, \{b_{xj}, c_{xj}\}, \{a_{xj}\}>, <X_J, \{b_{xj}\}, \phi>, <X_J, \{a_{xj}\}, \{b_{xj}, c_{xj}\}>, <X_J, \{c_{xj}\}, \phi>, <X_J, \{b_{xj}\}, \{a_{xj}, c_{xj}\}>, <X_J, \{a_{xj}, b_{xj}\}, \phi>, <X_J, \{b_{xj}, c_{xj}\}, \phi>\}$. Define a map $f_J : (X_J, \tau_{IT}) \rightarrow (\grave{Y}_J, \sigma_{IT})$ by, $f_J(a_{xj}) = a_{yj}$, $f_J(b_{xj}) = b_{yj}$, $f_J(c_{xj}) = c_{yj}$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<\grave{Y}_J, \{a_{yj}\}, \{b_{yj}, c_{yj}\}>) = <X_J, \{a_{xj}\}, \{b_{xj}, c_{xj}\}>$ are $\mathcal{J}PC$ sets in X_J. Therefore f_J is Contra $\mathcal{J}P$- continuous map but $f_J^{-1}(<\grave{Y}_J, \{a_{yj}\}, \{b_{yj}, c_{yj}\}>) \notin \mathcal{J}P^*C(X_J)$. Therefore f_J is not a Contra $\mathcal{J}P^*$- continuous map.

Theorem 4.8 If $f_J : (X_J, \tau_{IT}) \rightarrow (\grave{Y}_J, \sigma_{IT})$ is Contra $\mathcal{J}P^*$- continuous and $g_J : (\grave{Y}_J, \sigma_{IT}) \rightarrow (Z_J, \mu_{IT})$ is $\mathcal{J}$- continuous map then

a. $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z_J, \mu_{IT})$ is Contra $\mathcal{J}P^*$- continuous.
b. $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z_J, \mu_{IT})$ is Contra $\mathcal{J}P$- continuous.

Proof: (a) Suppose ω_J be $\mathcal{J}OS$ in Z_J then $g_J^{-1}(\omega_J)$ is $\mathcal{J}OS$ in $\grave{Y}_J$. Therefore $f_J^{-1}(g_J^{-1}(\omega_J)) = (g_J \circ f_J)^{-1}(\omega_J)$ is $\mathcal{J}P^*CS$ in X_J. Therefore, $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z_J, \mu_{IT})$ is Contra $\mathcal{J}P^*$- continuous map. (b) By theorem – 5.4, $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z_J, \mu_{IT})$ is Contra $\mathcal{J}P$- continuous map.

Remark 4.9 If $f_J : (X_J, \tau_{IT}) \rightarrow (\grave{Y}_J, \sigma_{IT})$ and $g_J : (\grave{Y}_J, \sigma_{IT}) \rightarrow (Z_J, \mu_{IT})$ are Contra $\mathcal{J}P^*$- continuous but $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z_J, \mu_{IT})$ is no need to Contra $\mathcal{J}P^*$- continuous.

Example 4.10 Let $X_J = \{a_{xj}, b_{xj}, c_{xj}\}$, $\grave{Y}_J = \{a_{yj}, b_{yj}, c_{yj}\}$ and $Z_J = \{a_{zj}, b_{zj}, c_{zj}\}$. Consider the IT"s $\tau_{IT} = \{X_I, \phi_I, <X_J, \{a_{xj}\}, \{b_{xj}, c_{xj}\}>, <X_J, \{b_{xj}\}, \{a_{xj}, c_{xj}\}>, <X_J, \{a_{xj}, b_{xj}\}, \{c_{xj}\}>\}$, $\sigma_{IT} = \{Y_I, \phi_I, <\grave{Y}_J, \{a_{yj}\}, \{b_{yj}, c_{yj}\}>\}$ and $\mu_{IT} = \{Z_I, \phi_I, <Z_J, \{a_{zj}, c_{zj}\}, \{b_{zj}\}>\}$ then $\mathcal{J}P^*C(X_J) = \{X_I, \phi_I, <X_J, \{b_{xj}, c_{xj}\}, \{a_{xj}\}>, <X_J, \{a_{xj}, c_{xj}\}, \{b_{xj}\}>, <X_J, \{c_{xj}\}, \{a_{xj}, b_{xj}\}>\}$ and $\mathcal{J}P^*C(\grave{Y}_J) = \{Y_I, \phi_I, <\grave{Y}_J, \{b_{yj}, c_{yj}\}, \{a_{yj}\}>\}$. Define a maps $f_J : (X_J, \tau_{IT}) \rightarrow (\grave{Y}_J, \sigma_{IT})$ by $f_J(a_{xj}) = c_{yj}$, $f_J(b_{xj}) = b_{yj}$, $f_J(c_{xj}) = a_{yj}$ and $g_J : (\grave{Y}_J, \sigma_{IT}) \rightarrow (Z_J, \mu_{IT})$ by $g_J(a_{yj}) = b_{zj}$, $g_J(b_{yj}) = a_{zj}$, $g_J(c_{yj}) = c_{zj}$. Therefore $g_J \circ f_J : (X_J, \tau_{IT}) \rightarrow (Z, \mu_{IT})$. Here, $f_J^{-1}(Y_I) = X_I$, $f_J^{-1}(\phi_I) = \phi_I$, $f_J^{-1}(<\grave{Y}_J, \{a_{yj}\}, \{b_{yj}, c_{yj}\}>) = <X_J, \{c_{xj}\}, \{a_{xj}, b_{xj}\}>$ are $\mathcal{J}P^*C$ sets in X_J. Therefore f_J is Contra $\mathcal{J}P^*$- continuous map and $g_J^{-1}(Z_I) = Y_I$, $g_J^{-1}(\phi_I) = \phi_I$, $g_J^{-1}(<Z, \{a_{zj}, c_{zj}\}, \{b_{zj}\}>) = <\grave{Y}_J, \{b_{yj}, c_{yj}\}, \{a_{yj}\}>$ are $\mathcal{J}P^*C$ sets in $\grave{Y}_J$. Therefore g_J is Contra $\mathcal{J}P^*$- continuous map but $(g_J \circ f_J)^{-1}(<Z_J, \{a_{zj}, c_{zj}\}, \{b_{zj}\}>) = f_J^{-1}(g_J^{-1}(<Z_J, \{a_{zj}, c_{zj}\}, \{b_{zj}\}>) = f_J^{-1}(<\grave{Y}_J, \{b_{yj}, c_{yj}\}, \{a_{yj}\}>) = <X_J, \{a_{xj}, b_{xj}\}, \{c_{xj}\}> \notin \mathcal{J}P^*C(X_J)$. Therefore $g_J \circ f_J$ is not a Contra $\mathcal{J}P^*$- continuous map.

CONCLUSIONS

We discussed the $\mathcal{J}P^*$- Continuous maps and Contra $\mathcal{J}P^*$- Continuous maps in this paper. We intend to conduct research in the future on Quasi $\mathcal{J}P^*$- Continuous maps, Perfectly $\mathcal{J}P^*$- Continuous maps, Totally $\mathcal{J}P^*$- Continuous maps, Strongly $\mathcal{J}P^*$- Continuous maps and so on.

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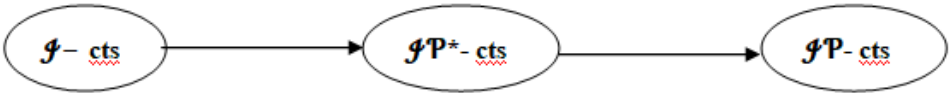


Fig.1: The link between $\mathcal{J}$P*- continuous map,$\mathcal{J}$- continuous and $\mathcal{J}$P- continuous maps

REVIEW ARTICLE

Nanocomposite for Photocatalytic Degradation of Antibiotics

Ankita Mishra[1] and Anurag Jyoti[2]*

[1]M.Sc. Student, Amity Institute of Biotechnology, Amity University, Madhya Pradesh, Gwalior, Madhya Pradesh, India.
[2]Associate Professor, Amity Institute of Biotechnology, Amity University, Madhya Pradesh, Gwalior, Madhya Pradesh, India.

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***Address for Correspondence**
Anurag Jyoti
Associate Professor,
Amity Institute of Biotechnology,
Amity University, Madhya Pradesh, Gwalior,
Madhya Pradesh, India.
E.Mail: ajyoti@gwa.amity.edu

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ABSTRACT

In recent decades the usage of antibiotics has increased immensely in clinical settings. These antibiotics are discharged into the environment, particularly aquatic bodies, through various means. As a result, they remain persistent in the environment for a longer time. The occurrence of antibiotics in aquatic ecosystems often leads to the emergence of antibiotic resistance in pathogens. Once the population is exposed to water, they face various health issues. Traditional methods of wastewater treatment often fail to adequately degrade these emerging contaminants. Nanomaterials owing to their small size and high surface area possess excellent properties and have numerous applications. The present review focuses on antibiotic pollution in surface water and antimicrobial resistance. In addition to that, the applications of nanocomposites for the effective degradation of antibiotics have been mentioned. Nanomaterials pose excellent photocatalytic properties for the uptake and degradation of antibiotics. These nanomaterials have immense potential for the sustainable remediation of antibiotic pollution.

Keywords: Antibiotic resistance, Wastewater treatment, Nanomaterials, Antibiotic degradation,

INTRODUCTION

Confronting the Global Threat of Anti-Microbial Resistance

Rapid industrialization and development have often led to the exploitation of environmental reservoirs. As a result, various contaminants have emerged in the environment [1]. The World Health Organization (WHO) lists Anti-Microbial Resistance (AMR) as one of the top 10 global public health threats that humanity faces. This growing problem significantly hinders efficient treatment of a wide range of diseases caused by bacteria, viruses, fungus, and

parasites. Recent estimates indicate that bacterial AMR has a worrisome impact on global health, causing approximately 5 million deaths annually worldwide. These figures show how frequently resistant microbes cause standard treatments to fail, resulting in longer hospital stays, higher medical costs, and higher death rates. If swift and coordinated action is not taken, WHO warns of even more disastrous consequences. By 2050, AMR may become responsible for up to 10 million deaths annually, surpassing the current global cancer mortality rates. As this potential future demonstrates, increasing surveillance, using antibiotics responsibly, investing in new antimicrobial therapies, and fostering international collaboration are all vital to slowing the spread and consequences of AMR [2].

Role of Healthcare and Improper Disposal

The bulk of antibiotics discovered in hydrosphere wastewater are linked to inappropriate disposal procedures as well as individual and medical professional use. The main source of antibiotic contamination in these circumstances has been shown to be hospitals [3]. A lack of knowledge about microbiology, the use or lack of treatment guidelines, the belief that antibiotics will be administered during an appointment, and restricted access to diagnostic tools are some of the factors that have been linked to unnecessary antibiotic use in healthcare settings. A wide range of social and cultural factors also affect antibiotic abuse, including public perceptions of antibiotic use as well as prescribing practices [4]. The main sources of antibiotic pollution in the environment include runoff from farms, hospital and slaughterhouse effluents, and household wastewater. Human and animal consumption of antibiotics can lead to their entry into water systems, where they remain and encourage the growth of resistant bacteria, due to improper disposal and insufficient treatment. The excretion of veterinary medications from slaughterhouses contributes to the high concentrations of medicines and resistance genes released by hospitals. Antibiotics from untreated waste and manure are carried into surface and groundwater by agricultural runoff, particularly during rainy seasons. Antibiotic resistance is facilitated by this pervasive pollution, which poses major threats to ecosystems and human health[5].

Emergence of Ciprofloxacin Resistance

A member of the fluoroquinolone (FQ) class of antibiotics, ciprofloxacin works well against a wide range of bacteria, both Gram-positive and Gram-negative. Ciprofloxacin resistance in a number of bacterial species, such as *Bacillus anthracis, Pseudomonas aeruginosa, Neisseria gonorrhoeae, Enterococci, Escherichia coli,* and *Klebsiella pneumoniae,* have been reported in increasing numbers [6]. A higher percentage of treatment failures and mortality is linked to the development of ciprofloxacin resistance in cases of persistent infections. Changes that prevent drug accumulation, plasmid-mediated defense that negates ciprofloxacin's bactericidal effect, and target site alterations in DNA gyrase and topoisomerase IV are some of the processes via which resistance arises[7].

Tracing Antibiotic Contamination Across South Asia's Major Rivers

Due to inadequate sewage treatment facilities in India, antibiotics are released into natural water bodies, which exacerbates the environmental problem of antimicrobial resistance (AMR). Though they make up only 3.5% of the world's surface area, South Asian countries are among the most densely populated areas and are sometimes referred to as the water-stressed zone because of their high demand and limited supply of water. High population density and insufficient wastewater treatment make it more difficult for antibiotic resistance to spread through contaminated water systems. The worrisome levels of antibiotic contamination in India's major rivers are shown in Table 1, demonstrating the seriousness of this problem [8].

Nanoparticle application in removal of organic pollutant

Every day, a range of industrial and non-commercial methods are used, but nanotechnology is one of the most novel approaches for handling water and wastewater. Research in nanoscience has led to an array of cost-effective and environmentally responsible treatment techniques that effectively clean water and meet high water quality requirements [16]. Fluoroquinolones, a major class of quinolone antibiotics including ciprofloxacin, levofloxacin, and ofloxacin, are commonly found as traces in wastewater. Recently, nanoparticles have been employed for their effective degradation. For instance, zinc oxide (ZnO) nanoparticle aggregates achieved 88.2% degradation of ciprofloxacin within 4 hours [17]. Nano graphene oxide magnetite demonstrated a 97% degradation efficiency for ofloxacin in just 2 hours [18].

From Nature to Nanotech: Green Synthesis of Metal Nanoparticles for a Cleaner Environment

Two separate basic synthesis principles-the top-down and bottom-up approaches-have been examined in the literature to produce nanomaterials with the required sizes, shapes, and functions. Reaction conditions and precursor concentrations can be modified to regulate the size and shape of nanoparticles. Instability, ambiguous mechanisms, possible toxicity, and recycling difficulties are obstacles to use in practice, though. Complex analysis is often needed, as are skilled operators. These restrictions point to important areas of unmet need. However, they also present intriguing chances for nanoscale innovation. Green synthesis is an environmentally friendly method of creating nanoparticles that scientists are using to deal with these problems. Both plant-mediated and microbial-mediated methods are used to create materials at the nanoscale. Construction products mediated by microbial use their naturally complex metabolic machinery in order to generate multiple nanoparticles with varying chemical compositions [19]. However, when it comes to microbiological preparations, scaling up can occasionally be difficult. Plant-based extracts provide a simple solution to this problem, and as a result, production rates may be increased.

Green Fabrication of Nanoparticles Using Botanical Resources

Microbes are less effective than plant extracts in terms of production rate. Compared to microorganisms, they reduce metal ions more quickly and create extremely stable nanomaterials [20]. Because plant extracts are widely available, inexpensive, and safe, they have become a crucial technique in the production of nanoparticles. Plant extracts are also abundant in a variety of phytochemicals that serve as stabilizers and reducing agents while plant-based nanoparticles are being formed [21]. The bulb extract of *U. epigea* has been used to successfully prepare Ag/ZnO nanocomposite in a cost-effective, non-toxic, simple, and environmentally friendly manner [22]. Several studies have explored the synthesis and functional evaluation of silver oxide-doped zinc oxide nanoparticles (Ag_2O–ZnO NPs) due to their enhanced photocatalytic and antioxidant properties. One such study reported the use of *Croton macrostachyus* leaf extract as a green synthesis agent, acting simultaneously as a reducing, stabilizing, and capping agent [23].

Hybrid Nanocomposite Photocatalysts: A New Frontier in Antibiotic Degradation

For photocatalytic degradation, nanomaterials are very effective because of their active sites and vast surface area. They frequently suffer from quick charge recombination and limited absorption of solar energy, though. These problems lower their photocatalytic performance and overall stability. The creation of hybrid nanocomposite photocatalysts has been widely adopted by researchers as a solution to these restrictions. Two or more nanomaterials with complementary qualities are combined to create these. These heterostructures improve overall stability and efficiency of photocatalysis [24]. Fe/Cu bimetallic nanoparticles supported on glass (G-Fe/Cu) were created by Ibrahim *et al* (2024) with the aid of grape leaf extract, a natural reducing agent [25]. This supports the effectiveness of plant-based synthesis techniques demonstrated by previous studies. When circumstances were optimized, the nanocomposite demonstrated a high ciprofloxacin (CIP) elimination effectiveness of 99% (Table 2).

Applications of ZnO/Ag_2O Nanocomposites for antibiotic degradation

In environmental treatment, ZnO/Ag_2O NPs are highly valued for their photocatalytic properties. These nanoparticles are capable of efficiently breaking down organic pollutants, and wastewater dyes can cleanse contaminated water sources when exposed to sunshine. Their ability to generate reactive oxygen species in response to UV-vis light helps to break down a variety of environmental pollutants, leading to cleaner water and air [32]. A study looks at the production of pure ZnO nanoparticles (NPs) and Ag/ZnO nanocomposites (NCs) with silver added at varying quantities (0.5%, 1.0%, and 2.0%) utilizing seed extract from *Ocimum tenuiflorum* (Tulsi). Comprehensive tests were conducted to evaluate the antibacterial properties and dye degradation capabilities of the green-synthesized ZnO-NPs and Ag/ZnO-NCs at different silver loadings [33]. In several studies, lemon peel extract has been utilized to successfully produce environmentally friendly ZnO nanoparticles and ZnO/Ag nanocomposite heterostructures. The sustained production of nanomaterials for environmental restoration using plant-based methods is demonstrated by this eco-friendly approach. In just 12 minutes, the synthesized nanomaterials demonstrated exceptional photocatalytic efficacy in natural sunshine, degrading 90% of ciprofloxacin and 98.8% of metronidazole. Their favourable physicochemical characteristics, such as low bandgap energies and high degradation efficiencies, lend them promise as practical and long-term solutions for water contamination [34].

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Table 1. Rivers in Crisis: Antibiotic Contamination and its Levels across India

River	Antibiotic	Concentration	References
Kshipra River	Sulfamethoxazole, ofloxacin, norfloxacin	0.66 μg l^{-1}-2.75 μg l^{-1}	[9]
Ganga river	Ciprofloxacin, Ofloxacin, Erythromycin	8.62 μg l^{-1} - 1.36 μg l^{-1}	[10, 11]
Yamuna river	Kanamycin, Ciprofloxacin, Amoxicillin, Ofloxacin	0.06 μg l^{-1} - 2.00 μg l^{-1}	[12, 13]
Gomti river	Ciprofloxacin,Tetracycline	1.71 μg l^{-1} - 1.80 μg l^{-1}	[14, 15]

Table 2. Efficiency of Advanced Nanostructured Photocatalysts in Antibiotic Degradation

Photocatalyst	Pollutant	Degradation Efficiency	Time	Light Source	References
AgI/$Bi_2Sn_2O_7$ Z-scheme nanojunction hybrid	Tetracycline	~83.0%	50 min	300 W Xe lamp (visible light)	[26]
CuO/ZnO hollow structure composite	Tetracycline	~87.0%	60 min	Visible light (λ > 420 nm)	[27]
Ag/ZnO/C composite	Tetracycline hydrochloride	95.8% (UV), 90.6% (visible light)	35 min (UV), 280 min (visible)	UV and visible light	[28]
Green 5% Hy-Co-ZnO	ciprofloxacin	99%	120 minutes	Visible LED light	[29]
Green synthesized Ag/Fe/ZnO NCs	Amoxicillin	98.5%	180 minutes	visible light	[30]
ZnO/GO nanocomposites	ciprofloxacin	~100%	85 min	Direct sunlight	[31]

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RESEARCH ARTICLE

Analytical Solution for Fourth Order Variable Coefficient's By using STHPM

Pradip R. Bhadane[1]* and Sharad B. Ugale[2]

[1]Assistant Professor, Department of Mathematics, Shrimati Vimlaben Khimji Tejookaya Arts, Science and Commerce College, Nashik, (Affiliated to Savitribai Phule Pune University, Pune) Maharashtra, India.
[2]Research Scholar (Reg.No.: 553171), Department of Mathematics, K.T.H.M. College, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra, India.

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***Address for Correspondence**
Pradip R. Bhadane
Assistant Professor,
Department of Mathematics,
Shrimati Vimlaben Khimji Tejookaya Arts, Science and Commerce College, Nashik,
(Affiliated to Savitribai Phule Pune University, Pune) Maharashtra, India.
E.Mail: prbhadane66@gmail.com

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ABSTRACT

In this work, we apply a novel method for solving one-dimensional fourth-order parabolic linear partial differential equations with variable coefficients (FOPPDEs) that combines the new integral transform ST and the HPM, commonly known as the Shehu transform homotopy perturbation method (STHPM).Some instances of one-dimensional FOPPDEs are solved to demonstrate the capability and reliability of the aggregate of ST and HPM. We have, in comparison, the acquired analytical solution with the available solutions, which is determined to be exactly equal. The results display that the mixture of ST and HPM is quite successful, nicely suitable for use in such problems.

Keywords: hehu Transform (ST), Homotopy Perturbation Method (HPM), Fourth Order Parabolic Partial Differential Equations (FOPPDE's), Analytical Solution.
Article type: Research article
2010 AMS Classification: 65M99, 65Mxx, 35F25, 35G25, 74H10

INTRODUCTION

ST was first delivered by a China Mathematician Shehu Maitama and Weidong Zhao in2019's [17]. ST is an integral transform method, which is very effective and beneficial for fixing ordinary and partial differential equations [2], [3], [4], [9]. But the ST is completely incapable of coping with nonlinear equations. It is due to the fact the difficulties, which are resulting from the nonlinear terms and lots of physical phenomena that are controlled with the aid of linear or nonlinear differential equations. Due to this motive, many researchers desired to use the HPM to handle

nonlinear equations. The technique gives a completely speedy convergence of the solution series inside the maximum instances. The HPM became first proposed by means of the Chinese mathematician He's in 1999 [13] and became similarly improved through He's [14], [15].Remarkable research work has been advanced through the use of the HPM to the linear and non-linear equations [1,6,7,8].Since this technique is prolonged and difficult for fixing those varieties of numerical problems. The proposed approach via Shehu Maitama and W. Zhao [18], is a coupling of the ST and HPM. Due to the functionality of combining those two effective gears, the STHPM is discovered very powerful approach. In current years, some authors have paid attention to take a look at the answers of linear and nonlinear partial differential equations using HPM with the ST [19]. Fundamentally there are three types of PDEs-Hyperbolic, Parabolic, and Elliptic PDEs [22][24]. Many problems of physical interest are described by linear PDEs with initial and boundary conditions. One of them is FOPPDE's with variable coefficients. Previously many analytical techniques were used for finding the solution for FOPPDE's, due to its significance inside the fields of engineering, But the numerical answers of the FOPPDE's are very hard due to the nonlinear terms. Therefore, plenty attention has been paid with the aid of researcher's the use of numerous methods for FOPPDE's. In 2001, Abdul-Majid Wazwaz [20][21] found new magnificence of answers for FOPPDE's. Also in 2009, M. Dehghan and J. Manafian [10] used HPM to obtain an approximate solution. Later on M. Khan at el.[16] used Laplace decomposition Method (2011).V. G. Gupta and S. Gupta [11] used HPM. P. G. Bhadane and V. H. Pradhan [5] used coupling of Elzaki and HPM (2013). Khalid S. Aboodh used Aboodh and HPM [23] in (2015) and M. A. AL. Jawary [12] used for solving FOPPDE's (2015). In the existing paper, a new STHPM is used and proposed to resolve the one dimensional FOPPDE's with variable coefficients. This method offers the answer in a rapid convergent series, which leads the solution in a closed form.

Basic definition's and Fundamental Facts

Definition: 2.1 *A new transform called the Shehu transform defined for function of exponential order, we Consider functions in the set A defined by*

$$A=\left\{v(t)|\ \exists N,\lambda_1,\lambda_2>0,|v(t)|<Ne^{\frac{|t|}{\lambda_4}},if\quad t\in(-1)'-X[0,\infty)\right\}$$

In the set A, the constant N is finite number and λ_1,λ_2 are finite or infinite The Shehu transform denoted by the operator $S(.)and$ defined by the integral equation.

$$S[v(t)]=V(s,u)=\int_0^{\infty}\exp\left(\frac{-st}{u}\right)v(t)dt$$

The inverse Shehu transform is given by

$S^{-1}[V(s,t)]=v(t),\text{for}t\geq 0$

where s and u are the Shehu transform variables.

Shehu Transform for Simple Functions

We have $S[v(t)]=V(s,u)=\int_0^{\infty}v(t)\left(e^{\frac{-st}{u}}\right)dt$

1. If v(t) = 1, then $S[1]=V(s,u)=\int_0^{\infty}(1)\left(e^{\frac{-st}{u}}\right)dt=\frac{u}{s}$
2. If v(t)=t, then $S[t]=V(s,u)=\int_0^{\infty}(t)\left(e^{\frac{-st}{u}}\right)dt=\frac{u^2}{s^2}$

More generally $S[t^n]=V(s,u)=\int_0^{\infty}(t^n)\left(e^{\frac{-st}{u}}\right)dt=n!\frac{u^{n+1}}{s^{n+1}}$

3. If v(t) = exp(αt), then $S[e^{\alpha t}]=V(s,u)=\int_0^{\infty}(e^{\alpha t})\left(e^{\frac{-st}{u}}\right)dt=\frac{u}{s-\alpha u}$

Theorem: 2.1 *Let $V(s,u)$ is Shehu transform of $v(t)$ and $S[v(t)]=V(s,u)$, Then*

1. $S\left[\frac{d}{dt}(v(t))\right]=S[v'(t)]=\int_0^{\infty}(v'(t))\left(e^{\frac{-st}{u}}\right)dt$

Integrating by parts, we find $S[v'(t)]=V(s,u)-v(0)$

2. $S\left[\frac{d^2}{dt^2}(v(t))\right]=S[v''(t)]=\int_0^{\infty}(v''(t))\left(e^{\frac{-st}{u}}\right)dt$ then

$$S[v^*(t)]=\frac{s^2}{u^2}V(s,u)-\frac{s}{u}v(0)-v'(0)$$

3. $S\left[\frac{d^n}{dt^n}(v(t))\right]=S[v^n(t)]=\int_0^{\infty}(v^n(t))\left(e^{\frac{-st}{u}}\right)dt$, then $S[v^n(t)]=\frac{s^n}{u^n}V(s,u)-\sum_{k=0}^{n-1}\left(\frac{s}{u}\right)^{n-(k+1)}$

Convergence Theorem and Examples

We would study convergence theorem on series solution.

Theorem: 3.1 *Shehu transform magnify the coefficient's of the power series function*

$v(t) = \sum_{n=0}^{\infty} b_n t^n$

is $S[v(t)] = V(s,u) = \sum_{n=0}^{\infty} n!\, b_n \frac{u^{n+1}}{s^{n+1}}$.

Proof:- Let v(t) be in A when $v(t) = \sum_{n=0}^{\infty} b_n t^n$, then by Taylors series expansion $v(t) = \sum_{n=0}^{\infty} \frac{v^n(0)}{n!} t^n$ by definition of Shehu transform, we have $S[v(t)] = \int_0^{\infty} \sum_{n=0}^{\infty} \frac{v^n(0)}{n!} t^n \left(e^{\frac{-st}{u}}\right) dt = \sum_{n=0}^{\infty} \frac{v^n(0)}{n!} \int_0^{\infty} \left(e^{\frac{-st}{u}}\right) t^n dt$,

Integratingusingbypartstheorem, onceobtain

$$= \sum_{n=0}^{\infty} \frac{v^n(0)}{n!} \left[\frac{u^{n+1}}{s^{n+1}}\right] n! = \sum_{n=0}^{\infty} v^n(0) \left[\frac{u^{n+1}}{s^{n+1}}\right] = \sum_{n=0}^{\infty} n!\, b_n \frac{u^{n+1}}{s^{n+1}}$$

Consequently

$$S[(1+t)^m] = S\left(\sum_{n=0}^{\infty} C_n^m t^n\right) = \sum_{n=0}^{\infty} C_n^m\, S(t^n) = \sum_{n=0}^{\infty} \frac{m!}{n!\,(m-n)!} \left(n! \frac{u^{n+1}}{s^{n+1}}\right)$$

$$= \sum_{n=0}^{\infty} \frac{m!}{(m-n)!} \left(\frac{u^{n+1}}{s^{n+1}}\right) = \sum_{n=0}^{\infty} P_n^m \left(\frac{u^{n+1}}{s^{n+1}}\right)$$

ST sends combination C_n^m into permutation P_n^m. Also $S[v(t)]$ converges in an interval by $v = 0$ as $v^n(0) \to 0$, as $n \to \infty$, and $\lim_{n\to\infty} \left|\frac{v^{n+1}(0)}{v^n(0)} \left(\frac{u}{s}\right)\right| < 1$..

This suggests that the convergence of $S[v(t)]$ relies upon on the convergence of $v^n(0)$.

Example 3.1 *consider the function*

$v(t) = \ln(t+1), t \in (-1,1) = 0,$

otherwise

since $v(t) = \sum_{n=0}^{\infty} (-1)^{n-1} \frac{t^n}{n}$, except for $v = 0$

$$S[v(t)] = \sum_{n=0}^{\infty} \frac{(-1)^{n-1}}{n} \left[\frac{u^{n+1}}{s^{n+1}}\right] n! = \sum_{n=0}^{\infty} (-1)^{n-1} (n-1)! \left[\frac{u^{n+1}}{s^{n+1}}\right]$$

This series diverges throughout, because its convergences only when

$$\lim_{n\to\infty} \left|\frac{(-1)^{n-1}(n-1)!}{(-1)^n (n)!}\right| = \lim_{n\to\infty} \frac{1}{n} = 0$$

Algorithm of the STHPM

Consider a one dimensional linear non homogeneous FOPPDE with variable coefficients of the form.

$\frac{\partial^2 \eta}{\partial t^2} + \psi(z) \frac{\partial^4 \eta}{\partial z^4} = \phi(z,t)$ (1)

where $\psi(z)$ is a variable coefficient with the subsequent initial conditions.

$\eta(z,0) = f(z)$ and $\frac{\partial \eta}{\partial t}(z,0) = h(z)$ (2)

and boundary conditions are

$\frac{\partial^2 \eta}{\partial z^2}(a,t) = \mu_3(t), \frac{\partial^2 \eta}{\partial z^2}(b,t) = \mu_4(t)$ (3)

By ST on both sides of equation (1)

$S\left[\frac{\partial^2 \eta}{\partial t^2} + \psi(x) \frac{\partial^4 \eta}{\partial z^4}\right] = S[\phi(z,t)]$ (4)

But ST of partial derivative is given by

$$S\left[\frac{\partial^2 \eta}{\partial t^2}\right] = \frac{s^2}{\eta^2} S[f(z,t)] - \frac{s}{\eta}[f(z,0)] - \frac{\partial f}{\partial t}(\boldsymbol{z}, 0)$$

using this property Eq. (3) may be written as

$$\frac{s^2}{\eta^2}\,\mathrm{S}[\eta(z,t)] - \frac{s}{\eta}[\eta(z,0)] - \frac{\partial\eta}{\partial t}(z,0) = \mathrm{S}[\phi(z,t)] - \mathrm{S}\left[\psi(z)\frac{\partial^4\eta}{\partial z^4}\right] \quad (5)$$

put the values preliminary conditions in eq (5), once obtain

$$\frac{s^2}{\eta^2}\,\mathrm{S}[\eta(z,t)] - \frac{s}{\eta}f(z) - h(z) = \mathrm{S}[\phi(z,t)] - \mathrm{S}\left[\psi(z)\frac{\partial^4\eta}{\partial z^4}\right]$$

by simple calculations, we have

$$\mathrm{S}[\eta(z,t)] = g(z,v) - \frac{\eta^2}{s^2}\,\mathrm{S}\left[\psi(z)\frac{\partial^4\eta}{\partial z^4}\right] \quad (6)$$

where

$$g(z,v) = \frac{\eta}{s}f(z) - \frac{\eta^2}{s^2}h(z) + \mathrm{S}[\phi(z,t)]$$

Performing inverse ST on both sides of eq. (6), we get

$$\eta(z,t) = k(z,t) - \mathrm{S}^{-1}\left[\frac{\eta^2}{s^2}\,\mathrm{S}\left[\psi(z)\frac{\partial^4\eta}{\partial z^4}\right]\right] \quad (7)$$

where $k(z,t) = \mathrm{S}^{-1}[g(z,v)]$ represents the source term and the prescribed initial conditions. Now we apply the HPM.

where $k(z,t) = \mathrm{S}^{-1}[g(z,v)]$ represents the source term and the prescribed initial conditions. Now we apply the HPM

$$\eta(\boldsymbol{z},t) = \sum_{n=0}^{\infty} P^n\eta_n(\boldsymbol{z},t) \quad (8)$$

By substituting eq. (8) into eq. (7), we get

$$\sum_{n=0}^{\infty} P^n\eta_n(z,t) = k(z,t) - p\mathrm{S}^{-1}\left\{\left[\frac{\eta^2}{s^2}\,\mathrm{S}[\psi(z)(\sum_{n=0}^{\infty} P^n\eta_n(z,t))_{zzzz}]\right]\right\} \quad (9)$$

This is coupling of the ST and HPM.

Comparing the coefficients of like powers of p, the following approximations are obtained

$$P^0{:}\,\eta_0(z,t) = k(z,t)$$

$$P^1{:}\,\eta_1(z,t) = -\mathrm{S}^{-1}\left\{[\frac{\eta^2}{s^2}\,\mathrm{S}[\psi(z)\eta_{0zzzz}(z,t)]]\right\}$$

$$P^2{:}\,\eta_2(z,t) = -\mathrm{S}^{-1}\left\{[\frac{\eta^2}{s^2}\,\mathrm{S}[\psi(\boldsymbol{z})\eta_{1zzzz}(\boldsymbol{z},t)]]\right\}$$

In general recursive relation is given by

$$P^n{:}\,\eta_n(z,t) = -\mathrm{S}^{-1}\left\{\left[\frac{\eta^2}{s^2}\,\mathrm{S}\left[\psi(z)\eta_{(n-1)zzzz}(z,t)\right]\right]\right\}$$

Then the solution is given by

$$\eta(z,t) = \eta_0(z,t) + \eta_1(z,t) + \eta_2(z,t) + \eta_3(z,t) + \text{- - - - - - - -} \quad (10)$$

Application's

To show the effectiveness of the technique we've solved homogeneous and nonhomogeneous one dimensional linear FOPPDE's with initial and boundary situations. Example 5.1 *Consider fourth order homogeneous PDE as*

$$\frac{\partial^2\eta}{\partial t^2} + \left(\frac{1}{z} + \frac{z^4}{120}\right)\frac{\partial^4\eta}{\partial z^4} = 0, \frac{1}{2} < z < 1, t > 0 \quad (11)$$

with the subsequent initial conditions

$$\eta(z,0) = 0, and\, \frac{\partial\eta}{\partial t}(z,0) = 1 + \frac{z^5}{120} \quad (12)$$

and boundary conditions

$$\eta(0.5,t) = \left(\frac{1+(0.5)^5}{120}\right)\mathrm{sin}t, \text{and } \eta(1,t) = \frac{121}{120}\mathrm{sin}t$$

$$\frac{\partial^2\eta}{\partial z^2}(0.5,t) = 0.02084\mathrm{sin}t, \frac{\partial^2\eta}{\partial z^2}(1,t) = \frac{1}{6}\mathrm{sin}t \quad (13)$$

Applying ST to Eq. (11), we get

$$\mathrm{S}\left[\frac{\partial^2\eta}{\partial t^2}\right] + \mathrm{S}\left[\left(\frac{1}{z} + \frac{z^4}{120}\right)\frac{\partial^4\eta}{\partial z^4}\right] = 0$$

$$\frac{s^2}{\eta^2}\,\mathrm{S}[\eta(z,t)] - \frac{s}{\eta}\eta(z,0) - \frac{\partial\eta}{\partial t}(z,0) + \mathrm{S}\left[\left(\frac{1}{z} + \frac{z^4}{120}\right)\frac{\partial^4\eta}{\partial z^4}\right] = 0 \quad (14)$$

Using initial conditions from Eq. (12), we get

$$S[\eta(z,t)] = \frac{\eta^2}{s^2}\left[1+\frac{z^5}{120}\right] - \frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)\frac{\partial^4\eta}{\partial z^4}\right] \quad (15)$$

Now taking inverse ST on both sides of Eq. (15), we have

$$\eta(z,t) = \left[1+\frac{z^5}{120}\right]t - S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)\frac{\partial^4\eta}{\partial z^4}\right]\right\} \quad (16)$$

Now, we apply the HPM.

$$\eta(z,t) = \sum_{n=0}^{\infty} P^n\eta_n(z,t)$$

Putting this value of $\eta(z,t)$ into Eq. (16), we get

$$\sum_{n=0}^{\infty} P^n\eta_n(z,t) = \left[1+\frac{z^5}{120}\right]t - pS^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)\left(\sum_{n=0}^{\infty} P^n\eta_n(z,t)\right)_{zzzz}\right]\right\} \quad (17)$$

Comparing the coefficient of like powers of p, in Eq. (17) the following approximations are obtained.

$$P^0{:}\,\eta_0(z,t) = k(z,t) = \left[1+\frac{z^5}{120}\right]t$$

$$P^1{:}\,\eta_1(z,t) = -S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)(\eta_{0zzzz}(z,t))\right]\right\}$$

$$= -S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)zt\right]\right\}$$

$$= -\left(1+\frac{z^5}{120}\right)\frac{t^3}{3!}$$

$$P^2{:}\,\eta_2(z,t) = -S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)(\eta_{1zzzz}(z,t))\right]\right\}$$

$$= \left(1+\frac{z^5}{120}\right)\frac{t^5}{5!}$$

$$P^3{:}\,\eta_3(z,t) = -S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)(\eta_{2zzzz}(z,t))\right]\right\}$$

$$= -\left(1+\frac{z^5}{120}\right)\frac{t^7}{7!}\vdots$$

$$P^n{:}\,\eta_n(z,t) = -S^{-1}\left\{\frac{\eta^2}{s^2}\,S\left[\left(\frac{1}{z}+\frac{z^4}{120}\right)(\eta_{(n-1)zzzz}(z,t))\right]\right\}$$

$$= (-1)^n\left(1+\frac{z^5}{120}\right)\frac{t^{2n+1}}{(2n+1)!}, n = 1,2,3\ldots$$

So on within the equal way the rest of the components of iteration method can be received and consequently answer may be written in closed form as

$$\eta(z,t) = \left(1+\frac{z^5}{120}\right)t - \left(1+\frac{z^5}{120}\right)\frac{t^3}{3!} + \left(1+\frac{z^5}{120}\right)\frac{t^5}{5!} - \left(1+\frac{z^5}{120}\right)\frac{t^7}{7!} + ------$$

$$\eta(z,t) = \left(1+\frac{z^5}{120}\right)\left(t-\frac{t^3}{3!}+\frac{t^5}{5!}-\frac{t^7}{7!}+------\right)$$

$$\eta(z,t) = \left(1+\frac{z^5}{120}\right)\sin t \quad (18)$$

Which is an exact solution of Eq. (11) and it is the same results obtained by existing methods. The following plot is obtained using wolfram Alpha software

3D behavior of an approximate solution of (11) (Figure 1)

Example 5.2 *Consider fourth order homogeneous PDE as*

$$\frac{\partial^2\eta}{\partial t^2} + \left(\frac{z}{\sin z}-1\right)\frac{\partial^4\eta}{\partial z^4} = 0, 0 < \boldsymbol{z} < 1, t > 0 \quad (19)$$

with the following initial conditions

$$\eta(z,0) = z - \sin z, \text{and}\,\frac{\partial\eta}{\partial t}(z,0) = -z + \sin z \quad (20)$$

and boundary conditions

$$\eta(0,t) = 0, \text{and} \qquad \eta(1,t) = e^{-t}(1-\sin 1)$$

$\frac{\partial^2\eta}{\partial z^2}(0,t)=0, \frac{\partial^2\eta}{\partial z^2}(1,t)=e^{-t}\sin 1$ (21)

Applying ST to Eq. (19), we get

$$S\left[\frac{\partial^2\eta}{\partial t^2}\right]+S\left[\left(\frac{z}{\sin z}-1\right)\frac{\partial^4\eta}{\partial z^4}\right]=0$$

$$\frac{s^2}{\eta^2}S[\eta(z,t)]-\frac{s}{\eta}\eta(z,0)-\frac{\partial\eta}{\partial t}(z,0)+S\left[\left(\frac{z}{\sin z}-1\right)\frac{\partial^4\eta}{\partial z^4}\right]=0 \quad (22)$$

Using initial conditions from Eq. (20), we get

$$S[\eta(z,t)]=\frac{\eta}{s}(z-\sin z)+\frac{\eta^2}{s^2}(-z+\sin z)-\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)\frac{\partial^4\eta}{\partial z^4}\right] \quad (23)$$

Now taking inverse ST on both sides of above Eq. (23), we have

$$\eta(z,t)=(z-\sin z)+(-z+\sin z)t-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)\frac{\partial^4\eta}{\partial z^4}\right]\right\} \quad (24)$$

Now, we apply the HPM.

$$\eta(z,t)=\sum_{n=0}^{\infty}P^n\eta_n(z,t)$$

Putting this value of $\eta(z,t)$ into Eq. (24), we get

$$\sum_{n=0}^{\infty}P^n\eta_n(z,t)=(z-\sin z)+(-z+\sin z)t$$

$$-pS^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)\left(\sum_{n=0}^{\infty}P^n\eta_n(z,t)\right)_{zzzz}\right]\right\} \quad (25)$$

Comparing the coefficient of like powers of p, in Eq. (25) the following approximations are obtained.

$$P^0:\eta_0(z,t)=k(z,t)=(z-\sin z)+(-z+\sin z)t$$

$$P^1:\eta_1(z,t)=-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)(\eta_{0zzz}(z,t))\right]\right\}$$

$$=-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)(t-1)\sin z\right]\right\}$$

$$=-(z-\sin z)S^{-1}\left\{\frac{\eta^4}{s^4}-\frac{\eta^3}{s^3}\right\}$$

$$=(z-\sin z)\left(\frac{t^2}{2!}-\frac{t^3}{3!}\right)$$

$$P^2:\eta_2(z,t)=-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)(\eta_{1zzzz}(z,t))\right]\right\}$$

$$=(z-\sin z)\left(\frac{t^4}{4!}-\frac{t^5}{5!}\right)$$

$$P^3:\eta_3(z,t)=-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)(\eta_{2zzzz}(z,t))\right]\right\}$$

$$=(z-\sin z)\left(\frac{t^6}{6!}-\frac{t^7}{7!}\right)$$

$$\vdots$$

$$P^n:\eta_n(z,t)=-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[\left(\frac{z}{\sin z}-1\right)\left(\eta_{(n-1)zzzz}(z,t)\right)\right]\right.$$

$$=(z-\sin z)\left(\frac{t^{2n}}{2n!}-\frac{t^{2n+1}}{2n+1!}\right), n=1,2,3\ldots$$

So on within the equal way the rest of the components of iteration method can be received and consequently answer may be written in closed form as

$$\eta(z,t)=(z-\sin z)+(-z+\sin z)t+(z-\sin z)\left(\frac{t^2}{2!}-\frac{t^3}{3!}\right)$$

$+(z-\sin z)\left(\frac{t^4}{4!}-\frac{t^5}{5!}\right)+(z-\sin z)\left(\frac{t^6}{6!}-\frac{t^7}{7!}\right)+-----$

$$\eta(z,t)=(z-\sin z)\left(1-t+\frac{t^2}{2!}-\frac{t^3}{3!}+\frac{t^4}{4!}-\frac{t^5}{5!}+\frac{t^6}{6!}-\frac{t^7}{7!}-------\right)$$

$$\eta(z,t)=(z-\sin z)e^{-t} \qquad (26)$$

Which is an exact solution of Eq. (19) and it is the same results obtained by existing methods. The following plot is obtained using wolfram Alpha software. 3D behavior of an approximate solution of (19) (Figure 2)

Example 5.3 *Consider non homogeneous FOPDE as*

$$\frac{\partial^2\eta}{\partial t^2}+(1+z)\frac{\partial^4\eta}{\partial z^4}=\left(z^4+z^3-\frac{6}{7!}z^7\right)\cos t, 0<z<1, \quad t>0 \qquad (27)$$

with the following initial conditions

$$\eta(z,0)=\frac{6}{7!}z^7, \text{and}\frac{\partial\eta}{\partial t}(z,0)=0 \qquad (28)$$

and boundary conditions

$$\eta(0,t)=0, \text{and } \eta(1,t)=\frac{6}{7!}\cos t$$

$$\frac{\partial^2\eta}{\partial z^2}(0,t)=0, \frac{\partial^2\eta}{\partial z^2}(1,t)=\frac{1}{20}\cos t \qquad (29)$$

Applying ST to Eq. (27), we get

$$S\left[\frac{\partial^2\eta}{\partial t^2}\right]+S\left[(1+z)\frac{\partial^4\eta}{\partial z^4}\right]=S\left[\left(z^4+z^3-\frac{6}{7!}z^7\right)\cos t\right]$$

Using initial conditions from Eq. (28), we get

$$S[\eta(z,t)]=\frac{\eta}{s}(0)+\frac{\eta^2}{s^2}S\left[\left(z^4+z^3-\frac{6}{7!}z^7\right)\cos t\right]-\frac{\eta^2}{s^2}S\left[(1+z)\frac{\partial^4\eta}{\partial z^4}\right]$$

$$S[\eta(z,t)]=\frac{\eta}{s}\frac{6}{7!}z^7+\frac{\eta^2}{s^2}\left(z^4+z^3-\frac{6}{7!}z^7\right)S[\cos t]-\frac{\eta^2}{s^2}S\left[(1+z)\frac{\partial^4\eta}{\partial z^4}\right]$$

$$S[\eta(z,t)]=\frac{\eta}{s}\frac{6}{7!}z^7+\left(z^4+z^3-\frac{6}{7!}z^7\right)\frac{\eta^3}{s(s^2+\eta^2)}-\frac{\eta^2}{s^2}S\left[(1+z)\frac{\partial^4\eta}{\partial z^4}\right] \qquad (30)$$

Now taking inverse ST on both sides of above Eq. (30), we have

$$\eta(z,t)=\frac{6}{7!}z^7+\left(z^4+z^3-\frac{6}{7!}z^7\right)(1-\cos t)-S^{-1}\left\{\frac{\eta^2}{s^2}S\left[(1+z)\frac{\partial^4\eta}{\partial z^4}\right]\right\} \qquad (31)$$

Now, we apply the HPM.

$$\eta(z,t)=\sum_{n=0}^{\infty}P^n\eta_n(z,t)$$

Putting this value of $\eta(z,t)$ into Eq. (31), we get

$$\left.\begin{aligned}\textstyle\sum_{n=0}^{\infty}P^n\eta_n(z,t)=\frac{6}{7!}z^7+\left(z^4+z^3-\frac{6}{7!}z^7\right)(1-\cos t)\\ -pS^{-1}\{\tfrac{\eta^2}{s^2}S[(1+z)(\textstyle\sum_{n=0}^{\infty}P^n\eta_n(z,t))\}_{zzzz}]\end{aligned}\right\} \qquad (32)$$

we take

$$k(z,t)=\frac{6}{7!}z^7+\left(z^4+z^3-\frac{6}{7!}z^7\right)(1-\cos t)$$

$$=\frac{6}{7!}z^7\cos t+(z^4+z^3)(1-\cos t)$$

$$=k_0(z,t)+k_1(z,t)$$

Where

$$k_0(z,t)=\frac{6}{7!}z^7\cos t \text{ and } k_1(z,t)=(z^4+z^3)(1-\cos t)$$

Comparing the coefficient of like powers of p, in Eq. (32) the following approximations are obtained.

$P^0: \eta_0(z,t) = \frac{6}{7!} z^7 \cos t$

$P^1: \eta_1(z,t) = (z^4 + z^3)(1 - \cos t) - \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}[(1+z)(\eta_{0zzz}(z,t))]\right\}$

$= (z^4 + z^3)(1 - \cos t) - \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}[(1+z)z^3 \cos t]\right\}$

$= (z^4 + z^3)(1 - \cos t) - (z^3 + z^4)\mathrm{S}^{-1}\left\{\frac{\eta^3}{s(s^2+\eta^2)}\right\}$

$= (z^4 + z^3)(1 - \cos t) - (z^3 + z^4)(1 - \cos t) = 0$

$P^2: \eta_2(z,t) = -\mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}[(1+z)(\eta_{1zazz}(z,t))]\right\}$

$= 0$

So on within the equal way the rest of the components of iteration method can be received and consequently answer may be written in closed form as

$P^n: \eta_n(z,t) = 0 \quad for \quad \mathrm{n} \geq 1.$

$\eta(z,t) = \frac{6}{7!} z^7 \cos t$ (33)

Which is an exact solution of Eq. (27) and it is the same results obtained by existing methods. The following plot is obtained using wolfram Alpha software. 3D behavior of an approximate solution of (27) (Figure 3)

Example 5.4 *Consider non-homogeneous FOPDE as*

$\frac{\partial^2 \eta}{\partial t^2} + z\eta^2 \frac{\partial^4 \eta}{\partial z^4} = 24 z^9 t^3, 0 < z < 1, t > 0$ (34)

with the following initial conditions

$\eta(z,0) = 0, \text{and} \frac{\partial \eta}{\partial t}(z,0) = \boldsymbol{z}^4$ (35)

and boundary conditions

$\eta(0,t) = 0, \text{and} \quad \eta(1,t) = t$

$\frac{\partial^2 \eta}{\partial z^2}(0,t) = 0, \frac{\partial^2 \eta}{\partial z^2}(1,t) = 12t$ (36)

Applying ST to Eq. (34), we get

$\mathrm{S}\left[\frac{\partial^2 \eta}{\partial t^2}\right] + \mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right] = \mathrm{S}[24 z^9 t^3]$

$\frac{s^2}{\eta^2}\,\mathrm{S}[\eta(z,t)] - \frac{s}{\eta}\eta(z,0) - \frac{\partial \eta}{\partial t}(z,0) + \mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right] = \mathrm{S}[24 z^9 t^3]$

Using initial conditions from Eq. (35), we get

$\mathrm{S}[\eta(z,t)] = \frac{\eta^2}{s^2} z^4 + \mathrm{S}[24 z^9 t^3] - \frac{\eta^2}{s^2}\,\mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right]$

$\mathrm{S}[\eta(z,t)] = \frac{\eta^2}{s^2} z^4 + 24 z^9 \left(3! \frac{s^4}{\eta^4}\right) - \frac{\eta^2}{s^2}\,\mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right]$ (37)

Now taking inverse ST on both sides of above Eq. (37), we have

$\eta(z,t) = z^4 t + 24 z^9 (3!)\mathrm{S}^{-1}\left(\frac{s^4}{\eta^4}\right) - \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right]\right\}$

$\eta(z,t) = z^4 t + 24 z^9 t^3 - \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}\left[z\eta^2 \frac{\partial^4 \eta}{\partial z^4}\right]\right\}$ (38)

Now, we apply the HPM.

$\eta(z,t) = \sum_{n=0}^{\infty} P^n \eta_n(z,t)$

Putting this value of $\eta(z,t)$ into Eq. (38), we get

$\sum_{n=0}^{\infty} P^n \eta_n(z,t) = z^4 t + 24 z^9 t^3 - p\mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}[z\eta^2(\sum_{n=0}^{\infty} P^n \eta_n(z,t))_{zzzz}]\right\}$ (39)

we take

$k(z,t) = z^4t + 24z^9t^3 = k_0(z,t) + k_1(z,t)$

Where

$k_0(z,t) = z^4t$ and$k_1(z,t) = 24z^9t^3$

Comparing the coefficient of like powers of p, in Eq. (39) the following approximations are obtained.

$P^0{:}\eta_0(z,t) = z^4t$

$P^1{:}\eta_1(z,t) = 24z^9t^3 - \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}\left[(z\eta^2)\left(\eta_{0,ezz}(z,t)\right)\right]\right\}$

$= 24z^9t^3 - z\mathrm{S}^{-1}\left\{\frac{\eta^4}{s^2}\,\mathrm{S}[(24t)]\right\}$

$= 24z^9t^3 - 24z\mathrm{S}^{-1}\left\{\frac{\eta^4}{s^2}\,\mathrm{S}[t]\right\}$

$= 24z^9t^3 - 24z\mathrm{S}^{-1}\left\{\frac{\eta^6}{s^4}\right\}$

$= 24z^9t^3 - 24z^9t^3 = 0$

$P^2{:}\eta_2(z,t) = \mathrm{S}^{-1}\left\{\frac{\eta^2}{s^2}\,\mathrm{S}[(z\eta^2)(\eta_{0zzz}(z,t))]\right\}$

$= 0$

So on within the equal way the rest of the components of iteration method can be received and consequently answer may be written in closed form as

$P^n{:}\eta_n(z,t) = 0 \;\; for \quad \mathrm{n} \geq 1$

$\eta(z,t) = z^4t$ (40)

Which is an exact solution of Eq. (34) and it is the same results obtained by existing methods.

The following plot is obtained using wolfram Alpha software.

3D behavior of an approximate solution of (34) (Figure 4).

CONCLUSION

The major concern of this article is to construct an analytic answer for FOPPDEs with variable coefficients. We have finished this purpose by making use of STHPM. The STHPM is simple to understand and easy to implement, and does not require any restrictive assumptions as required by some existing methods. The non-homogeneous problems were successfully handled by HPM. The obtained exact solution of applying the STHPM is in full agreement with the obtained solutions with those methods available in the literature, such as ADM, DJM, and VIM. Finally, we conclude that STHPM may be considered as a pleasant refinement in the present numerical technique and can locate wide programs in exclusive fields of science.

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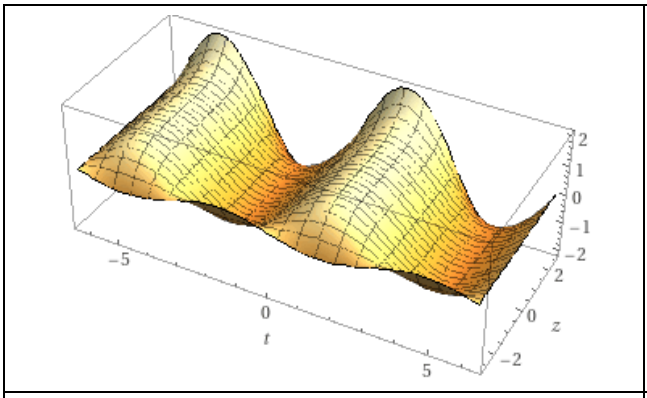


Figure 1: 3D behavior of an approximate solution of (11)

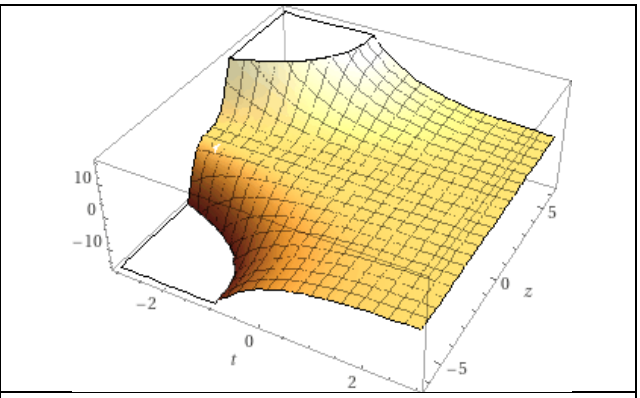


Figure 2: 3D behavior of an approximate solution of (19)

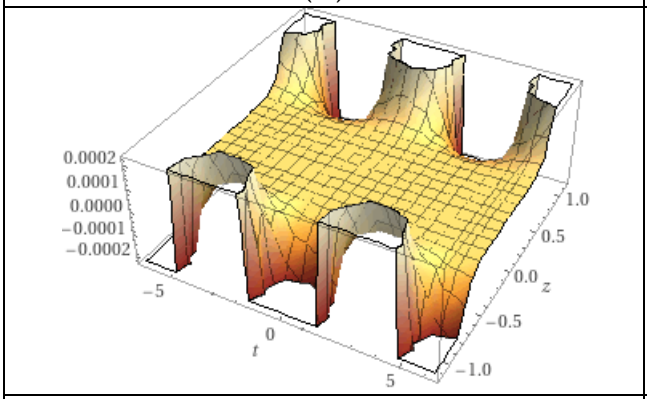


Figure 3: 3D behavior of an approximate solution of (27)

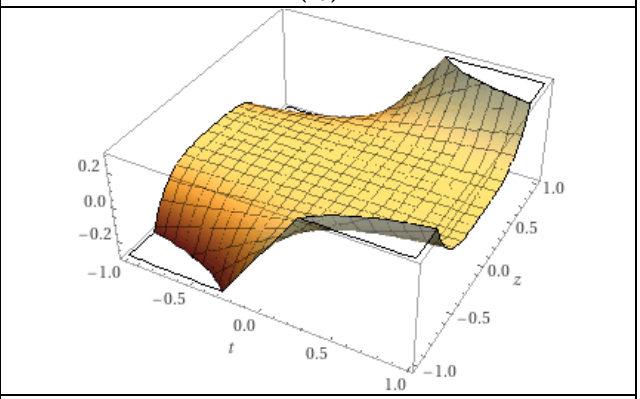


Figure 4: 3D behavior of an approximate solution of (34).

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RESEARCH ARTICLE

Quality of Life of Parents with Children with Special Needs

P Karthik[1]* and (Sr.) Lourdu Mary[2]

[1]Coordinator, RESCAPES and CRDE, Holy Cross College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.
[2]Assistant Professor (S.G) and Research Advisor, Department of Rehabilitation Science, Holy Cross College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
P Karthik
Coordinator,
RESCAPES and CRDE,
Holy Cross College, (Autonomous),
(Affiliated to Bharathidasan University),
Tiruchirappalli, Tamil Nadu, India.
E.Mail: mail4karthikpn@gmail.com

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ABSTRACT

Parents who have children with disabilities are often reported to have physical and psychological distress related to caring for their children, thus affecting their quality of life (QOL). This study explored the FQOL among parents who have children with or without disabilities. A total of 64 parents were recruited for the study using convenience sampling. The Family Quality of Life Scale (FQOL) is a standardised tool designed to measure the overall quality of life experienced by families, particularly those with a member who has a disability. It provides insights into how families perceive their well-being across multiple domains used to measure the QOL among the parents (Bonomi, Patrick, Bushnell *et al*., 2000; Leung, Tay, Cheng *et al*., 1997). Other demographic data, such as financial conditions and family background, were also recorded. FQOL showed a slight but significant decline with increased years of parenting ($r = -0.146$, $p < 0.05$). Male caregivers reported higher FQOL than females ($Z = 3.121$, $p < 0.05$). No link was found between birth order and FQOL. ANOVA revealed significant FQOL differences across disability types ($F = 3.341$, $p < 0.05$), with higher scores for mental handicap and cerebral palsy. The findings highlight that FQOL is influenced by care giving duration, gender, and the child's type of disability. These insights underscore the need for targeted interventions that address long-term caregiver stress, promote gender equity in support systems, and provide condition-specific resources to enhance overall family well-being.

Keywords: Disability, Family Quality of Life, Burden, Social support, Parents with disabled children

INTRODUCTION

In recent years, the term *quality of life* (QOL) has gained increasing attention across various sectors and disciplines. It is recognised as a multidimensional, subjective, and evolving concept that is challenging to define and measure precisely (Dellasoudas, 2006). Quality of life encompasses both material and non-material aspects, including an individual's standard of living, education, health, emotional well-being, interpersonal relationships, social inclusion, opportunities for leisure, and the broader social environment (Fallowfield, 1990; Schalock, Gardner, and Bradley, 2007; Majnemer *et al*., 2008). According to Wehmeyer and Schalock (2001), the core dimensions of quality of life include emotional well-being, material well-being, interpersonal relations, personal development, physical well-being, self-determination, social inclusion, and rights. Building on this broader framework, the concept of *Family Quality of Life* (FQOL) has emerged, particularly in discussions related to families of individuals with disabilities. FQOL is defined as the extent to which families can meet their basic needs, enjoy their time together, participate in leisure and community activities, and experience emotional and physical well-being (Park *et al*., 2003). It reflects family satisfaction based on internal strengths and external support systems (Gardiner and Iarocci, 2012). The presence of a child with a disability in a family inevitably affects this dynamic, often in profound ways. According to Kotzampopoulou (2014), families may experience both positive transformations and considerable challenges. On the negative side, the demands of caregiving can lead to sleep deprivation, increased stress, and a lack of personal time for parents (Dardas and Ahmad, 2014). The initial diagnosis of a disability often triggers complex emotional responses such as denial, guilt, embarrassment, anxiety, or even depression (George and Floyd, 2006). Research consistently demonstrates that parents of children with disabilities report significantly higher levels of stress compared to those with non-disabled children (Dardas and Ahmad, 2014; Hsiao, 2017). This elevated stress can contribute to long-term physical and psychological health concerns, diminishing their quality of life (Leung and Li-Tsang, 2003). Numerous variables influence how deeply a child's disability affects family life and parental well-being. These include the child's personality, the nature and severity of the disability, the family's socio-economic background and parenting style (Mulroy *et al*., 2008; Hu, Wang, and Fei, 2012; Wang *et al*., 2004). Environmental and social contexts, along with the availability of community and institutional support, also play critical roles. As outlined in the *Developmental Disabilities Assistance and Bill of Rights Act* (1991), developmental disabilities are severe, chronic conditions that originate before the age of 22 and are expected to be lifelong. These impairments significantly restrict functioning in key areas such as self-care, language, learning, mobility, and independent living, thereby requiring extensive and often lifelong support and care. For parents, particularly primary caregivers, this often results in immense caregiving responsibilities that limit social interaction, employment, and recreational opportunities, ultimately compromising their own QOL (Azaula, Msall, Buck *et al*., 2000)(Ellis, Upton and Thompson, 2000; Unalan *et al*., 2001). Understanding the impact of disabilities on family life is critical for developing meaningful interventions. Children with disabilities bring unique challenges that necessitate comprehensive and continuous support. Rehabilitation professionals, policymakers, and service providers must be attuned to the needs of both the children and their families. By doing so, they can help enhance FQOL and create systems that promote resilience, inclusion, and sustained well-being across the lifespan.

METHODS

This study used a cross-sectional study design based on power analysis, and a sample size of 64 parents with children with disabilities was recruited for the study from a Special school at Tiruchirappalli. The children were 6 to 18 years of age. Approval from the Ethical Research Committee of The special school was granted prior to the study. All parents signed the informed consent form prior to the commencement of the assessments and interviews. The Family Quality of Life Scale (FQOL) items were collected through interviews from the parents for completion. The Family Quality of Life Scale (FQOL) was developed by Jean Ann Summers and her colleagues at the Beach Center on Disability, University of Kansas. The Family Quality of Life Scale (FQOL) is a standardized tool designed to measure the overall quality of life experienced by families, especially those with a member who has a disability. It provides valuable insights into how families perceive their well-being across multiple domains. The primary purpose of the

FQOL Scale is to assess family well-being in a holistic manner, understand family needs and strengths, inform service planning and policy development, and evaluate the impact of interventions and support programs. The scale focuses on five core domains: Family Interaction, which assesses the quality of relationships, communication, and mutual support; Parenting, which examines how parents support and guide their children, including caregiving and developmental responsibilities; Emotional Well-being, which includes stress levels, mental health, and emotional support within the family; Physical/Material Well-being, which covers financial resources, housing, food security, and health; and Disability-related Support, which evaluates access to services, support systems, and accommodations necessary for a family member with a disability. (Table: 1)

Pearson coefficient of correlation (Table: 2)

The Pearson correlation coefficient between years of parenting and overall Family Quality of Life (FQOL) is -0.146, indicating a weak negative correlation, meaning that as the number of years in parenting increases, the perceived FQOL slightly decreases. Although the strength of the relationship is low, the result is statistically significant ($P < 0.05$), suggesting that this negative association is unlikely to be due to chance. This may reflect cumulative stress, caregiver fatigue, or emotional and practical burdens that tend to build over time in long-term parenting roles. The finding highlights the importance of providing sustained support systems and interventions to promote and maintain FQOL among parents, particularly those engaged in long-term caregiving.

Z test between the Gender of the Respondents with regard to FQOL (Table: 3)

The Z-test analysis reveals a statistically significant difference in the Overall Family Quality of Life (FQOL) between male and female respondents, with a Z value of 3.121 and $P < 0.05$, indicating that the observed difference is unlikely due to chance. Males reported a higher mean FQOL score (27.62) compared to females (26.00), with standard deviations of 4.146 and 4.463 respectively. This suggests that male respondents perceive a relatively better quality of family life than their female counterparts, possibly due to variations in role expectations, caregiving responsibilities, or access to support systems. The findings highlight the need for gender-sensitive approaches in enhancing family well-being and addressing the specific challenges faced by women in maintaining FQOL.

Association between Birth Order and FQOL (Table: 4)

The chi-square test results indicate that there is no statistically significant association between birth order and Family Quality of Life (FQOL) among the respondents, as the chi-square value is $\chi^2 = 4.260$ with 2 degrees of freedom and $P > 0.05$. This means that whether a person is first born, middle born, or last born does not significantly influence their reported levels of FQOL. Although variations are seen in the frequency distribution—for example, 17 first-born individuals reported high FQOL compared to 14 middle-born and 6 last-born—the differences are not large enough to be considered statistically meaningful. Hence, birth order does not appear to play a decisive role in determining family quality of life in this sample.

One Way Analysis of Variance among the Type of disability with regard to FQOL (Table 5)

The one-way ANOVA comparing overall Family Quality of Life (FQOL) scores across the four disability groups (G1: Cerebral Palsy, G2: Down Syndrome, G3: Mental Handicap, G4: Developmental Delay) yielded an F-statistic of 3.341 ($df = 3, 274$) with $P < 0.05$, indicating a significant difference among groups. The mean FQOL scores were highest for parents of children with Mental Handicap (G3 = 34.72) and Cerebral Palsy (G1 = 34.52), followed by Down Syndrome (G2 = 33.79), and lowest for Developmental Delay (G4 = 32.18). This suggests that the type of disability is significantly associated with family well-being, with parents of children with Mental Handicap and Cerebral Palsy reporting better overall quality of life than those caring for children with Down Syndrome or Developmental Delay.

RESULTS

The survey included 64 respondents, with data collected on various socio-demographic variables and types of disability among children. The majority of respondents (23.44%) were aged 26–30 years, and the sample included

slightly more females (54.69%) than males (45.31%). Most respondents were married (82.81%), indicating family-based caregiving dynamics. Regarding educational status, 28.13% were graduates and 23.44% had completed up to HSC, reflecting a moderate educational background overall. Employment data revealed that half of the participants (50%) were employed full-time, and about one-third (32.81%) were working part-time. In terms of income, 32.81% of families earned between ₹20,001 and ₹30,000 monthly, with 25% earning ₹30,001–₹40,000, suggesting a predominantly lower-middle-income group. The religious composition showed a majority of Hindus (65.63%), followed by Christians (23.44%) and Muslims (10.94%). Among the participants, 25% had less than 5 years of parenting experience, 21.88% had 5–10 years, while 26.56% each had 11–15 years and over 16 years. This indicates a fairly even distribution between newer and more experienced caregivers. Diagnostically, children with developmental delay (34.38%) and mental handicap (32.81%) made up the largest groups, followed by those with cerebral palsy (18.75%) and Down syndrome (14.06%). Birth order, revealed that nearly half were first-born (48.43%), about one-third were second-born (34.37%), and the remainder were third-born (17.18%). This distribution suggests that first-born individuals predominated in the caregiving roles sampled, with fewer participants from later birth positions. Such a pattern may reflect family size dynamics or cultural factors influencing parental responsibilities, and should be kept in mind when exploring how ordinal position might relate to family quality of life and caregiving experiences. The data points to the diverse yet predominantly middle-income, married, and moderately educated demographic background of caregivers. The diagnostic spread further highlights the relevance of support programs addressing developmental delays and mental handicaps. Statistical tests revels that A Pearson correlation analysis showed a weak negative correlation between years of parenting and Family Quality of Life (FQOL) ($r = -0.146$), which was statistically significant ($P < 0.05$). This suggests that FQOL slightly decreases as years of parenting increase, possibly due to accumulated stress or caregiver fatigue. The analysis revealed a statistically significant difference in Family Quality of Life (FQOL) between male and female respondents, with males reporting a higher mean FQOL (27.62) compared to females (26.00). The Z-test value was 3.121 with $P < 0.05$, indicating that the difference is unlikely due to chance. This suggests that gender plays a role in perceived FQOL, possibly due to differences in caregiving responsibilities or access to support. In contrast, the chi-square test examining the association between birth order and FQOL showed no statistically significant relationship ($\chi^2 = 4.260$, $df = 2$, $P > 0.05$). Although there were some variations in FQOL scores across birth order categories, the differences were not significant, indicating that birth order does not appear to influence FQOL among the respondents. A one-way ANOVA showed a significant difference in FQOL across disability groups ($F = 3.341$, $p < 0.05$). Higher FQOL scores were reported by parents of children with disability it indicates that disability type influences family well-being.

DISCUSSION

The findings confirm the influence of caregiving burden on Family Quality of Life (FQOL), echoing results from earlier research. The negative correlation between years of parenting and FQOL aligns with Ellis *et al*. (2000) and Elmstahl *et al*. (1996), who reported cumulative stress and caregiver fatigue as key contributors to declining caregiver well-being over time. The gender disparity in FQOL is consistent with Dardas and Ahmad (2014) and Leung and Li-Tsang (2003), who noted that female caregivers, often the primary caretakers, experience more psychological distress and lower well-being than males. Cultural expectations and unequal distribution of caregiving tasks may account for this gap. The non-significance of birth order reflects findings from Mulroy *et al*. (2008), who suggested that the influence of ordinal position is less crucial than the child's needs and family coping resources. The significant variation in FQOL across types of disabilities corroborates Hu *et al*. (2012) and Wang *et al*. (2004), who emphasized that severity and nature of the child's condition strongly shape family dynamics. Higher FQOL among caregivers of children with cerebral palsy and mental handicap may be due to better-established support systems for these conditions, as noted by Azaula *et al*. (2000). Overall, the results reinforce the multidimensional impact of caregiving on family life, highlighting the need for disability-specific support systems, long-term stress management resources, and gender-sensitive interventions. As suggested by Gardiner and Iarocci (2015) and Summers *et al*. (2005), FQOL is not only shaped by individual factors but also by broader social, economic, and institutional contexts. Overall, these

results reinforce the complex interplay of demographic, psychological, and diagnostic factors in shaping family quality of life and underscore the need for tailored, inclusive policy and practice interventions.

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Table 1

SL No	Variables	No of Respondents	percentage
1	Age		
	21-25	9	14.06
	26-30	15	23.44
	31-35	12	18.75
	36-40	13	20.31
	41-45	8	12.50
	46 and above	7	10.94
2	Gender		
	Female	35	54.69
	Male	29	45.31
3	Marital Status		
	Married	53	82.81
	Divorced	11	17.19
4	Education		
	Illiterate	1	01.56
	Primary	6	09.38
	Secondary	7	10.94
	Upto HSC	15	23.44
	Graduate	18	28.13
	Post Graduate	10	15.63
	Above PG	7	10.94
5	Work Status		
	Unemployed	11	17.19
	Full-Time	32	50.00
	Part-Time	21	32.81
6	Monthly Income (INR) ₹		
	Less than 10,000	10	15.63
	10,001 - 20,000	13	20.31
	20,001 – 30,000	21	32.81
	30,001 – 40,000	16	25.00
	Above 40,000	4	06.25
7	Religion		
	Christian	15	23.44
	Hindu	42	65.63
	Islam	7	10.94
8	Years of Parenting		
	Less than 5 years	16	25.00
	5-10 years	14	21.88
	11-15 years	17	26.56

	Above 16 years	17	26.56
9	Diagnosis		
	Cerebral palsy	12	18.75
	Down Syndrome	9	14.06
	Mental handicap	21	32.81
	Developmental Delay	22	34.38
10	Ordinal Position		
	First	31	48.43
	Second	22	34.37
	Third	11	17.18

Table 2: Pearson coefficient of correlation

FQOL	Correlation value	Statistical inference
Years of Parenting	-.146	P < 0.05 Significant

Table 3: Z test between the Gender of the Respondents with regard to FQOL

FQOL Gender	Mean	Standard Deviation	Statistical Inference
Male	27.62	4.146	Z=3.121 P<.05 Significant
Female	26.00	4.463	

Table 4: Association between Birth Order and FQOL

FQOL	Birth Order			Statistical Inference
	First Born	Middle Born	Last Born	
Low	14	08	5	χ^2 =4.260 df = 2 P >0.05 Not significant
High	17	14	6	

Table 5: One Way Analysis of Variance among the Type of disability with regard to FQOL

FQOL Variance	Sum of Squires	Mean Squires	df	Mean	Statistical Inference
FQOL				G1=34.52	F=3.341
Between Groups	207.183	69.060	3	G2=33.79	P<.05
Within Groups	5664.232	20.672	274	G3=34.72	Significant
				G4=32.18	

RESEARCH ARTICLE

DFT Analysis and ADMET Prediction of (1E, 2E) - 1- Benzylidene – 2 - (3 - Bromo Methoxy Benzylidene) Hydrazine

S. Rajapriya[1] and R. Arulmani[2]*

[1]Research Scholar, Department of Chemistry, Government Arts College, C-Mutlur, (Affiliated to Annamalai University, Annamalai Nagar), Chidambaram, Cuddalore, Tamil Nadu, India.
[2]Assistant Professor, Department of Chemistry, Government Arts College, C-Mutlur, (Affiliated to Annamalai University, Annamalai Nagar), Chidambaram, Cuddalore, Tamil Nadu, India.

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***Address for Correspondence**
R. Arulmani
Assistant Professor,
Department of Chemistry,
Government Arts College, C-Mutlur,
(Affiliated to Annamalai University, Annamalai Nagar),
Chidambaram, Cuddalore, Tamil Nadu, India.
E.Mail: drrarulmani@gmail.com

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ABSTRACT

In the present research, a novel unsyymetrical azine has been synthesised and its DFT study has been carried out using Gaussian09 programme. The structure of titled compound is optimized and determined as co-planar with c1-point group. Their bond angles, lenghths and torsional angles are determined and matched well with literature valued. The HOMO-LUMO analysis demostrated that present compound is highly recative due to its low energy gap. Its NBO, MEP and Mulliken charges are calculated. The ADMET parameters are predicted with the help of SwissADME web tool along with its Boiled-egg and Bioavailability radar model predictions. The results showed that titled compound has good bioavailability material.

Keywords: Unsymmetrical azine, DFT study, Optimization, NBO analysis, NLO property and ADMET prediction.

INTRODUCTION

Azines, a class of organic compounds containing a double bond between two carbon atoms and one nitrogen atom, are crucial intermediates in organic synthesis and materials chemistry [1,2]. Azines can be broadly categorized into symmetrical and unsymmetrical types, with unsymmetrical azines being characterized by the presence of two different substituents attached to the nitrogen or carbon atoms involved in the azine linkage [3]. These unsymmetrical azines, due to their unique structural features, offer distinct chemical reactivity and potential

applications, making them a topic of significant interest in contemporary chemical and pharmaceutical research [4]. The general formula for an unsymmetrical azine is $R_1C{=}NNCR_2$, where R_1 and R_2 are different substituents, either alkyl, aryl, or other functional groups [5]. The unsymmetry of the substituents impacts the electronic properties and reactivity of the molecule, leading to distinct steric and electronic interactions that govern their behavior in both synthetic and catalytic processes [6]. The nature of the substituents on the azine backbone affects the stability, nucleophilicity, and electrophilicity of the compound, which is crucial for tuning the reactivity for various applications [7]. These compounds have demonstrated significant biological activity, particularly as antitumor, antibacterial, and anti-inflammatory agents [8]. Their ability to interact with biological targets such as enzymes and receptors makes them potent candidates for drug development [9]. Due to their electron-rich nature, they have also found applications in organic electronics, such as organic light-emitting diodes (OLEDs) and organic solar cells [10]. Unsymmetrical azines can form stable complexes with metal ions, which can be exploited in catalysis and materials chemistry [3,6]. Density Functional Theory (DFT) analysis plays a crucial role in understanding the reactivity and stability of unsymmetrical azines. The electron density distribution, molecular orbitals, and energy levels can be computed to predict the most likely reaction pathways, stability, and electronic properties of these compounds [7]. Such analysis is pivotal for designing new azine derivatives with optimized properties for specific applications, such as drug design or material development [4,9]. For pharmaceutical applications of unsymmetrical azines, understanding their ADMET properties is crucial. ADMET studies predict the pharmacokinetic and toxicological behavior of compounds, which is essential for evaluating the drug-likeness of new molecules [10]. These properties are assessed through computational models and in vitro/in vivo experiments. Unsymmetrical azines may exhibit favorable ADMET characteristics, but these properties must be optimized for safe and effective therapeutic use [8,9]. Furthermore, molecular docking studies have become an essential tool for understanding the interactions between drug candidates, such as unsymmetrical azines, and their target proteins. Docking simulations allow researchers to predict the binding affinity, orientation, and interactions between a small molecule and its receptor [7]. This technique is particularly valuable in the early stages of drug discovery, as it can guide the design of azine-based molecules with improved specificity and potency for their biological targets [9,10].

Experimental

Synthesis of (1*E*,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine

About 0.01mol of 3-bromo-4-methoxy benzaldehyde and 5ml of hydrazine hydrate (0.01mol) were taken in a stoppered conical flask. The mixture was dissolved in ethanol and stirred well for about half an hour [11]. The formed hydrazine solid was separated as white solid and it was stirred with benzaldehyde (0.01 mol). The mixture was cooled and yellow solid mass separated out was removed by filtration, dried and recrystallized from ethanol. In every step, the completion of the reaction was monitored by TLC. The synthetic method is given in Scheme-1.

RESULTS AND DISCUSSION

Spectral data: mp:119C; MW, FT-IR: 3032cm^{-1}, 2983cm^{-1}, 1612cm^{-1}, 1544cm^{-1}, 912cm^{-1}, 532cm^{-1}; ^{1}H NMR (δppm): 5.028, 6.032-7.992, 8.124; ^{13}C NMR(δppm): 55.24, 119-138.34, 157.24.

DFT analysis

Molecular Geometry

The DFT calculations are carried out using Gaussian 09w software [12] with the help of Gauss view using B3LYP/611++G (d,p) basis set. From these calculations, the reactivity nature and reactivity sites are predicted. The Density Functional Theory (DFT) study of unsymmetrical azines involves computational methods to understand the electronic properties, structure and reactivity of azine molecules that lack symmetry in their molecular structure. Azines typically refer to heterocyclic compounds containing nitrogen atoms, often with a structure similar to pyridine or pyrimidine. Unsymmetrical azines are those where the substituents or the positions of nitrogen atoms are not identical, which can lead to varied electronic behaviors compared to their symmetrical counterparts. The titled compound is a non- planar molecule with C1 point group symmetry, as predicted by the DFT analysis and the

corresponding optimized structure and selected bond paramreters are shown in Figure-1 and Table-1 respectively. As shown in Table-1, the typical C–C aromatic bond lengths in the benzene rings range from 1.386 to 1.411 Å, which are in agreement with delocalized sp^2-hybridized carbon systems. Notably, the C3–Br3 bond measures 1.943 Å, consistent with literature-reported values for para-substituted aryl bromides [13]. The C4–O4 bond, representing the methoxy linkage, has a length of 1.391 Å, supporting partial double bond character due to resonance. The hydrazone linkage displays a N8–N9 bond length of 1.425 Å and a N9–C10 bond length of 1.298 Å, indicating partial double bond character in the latter due to π-conjugation, as also noted in structurally related hydrazones [14]. The C1–N8 distance is unusually long at 2.411 Å, possibly due to torsional strain or steric hindrance, which weakens π-overlap in that region. The aromatic ring bond angles cluster around 120°, consistent with trigonal planar carbon geometry. The N8–N9–C10 angle is 112.4°, slightly lower than the ideal trigonal planar value, likely due to lone pair–bond pair repulsion on the nitrogen. An unusual bond angle of 147.1° for C6–C1–N8 suggests significant deviation from planarity near the hydrazone bridge, possibly accommodating steric strain or conjugative effects. The majority of dihedral angles in the aromatic systems are close to 0° or 180°, confirming coplanarity and conjugation within the ring systems. The C1–N8–N9–C10 dihedral angle is 179.1°, indicating a planar and fully conjugated hydrazone bridge, which is essential for electronic delocalization. A similar observation is made for C10–C11–C12–C13 (180.0°) and C5–C6–C1–N8 (180.0°), further affirming the extended conjugation pathway across the molecule [15]. These structural parameters reveal that the molecule maintains an extended π-conjugated system through the central hydrazone moiety and substituted aromatic rings. The optimized geometry thus supports its potential in electronic, photophysical, and biological applications, including as an NLO material or antimicrobial agent [16, 17].

Frontier Molecular Orbbital Study

The Frontier Molecular Orbitals (FMO) study of an zzine compound using the given parameters involves the analysis of the Highest Occupied Molecular Orbital (HOMO) and the Lowest Unoccupied Molecular Orbital (LUMO). These orbitals shown in Figure-2 play a critical role in determining the chemical reactivity and electronic properties of a molecule. A smaller energy gap displayed in Table-2usually corresponds to a molecule that is more reactive, as it is easier to excite an electron from the HOMO to the LUMO. In this case, the energy gap of 3.990 eV suggests that the molecule has significantly higher reactivity and low stability. Ionization energy is higher and low electron affinity. The lower energy gap implies its reactivity which may be active against biological applications. The calculated ionization potential (I) and electron affinity (A) of 6.066 eV and 2.076 eV, respectively, are consistent with the FMO results, where a relatively low electron affinity and a high ionization potential suggest a resistance to electron acceptance but moderate susceptibility to electron loss. The chemical potential (μ) of -4.071 eV implies a moderate tendency of the molecule to release electrons, while the global hardness (η) of 1.995 eV and global softness (ν) of 0.501 eV^{-1} indicate that the molecule has a balanced stability and reactivity, suggesting its potential as a moderate electron donor or acceptor in chemical interactions [18, 19]. The electrophilicity index (ω) of 4.154 eV reveals the molecule's electrophilic character, quantifying its stabilization upon acquiring additional electronic charge. The chemical softness (s), calculated as 4.071 eV, further supports the molecule's moderate reactivity, particularly in electron-rich environments. These electronic descriptors suggest that the compound possesses a relatively moderate chemical hardness and a moderate HOMO-LUMO gap, indicating potential applications in optoelectronic devices or as a bioactive molecule where controlled reactivity is advantageous. The FMO analysis, in combination with the global reactivity parameters, illustrates the capacity of (1*E*,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine to participate in frontier orbital interactions, making it an interesting candidate for further theoretical and experimental studies of electron transfer processes [20, 21].

NBO analysis

The NBO analysis of (1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine is displayed in Table-3 and it reveals significant intramolecular charge transfer and stabilization through hyperconjugative interactions. Key donor orbitals, such as the bonding orbitals BD(2)C1-N17 and BD(2)C2-C4, donate electron density into antibonding acceptor orbitals BD(2)C2-C2*, BD(2)N19-C19* and BD(2)C3-C3*, with stabilization energies ranging from 39.5 to 94.8 kJ/mol. The largest stabilization occurs from BD(2)C2-C4 → BD(2)C3-C3* (94.8 kJ/mol), indicating strong delocalization of electron density across the aromatic system and enhancing π-conjugation. The lone pairs on

nitrogen (LP(1)N17 and LP(1)N19) also show interactions with σ* orbitals (BD(1)C1-H1*, BD(1)C20-H20*), suggesting hyperconjugative interactions that contribute to overall molecular stabilization. The oxygen lone pair (LP(2)O11) donating into BD(2)C7-C7* (36.4 kJ/mol) supports the electron-rich character of the methoxy-substituted ring, facilitating resonance-assisted stabilization and electronic delocalization [22, 23]. The donor-acceptor interplay in this system highlights its charge transfer capabilities, with notable stabilization energies observed for hyperconjugative interactions involving aromatic and heteroatomic centers. For instance, the BD(2)C7-C9 → BD(2)C2-C2* (88.6 kJ/mol) and BD(2)C26-C27 → BD(2)C22-C22* (89.2 kJ/mol) interactions reflect strong delocalization between adjacent rings and substituents, reinforcing molecular stability and electron density distribution. The involvement of nitrogen lone pairs (N17 and N19) in hyperconjugation with adjacent σ* orbitals indicates potential sites for nucleophilic attack and suggests a role in biological interactions or chemical reactivity. The electron delocalization across the azine bridge and aryl substituents contributes to the compound's unique electronic properties, essential for understanding its potential reactivity and interaction with external electrophiles or nucleophiles. These NBO-derived insights align with theoretical studies that emphasize the role of hyperconjugation and lone pair-π* interactions in modulating molecular reactivity and stability [24, 25].

MEP analysis

The Molecular Electrostatic Potential (MEP) analysis of (1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine, calculated using theDFT/B3LYP/6-311++G(d,p) level, offers valuable insights into the electronic distribution and potential reactive sites of the molecule. The MEP surface, as depicted in the Figure-3, illustrates regions of varying electrostatic potential where the most negative potentials (red regions) are localized around the electronegative oxygen atom of the methoxy group and nitrogen atoms of the azine moiety, indicating sites with high electron density and potential for electrophilic attack. Conversely, the positive electrostatic regions (blue) are observed near the hydrogen atoms and around the less electronegative carbons, suggesting susceptibility to nucleophilic attack. The MEP highlights the asymmetric electronic distribution arising from the bromine and methoxy substituents, reinforcing the molecule's polar nature. This analysis not only complements the FMO and NBO results by identifying reactive sites but also underscores the potential of the compound in interactions with biological targets or catalytic systems, where regions of varying electrostatic potential can play a crucial role in reactivity and binding affinity [26, 27].

Mulliken atomic chrges

The data shown in Table-4, reveal that the oxygen atom (O11) carries a significant negative charge (-0.549), reflecting its high electronegativity and strong electron-withdrawing capability. The nitrogen atoms (N17 and N19) also exhibit substantial negative charges (-0.281 and -0.282), highlighting their roles as potential sites for electrophilic attack. In contrast, several carbon atoms, such as C1 (0.176), C9 (0.310), and C13 (0.297), are positively charged, suggesting electron-deficient regions likely to attract nucleophilic species. The bromine atom (Br12) shows a moderate positive charge (0.153), indicating its involvement in modulating the electronic environment of the molecule. These charge distributions emphasize the polarized nature of the molecule, particularly around the methoxy and bromine-substituted regions, and align with the results from MEP and NBO analyses, confirming potential sites for chemical reactivity and molecular interactions [28, 29].

ADMET analysis

The ADMET evaluation of *(1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine* via the SwissADME platform as shown in Table-5 reveals its promising pharmacokinetic and drug-likeness characteristics. With a molecular weight of 317.18 g/mol, a low topological polar surface area (TPSA = 33.95 Å²), and zero hydrogen bond donors, the compound demonstrates high gastrointestinal (GI) absorption and is predicted to be blood-brain barrier (BBB) permeant, enhancing its central nervous system (CNS) accessibility [30]. It is not a substrate for P-glycoprotein (Pgp), indicating reduced risk of efflux-mediated drug resistance. The compound inhibits several cytochrome P450 enzymes, namely CYP1A2, CYP2C19, and CYP2C9, which could potentially lead to drug–drug interactions, whereas CYP2D6 and CYP3A4 inhibition was not observed, indicating partial metabolic stability [31].The compound exhibits moderate lipophilicity, with consensus Log P of 3.87, and individual values from five different models ranging from

3.22 to 4.91, suggesting favorable membrane permeability and bioavailability. Water solubility predictions from ESOL, Ali, and Silicos-IT methods categorize it as moderately soluble, with log S values between –4.37 to –6.06, which is acceptable for oral drug candidates. Importantly, no violations of Lipinski, Ghose, Veber, Egan, or Muegge rules were observed, implying excellent drug-likeness. The bioavailability score of 0.55 further supports its potential as a lead compound [32]. From a medicinal chemistry standpoint, the molecule is devoid of PAINS (Pan-Assay Interference Structures) alerts, indicating a low probability of false biological activity, although it presents one Brenk and one leadlikeness violation, possibly due to the bromine substituent or higher lipophilicity [33]. The synthetic accessibility score of 2.37 denotes that the compound is easy to synthesize, which is advantageous for future development.

Boiled-Egg prediction model

The Boiled-Egg model prediction for *(1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine* is illustrated in Figure-4, generated via the *SwissADME* web tool [30]. The compound (Molecule 1) is located within the yellow region (yolk) of the plot, which suggests a high probability of blood-brain barrier (BBB) penetration. Additionally, the molecule also falls within the white region, indicating favorable passive gastrointestinal absorption (HIA). The red circle denotes that the molecule is a PGP- substrate, meaning it is not actively effluxed by the P-glycoprotein transporter, potentially enhancing its CNS bioavailability. This dual prediction supports the compound's potential as a drug-like candidate with good oral bioavailability and central nervous system accessibility.

Bioavailablity Radar model

The SwissADME Bioavailability Radar model shown in Figure-5 provides a visual overview of drug-likeness for *(1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine* based on six physicochemical properties: lipophilicity (LIPO), size, polarity (POLAR), insolubility (INSOLU), insaturation (INSATU), and flexibility (FLEX). In the radar plot, the red line represents the molecular property values, while the pink area marks the optimal range for oral bioavailability. The molecule falls within the acceptable zone for lipophilicity, size, and polarity, indicating favorable membrane permeability and molecular weight. Slight deviations in flexibility and solubility suggest limited aqueous solubility and moderate conformational freedom, yet these are not significantly outside the ideal range. Overall, the radar plot suggests that the compound possesses satisfactory drug-like characteristics, aligning well with criteria for oral bioavailability [30].

CONCLUSION

In conclusion, the comprehensive investigations carried out on (1E,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine highlight its unique structural features and promising chemical profile. Spectral characterization confirmed the successful synthesis and molecular integrity of the compound, while DFT studies elucidated its non-planar geometry, extended π-conjugation, and the presence of distinct electronic domains arising from the unsymmetrical azine framework. The molecule exhibits notable intramolecular charge transfer and stabilization through hyperconjugative interactions, as revealed by NBO analysis, and its frontier molecular orbital characteristics point to a moderate HOMO-LUMO gap, supporting balanced chemical reactivity and potential for further electron transfer applications. MEP and Mulliken charge analyses identified key nucleophilic and electrophilic sites, underscoring the molecule's reactivity and scope for molecular interactions. ADMET predictions indicated high gastrointestinal absorption, blood-brain barrier permeability, favorable drug-likeness, and the capacity for central nervous system activity, alongside synthetic accessibility and moderate solubility. Overall, these synergistic structural, electronic, and pharmacokinetic features suggest that the titled compound is an excellent candidate for future exploration as a functional azine scaffold in electronic, photophysical and pharmacological domains.

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Table-1 Selected bond parameters of (1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine

Bond length		Bond angle		Dihedral angle	
C1-C2	1.410	C1-C2-C3	120.0	C1-C2-C3-C4	0.1
C2-C3	1.386	C1-C2-H2	119.1	C1-C2-C3-C4	0.4
C3-C4	1.405	C2-C3-C5	90.7	C1-C6-C5-H5	179.7
C1-C6	1.408	C2-C1-C6	118.8	C1-C2-C3-Br3	-177.9
C1-C7	1.459	C1-C6-H6	119.5	C1-C8-C7-H7	179.9
C2-H2	1.083	C2-C3-C4	121.4	C1-N8-N9-C10	179.1
C3-C5	2.412	C2-C3-Br3	118.5	C3-C2-C1-C6	0.3
C6-H6	1.086	C4-O20-C4'	117.2	C3-C1-C2-H2	-179.4
C5-H5	1.084	C5-C4-O4	118.2	C4-O4-C4'-H4(1)	178.1
C4-O4	1.391	C6-C1-C7	119.9	C4-O4-C4'-H4(2)	-63.1
C3-Br3	1.943	C6-C5-H5	121.3	C4-O4-C4'-H4(3)	59.3
O4-C4'	1.472	O4-C4'-H4(1)	105.0	C5-C6-C1-C7	179.8
C4'-H4(1)	1.089	O4-C4'-H4(2)	110.3	C5-C4-O4-C4'	110.0
C4'-H4(2)	1.095	O4-C4'-H4(3)	110.5	C5-C6-C1-N8	180.0
C4'-H4(3)	1.092	C6-C1-N8	147.1	C6-C1-N8-N9	-0.1
C1-N8	2.411	C8-C7-H7	120.1	C6-C5-C4-O4	177.2
C7-H7	1.092	C1-N8-N9	143.6	C7-C1-C6-H6	0.0
N8-N9	1.425	N8-N9-C10	112.4	N8-N9-C10-H10	0.0
N9-C10	1.298	N9-C10-H10	119.8	N8-N9-C10-C16	-179.9
C10-H10	1.093	N9-C10-C16	152.0	N8-N9-C10-C11	-179.9
C10-C16	2.474	N9-C10-C11	122.2	N9-C10-C11-C12	0.0
C10-C11	1.460	C10-C11-C12	121.7	C10-C11-C12-C13	180.0

C11-C12	1.411	C11-C12-C13	120.2	C11-C12-C13-C14	0.0
C12-C13	1.394	C12-C13-C14	120.3	C12-C13-C14-C15	0.0
C13-C14	1.404	C13-C14-C15	119.9	C9-C10-C16-H16	179.9
C14-C15	1.400	C10-C16-H16	88.4	C16-C11-C12-H12	-180.0
C16-H16	1.086	C11-C12-H12	118.6	C11-C12-C13-H13	-180.0
C12-H12	1.084	C12-C13-H13	119.8	C12-C13-C14-H14	-180.0
C13-H13	1.085	C13-C14-H14	120.0	C10-C16-C15-H15	179.9
C14-H14	1.085	C16-C15-H15	119.9		
C15-H15	1.085				

Table-2 Frontier Orbital analysis of (1E,2E)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine

Parameters	eV
E_{HOMO}	-6.006
E_{LUMO}	-2.076
Energy gap	3.990
Ionization energy(I)	6.066
Electron affinity(A)	2.076
Global hardness(η)	1.995
Chemical potential(μ)	-4.071
Electrophilicity index(ω)	4.154
Chemical softness(s)	4.071
global softness ν = 1/ η	0.501

Table-3 NBO analysis of (1*E*,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine

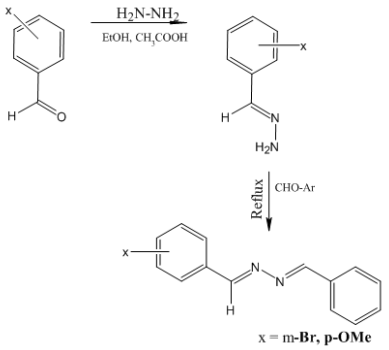
DONOR	ACCEPTOR	E2 (KJ/mol)
BD(2)C1-N17	BD*(2)C2-C2	39.497
BD(2)C1-N17	BD*(2)N19-C19	48.283
BD(2)C2-C4	BD*(2)C1-N1	84.308
BD(2)C2-C4	BD*(2)C3-C3	94.768
BD(2)C2-C4	BD*(2)C7-C7	86.358
BD(2)C3-C5	BD*(2)C2-C2	66.526
BD(2)C3-C5	BD*(2)C7-C7	86.902
BD(2)C7-C9	BD*(2)C2-C2	88.575
BD(2)C7-C9	BD*(2)C3-C3	77.948
BD(2)N19-C20	BD*(2)C1-N1	50.626
BD(2)N19-C20	BD*(2)C22-C22	38.451
BD(2)C22-C23	BD*(2)N19-C19	87.529
BD(2)C22-C23	BD*(2)C24-C24	80.082
BD(2)C22-C23	BD*(2)C26-C26	81.546
BD(2)C24-C25	BD*(2)C22-C22	81.337
BD(2)C24-C25	BD*(2)C26-C26	88.910
BD(2)C26-C27	BD*(2)C22-C22	89.203
BD(2)C26-C27	BD*(2)C24-C24	76.484
LP (2)O11	BD*(2)C7-C7	36.401
LP (1)N17	BD*(1)C1-H1	36.694
LP (1)N19	BD*(1)C20-H20	36.066

Table-4Mulliken charges of (1*E*,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene)hydrazine

Atoms	Mulliken charges	Atoms	Mulliken charges
1C	0.176	17N	-0.281
2C	0.090	19N	-0.282
3C	0.083	20C	0.176
4C	-0.015	22C	-0.016
5C	-0.304	23C	0.078
7C	0.028	24C	0.040
9C	0.310	25C	-0.001
11O	-0.549	26C	0.020
12Br	0.153	27C	-0.004
13C	0.297		

Table-5 ADMET parameters of (1*E*,2*E*)-1-benzylidene-2-(3-bromo-4-methoxybenzylidene) hydrazine

Physicochemical Properties		Pharmacokinetics	
Formula	$C_{15}H_{13}BrN_2O$	GI absorption	High
MW	317.18 g/mol	BBB permeant	Yes
Heavy atoms	19	Pgp substrate	No
Aromatic heavy atoms	12	CYP1A2 inhibitor	Yes
Fraction Csp3	0.07	CYP2C19 inhibitor	Yes
Rotatable bonds	4	CYP2C9 inhibitor	Yes
H-bond acceptors	3	CYP2D6 inhibitor	No
H-bond donors	0	CYP3A4 inhibitor	No
MR	82.26	log Kp (cm/s)	-5.42
TPSA	33.95	Druglikeness	
Lipophilicity		Lipinski violations	0
iLOGP	3.22	Ghose violations	0
XLOGP3	3.96	Veber violations	0
WLOGP	3.91	Egan violations	0
MLOGP	3.95	Muegge violations	0
Silicos-IT Log P	4.91	Bioavailability Score	0.55
Consensus Log P	3.87	Medicinal Chemistry	
Water Solubility		PAINS alerts	0
ESOL Log S	-4.5	Brenk alerts	1
ESOL Class	Moderately soluble	Leadlikeness violations	1
Ali Log S	-4.37	Synthetic Accessibility	2.37
Ali Class	Moderately soluble		
Silicos-IT LogSw	-6.06		
Silicos-IT class	Moderately soluble		



Scheme-1

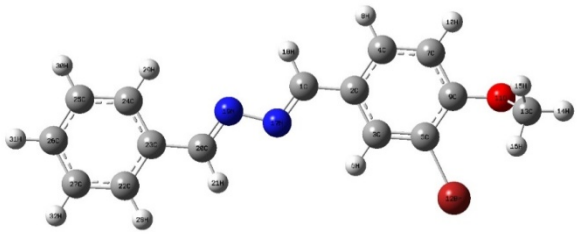
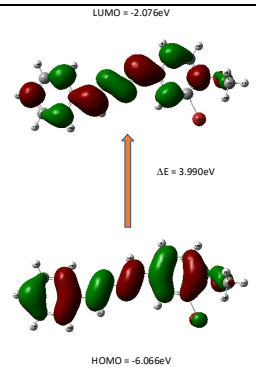


Figure-1: Optimized Structure



vFigure-2: HOMO-LUMO's of titled unsymmetrical azine

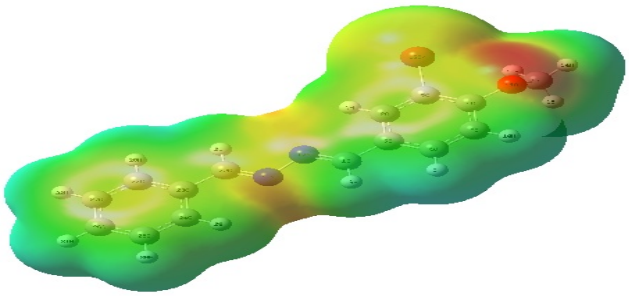


Figure-3: Molecular Electrostatoic Potential map of titled azine

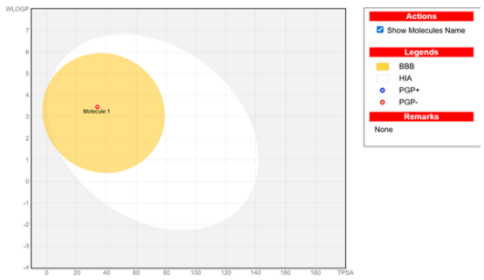


Figure-4: Boiled-Egg prediction model from Swiss ADME tool for titled azine

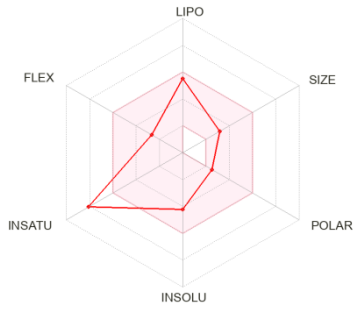


Figure-5: SwissADME Bioavailability Radar model for titled azine

RESEARCH ARTICLE

A Cross - Sectional Study on Assessment of Different Types of *Peenisam* (Sinusitis) Patients and their Body Constitution Based on the Siddha Concept among Patients Reporting at Ayothidoss Pandithar Hospital, National Institute of Siddha

Pushpalatha .N[1]*, Nalini Sofia .H[2] and Vetha Merlin Kumari .H[3]

[1]PG Scholar, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, (Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Guindy), Chennai, Tamil Nadu, India.
[2]Associate professor, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, (Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Guindy), Chennai, Tamil Nadu, India.
[3]Professor and Head of the Department, Department of Pothu Maruthuvam, National Institute of Siddha, Tambaram Sanatorium, (Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Guindy), Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Pushpalatha .N
PG Scholar,
Department of Maruthuvam,
National Institute of Siddha, Tambaram Sanatorium,
(Affiliated to The Tamil Nadu Dr. M.G.R Medical University, Guindy),
Chennai, Tamil Nadu, India.
E.Mail: pushpalathanagarajan07@gmail.com

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ABSTRACT

Siddhars categorized human diseases into 4448 categories. Among these, 86 are classified as nasal diseases, with *peenisam* being one of them. *Peenisam* is a prevalent condition that affects a substantial number of individuals each year. It can impact people of all ages and tends to worsen with the change of seasons, particularly in winter. This research investigates the factors that contribute to the worsening of the disease as outlined in *Siddha* texts, the various types of *peenisam* that affect a broader population, and the body constitution types that are more at risk for this specific condition. The study aims to ascertain the distribution of different types of *peenisam* mentioned in *Siddha* literature and to analyze the body constitutions of patients attending to the outpatient department at Ayothidoss Pandithar Hospital, NIS, along with their characteristics, causative factors, *Naadi*, and demographic details (including dietary habits, occupation, sex, and age). A total of 120 patients were evaluated based on defined inclusion and exclusion criteria, from which 100 individuals with *peenisam* (sinusitis) participated in the cross-sectional study, covering those aged over 15 years of all genders, including transgender individuals. Before joining the study, informed consent was obtained from every participant. Data was gathered and recorded using

a pre-designed questionnaire focusing on types of *peenisam* and *Dhega ilakanam*. The study revealed that *Neer Peenisam* accounted for 50% and *Mulai Peenisam* for 30% of cases, while *Pithakabam* (39%) and *Kabapitham* (27%) were the most common body constitutions affected. Key contributing factors identified included infrequent oil baths (93%), existing nasal ailments (88%), persistent sleeplessness (85%), nasal congestion caused by dust (81%), and the consumption of cold water (81%). The findings indicated that *Naadi* assessments predominantly identified *Pithakabam* (39%). The observed symptoms of the various *peenisam* types, along with *Naadi* and causative factors, closely align with descriptions found in *Siddha* literature.

Keywords: *Peenisam* (Sinusitis), *Siddha,* Cross-sectional study, *Dhega Ilakanam, Naadi*

INTRODUCTION

The *Siddha* system of medicine is an indigenous medicine. It has a holistic view of patients and maintains the mental as well as physical well-being of patients. Like the universe body is also made up of five elements. These five elements are the fundamentals of our body. Seasonal variations and environmental pollution reflect on our bodies because any change in the universe will be reflected in our bodies. According to the *Siddha* system of medicine, there is a close connection between the mind and body. So, mind and body health is maintained at a balanced level, which is very essential to protect our body and mind from disease. The physical function is maintained by three humours, they are *Vali, Azhal, and Iyam.* In the disease state, three humours are vitiated; they are called *Kutras*. *Siddhars* classified the diseases into 4448 types. Among them, the diseases of nasal Origin are 86, and one is *peenisam.* There are 9 Classifications of *peenisam* mentioned in *Yugivaithya Chinthamani*[1]. The signs and symptoms of *peenisam* are mentioned in *Yugivaithya Chinthamani,* such as headache, lacrimation, Nasal block, Nasal itching, ear discharge, running nose, cough with expectoration, absence of taste, which may be correlated with Maxillary sinusitis in biomedicine. The most frequently involved one is the maxillary sinus. *Peenisam* is otherwise known as *Mookadaippu* or *Neerkovai* as per *Siddha* Literature[2]. Intake of cold water, exposure to chillness, inhalation of smoke, harmful and Offensive gases, insomnia, speaking in a loud voice, using contaminated water for baths, Suppression of 14 natural urges (particularly vomiting &tears), Improper yoga practice Which increases the body heat are the triggering factors to cause this disease. The treatment of *Siddha* medicine is aimed at keeping the three humours in Equilibrium and maintaining the seven elements. So, proper diet, medicine, a Disciplined life, and medical advice for healthy living restore the equilibrium of humours in diseased conditions. The prevalence of sinusitis is estimated to be 14% of the global population. Women (5.7%) are highly affected compared to men (3.4%), and this increases with age. Approximately 0.5% to 2% of adults and 6% to 13% of children are complicated by bacterial Sinusitis. .1 in 8 Indians are hit by chronic sinusitis[3]. The prevalence of sinusitis in Tamil Nadu is 14% of the global population. Causes of sinusitis are bacterial, viral, and fungal infections, immune deficiencies, Congenital malformations, trauma, tumours, septal deviation, environmental factors and Recurrent usage of nasal drops. *Peenisam* is contrasted with the term sinusitis used nowadays. *Peenisam* is a common ailment that affects a significant portion of the Indian population each year. *Peenisam* affects one in every eight adults and worsens with the passing of the seasons, especially during winter [4]. This study aims to explore the causative factors that exacerbate the disease state as said in Siddha literature types of *peenisam* that impact a larger population and the body Constitution types that are more susceptible to *peenisam* and categorize the patients according to their body constitution which would suggest suitable and appropriate therapy in managing *peenisam (*sinusitis).

MATERIALS AND METHODS

Study Design: This was a cross-sectional study planned to assess the types of *peenisam* (sinusitis) and their body constitutions in sinusitis patients reported OPD of Ayothidoss Pandithar Hospital (APH), NIS, based on the *Siddha*

concept. Before starting the study, clearance was obtained from the Institutional Research Review Board (IRRB), followed by the Institutional Ethical Committee (IEC) with (IEC NO. NIS/24/IEC/2023/MP/42). The study was prospectively registered with reg.no (CTRI/2023/08/056947) registered on 25/08/2023. Then this study was carried out by screening 120 patients with *peenisam* (sinusitis visiting the OPD of APH NIS using the purposive sampling method. Among them, 100 patients aged above 15 years of both sexes were included in this study. Before being included in the study, informed consent was obtained from each patient. Data was collected and documented through a predesigned (type of *peenisam* and *thegailakanam*) questionnaire[5],[6].

Questioner Pattern

The questionnaire was divided into fundamental sections that include age, gender, Occupation, dietary habituation, causative factors, co-morbidity, alcohol, tobacco dependence, types of *peenisam*, inference of *Naadi* and *thegailakanam* among *peenisam* patients.

Study Enrollment

Patients reporting to Ayothidoss Pandithar Hospital will be enrolled in this study based on the inclusion and exclusion criteria. The patient will be informed about the purpose of the study. After consent is obtained from the patients, the data will be collected by using a predesigned questionnaire. Required information will be collected from each individual by using various data collection forms**.**

Data Analysis

All data collected will be entered into the computer using MS Excel software by the investigator. The data will be analysed using SPSS software under the guidance of SRO (Stat) NIS. The level of significance will be 0.05. A descriptive analysis will be made, and necessary tables/graphs generated to understand the profile of the patients included in the study. Then statistical analysis for the significance of different awareness characteristics will be done. The Chi-square goodness of fit test is to be performed for quantitative and qualitative data.

DISCUSSION

The Siddha system has a unique diagnostic method to identify the disease and causative agents. At the end of the study on *peenisam* and its types, we discuss significant facts about the disease with relevant justification from *Siddha* and modern literature based on observations and results. Sinusitis is a common disease with significant morbidity. No single etiological factor is responsible for this disease. Various allergic and infectious, & lifestyle changes have been held responsible. A higher incidence of sinusitis (30%) was observed in software engineers (IT professionals) who were affected more. Tailors (19%), housewives (23%) and students (15%). This observation indicated that the disease is associated with occupation; students and housewives are generally exposed to cold smoking and dust. In this study, the gender of females (75%) was found to be affected because half of the study population consisted of females. The majority of diseased cases. This study belongs to the 34% (17-30) years age group, and 25% age (31-40) years, this age group is normally a socially active group having more chances for exposure to exterior environment, it may be because of occupation dust mites, fast food. etc which are hazardous to health. Around 62(62%) were affected with chronic sinusitis, and of these patients were suffering from this disease for more than 12 weeks of duration, which indicates that the disease is chronic and resilient and progresses slowly. Regarding the presence of risk factors, developing sinusitis includes smoking, asthma, and allergies concluded by previous studies [7],[8]. In the present study population, about 50% of patients have a previous history of dust allergy, and smoking (23%), and 25% are affected with asthma. From this study is inferred that dust allergy and asthma increase the risk of sinusitis. 61 patients (61%) resist their urges (voluntary retention of stools and urine), as per *Siddha* literature, suppresses stool by counteracting the action of *Abana Vayu*, which has the function of pushing stool down. It increases and expels the accumulated faeces and causes *salathodam.* Previous research studies also speculate that the extrinsic upward pressure imposed on the diaphragm by a full bowel resulted in increased anteroposterior diameter, decreased lung height, and a reversible restrictive pattern on pulmonary function studies[9]. The benefits of the oil bath are

mentioned in *Siddha* literature, "*Pathartha guna chinthamani*". Taking an oil bath twice a week will prevent diseases. There will be a clear sensory function and prevention of head diseases [10]. Our study inferred that 93 patients had a lack of oil bath once in eight days, which may be one of the reasons for sinusitis. The relationship between sinusitis and asthma is unclear. Several theories have been proposed for a causal or shared association between sinusitis and asthma. Many research studies have suggested that the severity of nasal and sinus disease parallels that of the lower airway. This suggests that rhinitis, sinusitis and asthma are all manifestations of a single systemic disease [5]. Our study concludes that 25% of sinusitis patients are affected by asthma. Chronic rhinosinusitis (CRS)is associated with impaired sleep quality and excessive daytime sleepiness. Insomnia symptoms may be a risk factor for the development of Chronic rhinosinusitis (CRS) [11]. The study also inferred that 85 (85%) have an imbalance in sleep. Treating sleep imbalance may help in the treatment of sinusitis. When inhaled, the chemicals irritate our sinuses and cause a headache and mucosal congestion, potentially through mechanisms like vasomotor rhinitis, a condition where nasal passages become inflamed and congested in response to irritants [12]. The majority of cases had their diseases start in *Kaarkaalam and Koothirkaalam.* This implies that the monsoon and rain acted as triggering factors for the onset of the Condition of *peenisam.* When the body is exposed to cold, the risk of infection goes up. In a Cold environment, the blood vessels constrict and the immune system is weakened in lower temperatures. It might be because of the lowering of temperature, dampness of the Atmosphere, and waning immunity, triggering *Kabam* and thereby causing the Disease. So, during this season, drugs, diet, and hygienic practices that are specific to Strengthen *Udal Vanmai* can be advised. It is inferred that the rainy season has a great impact on the development of this *Peenisam*. So, proper precautionary measures like drinking hot water, intake of sour and salt foods which will increase *Vatha* and *Pitha* humour, intake of honey and external application of Musk, Camphor, and Sandal can be followed. Study results showed the proportion of *peenisam* types in this study among 100 patients: 10 *vali* peenisam,7 *azhal peenisam*,3 *Iya peenisam*,50 had *Neer peenisam,* and 30 patients had *mulai peenisam*. In *Siddha's* literature, there are 9 types of *peenisam* mentioned, but patients reported only under 5 categories. Other *peenisam* types, *kazhutu* and *ciray peenisam,* were not reported to have mucormycosis, aspergillosis, rhinosporidiosis, are come under nasal diseases. We need to concentrate on that disease, also based on our *Siddha* research. From the pulse diagnosis in 10 *vali peenisam,* 5 had *pithavadha naadi* and 5 had *pithakaba naadi;* predominantly, 7 patients had *azhal peenisam*. Out of seven, 6 patients had *pithakabam naadi*.3 patients had *Iya peenisam,* predominantly presenting naadi, *which* was predominantly found in the study. According to *Sathaga Naadi*, ***pithakaba Naadi*** and ***pithavadha Nadi*** are indicated for *peenisam* [13]. The study also explored the outcome of the *Dhegailakkanam* analysis according to the present observations. In *vali peenisam kabapitham*, *azhal peenisam vadha pitha dhegi* and *kabavadham*, In *Iya peenisam kabavadham*, *kabapitham* and *pithakabam dhegi* are frequently noted. In *Mulai Peenisam* majority of affected *dhegi* are *pithavadham* and *pithakabam dhegi*. In *Neer peenisam kabapitham* & *pithakabam dhegi*. Based on our study observations in all types of *peenisam* majority of the population, nearly 39% comes under ***pithakabam,*** and 27% comes under *kabapitha dhegi*. Much of the literature and Ayurveda articles concluded that those who have the *kabam* phenotype are more prone to having sinusitis. Disease prevention initiatives may benefit from an understanding of phenotypes, and the role of genetics will advance the *Siddha* medical system and lead to a tailor-made approach to preventive care.

CONCLUSION

The symptoms of *Peenisam* types and *Naadi* mentioned in *Yugi Vaithiya Chithamani* are similarly elicited in this study. Each individual has a unique combination of body constitution. It helps in tailoring lifestyle, diet and therapies to maintain balance and promote wellbeing. By understanding the body's constitution, we easily treat and reduce the burden of non-communicable diseases. *Naadi* (pulse) diagnosis is non-invasive and unique in our *Siddha* medicine. Previous study articles showed that *Naadi* is closely associated with (*Dhega ilakkanam*) one's body constitution [14]. *Naadi* is considered the current physical and diseased state of humour, body constitution defined as the relative presence of *Vatham*, *Pitham, and Kabam* at conception, and remains unaltered [15]. The preventable causative factors, attributed to the development of sinusitis as in *Siddha* literature, are scientifically justifiable.

OBSERVATION AND RESULTS

(Tables below)

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Table 1: Distribution of Occupation

OCCUPATION	NO.OF PATIENTS	PERCENTAGE
IT PROFESSIONALS	30	30%
HOME MAKER	23	23%
TAILORS	19	19%
CARPENTER	5	5%
FARMER	3	3%
BUILDING WORKER	3	3%
DRIVER	2	2%
STUDENT	15	15%
TOTAL	100	100%

Table 2: Age

AGE	VALI PEENISAM	AZHAL PEENISAM	IYA PEENISAM	NEER PEENISAM	MULAI PEENISAM	PERCENTAGE
17-30 YEARS	2	2	0	19	11	34%
31-40 YEARS	2	3	0	13	7	25%
41-50 YEARS	2	-	2	10	3	17%
51-60 YEARS	2	-	-	3	6	11%
60-70 YEARS	2	2	1	4	3	12%
71-80 YEARS	-	-	-	1	-	1%
TOTAL	10	7	3	50	30	100%

TABLE 3 GENDER DISTRIBUTION

GENDER	VALI PEENISAM	AZHAL PEENISAM	IYA PEENISAM	NEER PEENISAM	MULAI PEENISAM	GRAND TOTAL	PERCENTAGE
MALE	6	0	3	20	10	39	25%
FEMALE	4	7	0	30	20	61	75%
GRAND TOTAL	10	7	3	50	30	100	100%

TABLE 4: SOCIAL HABITS

SOCIAL HABITS	NO.OF.PATIENTS	PERCENTAGE
SMOKING	23	23%
SMOKING AND ALCOHOL	7	7%
ALCOHOL	2	2%
BETEL NUT	12	12%
NO HABITS	56	56%
TOTAL	100	100%

Table 5: Food Habits

DIETARY PATTERN	NO.OF.PATIENTS	PERCENTAGE
VEGETARIAN	6	6%
MIXED DIET	94	94%
TOTAL	100	100%

Table 6: Allergic History

ALLERGY	NO.OF.PATIENTS	PERCENTAGE
DUST ALLERGY	77	77%
FOOD ALLERGY	23	23%
TOTAL	100	100%

Table 7: Causative Factors

CAUSATIVE FACTORS		PEENISAM TYPES					Total
		VALI PEENISAM	AZHAL PEENISAM	IYA PEENISAM	NEER PEENISAM	MULAI PEENISAM	
ALLERGIC TO ANY SUBSTANCE	YES	8	6	3	47	27	91
	NO	2	1	0	3	3	9
DRINKING OF VERY COLD WATER	YES	9	7	3	45	22	86
	NO	1	0	0	5	8	14
EXPOSURE TO COLD AIR AND ATMOSPHERE	YES	10	5	3	41	28	87
	NO	0	2	0	9	2	13
INHALATION OF AIR POLLUTED WITH SMOKE AND DUST	YES	10	7	2	42	24	85
	NO	0	0	1	8	6	15
SMELLING THINGS THAT INDUCE SNEEZING	YES	9	7	2	47	27	92
	NO	1	0	1	3	3	8
EATING COLD FOODS	YES	8	6	3	44	26	87
	NO	2	1	0	6	4	13
WHEN THE BODY IS HEATED, TAKING A BATH IN COLD WATER	YES	9	7	3	43	28	90
	NO	1	0	0	7	2	10
DRINKING CONTAMINATED WATER	YES	0	0	0	7	6	13
	NO	10	7	3	43	24	87
SUPPRESSION OF SHEDDING TEARS AND VOMITING	YES	4	4	1	9	6	24
	NO	6	3	2	41	24	76
EXCESSIVE SLEEPLESSNESS/SLEEP	YES	10	6	3	40	26	85
	NO	0	1	0	10	4	15
LOUD SPEECH	YES	9	5	2	42	21	79
	NO	1	2	1	8	9	21
ASSOCIATION WITH VENEREAL DISEASES	YES	0	0	0	1	0	1
	NO	10	7	3	49	30	99
IN YOGA, BODY HEAT IS TRANSMITTED FROM THE *MOOLADHARAM* TO THE HEAD	YES	5	2	2	26	16	51
	NO	5	5	1	24	14	49
NASAL BLOCKAGE BY DUST	YES	9	6	3	40	23	81
	NO	1	1	0	10	7	19
VOLUNTARY RETENTION OF URINE AND STOOLS	YES	8	5	1	17	8	39
	NO	2	2	2	33	22	61
LACKING OF OIL BATH ONCE IN EIGHT DAYS	YES	10	7	3	45	28	93
	NO	0	0	0	5	2	7
DISEASE IN THE NOSE	YES	9	6	3	43	27	88
	NO	1	1	0	7	3	12

TAKING A BATH IN COLD WATER ONLY	YES	10	5	2	39	25	81
	NO	0	2	1	11	5	19
LYING IN AN UNCLEAN BED	YES	1	1	0	3	3	8
	NO	9	6	3	47	27	92

The chi-square goodness of fit test shows a significant p-value < 0.05 in most of the causative factors

Table 8: mode of onset

MODE OF ONSET	NO.OF.PATIENTS	PERCENTAGE
GRADUAL	2	2%
SEASONAL	32	32%
ALLERGIC EXPOSURE	65	65%
TOTAL	100	100%

Table 9: Duration

DURATION	NO.OF.PATIENTS	PERCENTAGE
ACUTE	38	38%
CHRONIC	62	62%
TOTAL	100	100%

Table 10: Co-Morbidities

CO-MORBIDITIES	NO.OF PATIENTS	PERCENTAGE
ASTHMA	25	25%
HYPERTENSION WITH ASTHMA	3	3%
CARDIOVASCULAR DISEASES WITH ASTHMA	1	1%
DIABETES WITH HYPERTENSION	2	2%
EPILEPSY	4	4%
DIABETES	8	7%
OTHERS	4	4%
NO CO MORBIDITIES	53	53%
TOTAL	100	100%

Table 11: Past History Related to Sinusitis

PAST HISTORY	NO.OF.PATIENTS	PERCENTAGE
NASAL POLYPH	11	11%
SEPTAL DEVIATION	39	39%
DUST ALLERGY	50	50%
TOTAL	100	100%

Table 12: Naadi

NAADI	VALI PEENISAM	AZHAL PEENISAM	IYA PEENISAM	NEER PEENISAM	MULAI PEENISAM	GRAND TOTAL
VADHAPITHAM	0	0	0	2	3	5
PITHAVADHAM	5	1	1	13	12	32
VADHAKABAM	0	0	0	3	1	4
KABAVADHAM	0	0	0	1	0	1
KABAPITHAM	0	0	2	4	3	10
PITHAKABAM	5	6	0	27	11	49
GRAND TOTAL	**10**	**7**	**3**	**50**	**30**	**100**

Table 13: Dhegi in Various Peenisam Types

DHEGI	VALI PEENISAM	AZHAL PEENISAM	IYA PEENISAM	NEER PEENISAM	MULAI PEENISAM	GRAND TOTAL	PERCENTAGE
VADHAPITHAM	2	2	0	9	2	15	4%
PITHAVADHAM	2	0	0	16	8	26	14%
VADHAKABAM	0	1	0	2	1	4	3%
KABAVADHAM	1	2	1	4	3	11	12%
KABAPITHAM	3	1	1	10	5	20	27%
PITHAKABAM	2	1	1	9	11	24	39%
GRAND TOTAL	10	7	3	50	30	100	100%

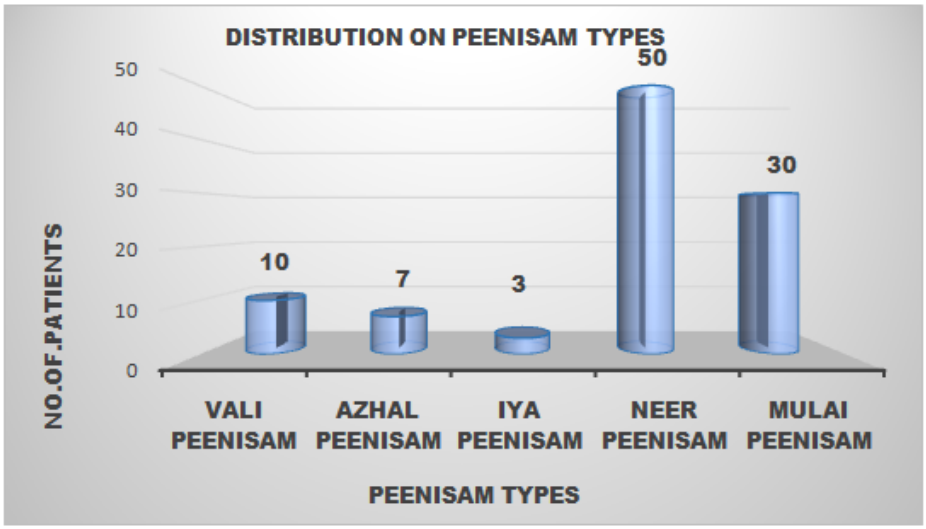


Figure 1: Peenisam Types

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RESEARCH ARTICLE

Wildlife Crimes in India : Challenges and Opportunities under the Wildlife Protection Act, 1972

Kaushik Chowdhury[1] and Shahnaz Mondal[2]*

[1]Assistant Professor, Department of Law, LJD Law College, (Affiliated to University of Calcutta), Kolkata, West Bengal, India.
[2]Assistant Professor, Department of Law, Brainware University, Barasat, Kolkata, West Bengal, India.

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***Address for Correspondence**
Shahnaz Mondal
Assistant Professor,
Department of Law,
Brainware University, Barasat, Kolkata,
West Bengal, India.
E.Mail: shahnazmondal655@gmail.com

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ABSTRACT

Wildlife crime poses a significant threat to biodiversity, conservation efforts, and the rule of law in India. Despite having a dedicated legal framework in the colour of the Wildlife Protection Act, 1972 (WPA), enforcement challenges, loopholes, and emerging threats such as organized wildlife trafficking networks have rendered many protections ineffective. This article critically examines the evolution, successes, and limitations of the WPA in addressing wildlife crime over the past five decades. It analyses key challenges, including administrative inefficiency, lack of inter-agency coordination, insufficient penalties, evidentiary difficulties, and socio-economic factors driving poaching and illegal trade. At the same time, the paper explores opportunities to strengthen wildlife protection through legislative amendments (such as the 2022 Amendment to the WPA), enhanced technological interventions (such as digital surveillance, forensic science, and AI-driven monitoring), community-based conservation models, and greater international cooperation. By adopting a doctrinal and empirical approach, this article aims to provide a comprehensive evaluation of the existing legal regime, draw comparisons with global best practices, and suggest actionable reforms to bridge the gap between law and effective conservation. The findings will contribute meaningfully to the ongoing scholarly discourse on environmental criminology and legislative reform in India.

Keywords: Biodiversity, Conservation, Ecosystem, Extinction, Protected Area, Wildlife Crime

INTRODUCTION

Biodiversity encompasses the total variety of life at all levels of biological organization, including genetic, species, and ecosystem diversity. Conservation efforts focus on protecting this diversity across all these levels. The term "biodiversity," which comes from "biological diversity," describes the range of plant and animal species found in a specific ecosystem or area. It includes all the different kinds of life on Earth, such as microorganisms, plants, and animals, as well as their various habitats. Because biodiversity is essential to ecosystem function, protecting it is extremely important for the environment and human welfare. Hence, conservation of biodiversity is necessary for a sustainable future. Species diversity is not evenly spread across the globe; it is typically prominent in tropical regions and less so toward the poles. Several factors contribute to the richness of species in the tropics, including a longer evolutionary history, a relatively stable environment, and higher solar energy that leads to higher productivity. Additionally, species richness is influenced by the size of a region[1].Ecosystems with high biodiversity are generally more stable, productive, and resilient to invasions by new species. The planet's rich biodiversity is essential for human survival. While Earth's fossil record shows several past mass extinction events, current extinction rates, mainly driven by human activities. Major causes of these elevated extinction rates include habitat loss and fragmentation, over-exploitation, invasive species, and co-extinctions. The reasons to conserve biodiversity are multifaceted, encompassing narrowly utilitarian benefits such as food, fibre, and medicines; broadly utilitarian benefits through ecosystem services like pollination, pest control, climate regulation, and flood management; and ethical considerations, where humanity holds a moral responsibility to protect and preserve this natural heritage for future generations. We depend on nature for many things, from the food we eat to the homes we live in and the clothes we wear. Wildlife, fish, and forests all provide for our needs. However, wildlife crime is putting at risk our ability to enjoy the benefits and beauty that wild plants and animals offer. Examples include killing an elephant to harvest its tusks for making jewelry, stealing turtle eggs to use in a special soup, and cutting down rosewood trees to create furniture. Wildlife crime involves illegal actions such as trading protected plants or animals, poaching wildlife, or damaging natural habitats unlawfully. Wildlife crime reduces the numbers of many species and can threaten some with extinction[2].The methods used by criminals can also harm ecosystems. For instance, fish poachers who use poison or explosives may destroy coral reefs that serve as homes for almost one-third of marine species. Illegal exploitation and trade of natural resources also cause significant economic losses, especially for areas that depend on income from legal wildlife trade and tourism. Moreover, wildlife crime threatens the livelihoods of many people who rely on wildlife for their survival and income. The Wildlife (Protection) Act of 1972 provides the legal foundation for protecting various wild animal and plant species, overseeing their habitats, and regulating the trade of wild animals, plants, and related products. It includes schedules that grant different levels of protection and government monitoring to specific species[3]. This Act also played a key role in enabling India's participation in the Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES). Earlier, the Act did not cover Jammu and Kashmir, but following the region's reorganization, it now applies there too. Unfortunately, Wildlife crime continues to pose a grave threat to India's biodiversity, conservation initiatives, and the integrity of its legal system. Although the Wildlife Protection Act, 1972 (WPA) serves as the cornerstone of India's legal framework for safeguarding wildlife, its effectiveness has been undermined by enforcement shortcomings, legal loopholes, and the rise of organized trafficking networks[4]. This article undertakes a critical assessment of the WPA's development, achievements, and limitations in addressing wildlife crime over the past fifty years. It identifies core challenges such as administrative inefficiencies, weak inter-agency coordination, inadequate punishments, evidentiary hurdles, and socio-economic drivers behind poaching and illegal wildlife trade. Simultaneously, the paper highlights potential avenues for reform, including recent legislative updates like the 2022 WPA Amendment, the adoption of advanced technologies (such as AI-based monitoring, forensic tools, and digital surveillance), community-led conservation strategies, and strengthened international collaboration. It proposes practical reforms to close the gap between statutory provisions and effective on-ground conservation. The study aims to enrich the broader academic discussion on environmental criminology and legal reform in the Indian context.

MATERIALS AND METHODS

On August 21, 1972, the Government of India enacted Act No. 53 of 1972, known as the Wildlife (Protection) Act, 1972, to safeguard wild animals, birds, and plants, thereby ensuring the ecological and environmental stability of the nation. Serving as a comprehensive or umbrella legislation, the Act encompasses various provisions aimed at the conservation of wildlife and their habitats. The Act consists of six schedules and is applicable across the entire country. It has undergone amendments in the years 2003, 2017 and 2022 to strengthen and update its provisions.

Constitutional Provisions Related to Wildlife Protection

Article 48A of the Indian Constitution mandates the State to endeavor to protect and enhance the environment, as well as conserve the country's forests and wildlife. Similarly, Article 51A outlines the fundamental duties of every citizen, which include the responsibility to protect and improve the natural environment, such as forests, rivers, lakes, and wildlife and to show compassion toward all living creatures.

Historical Legislation on Wildlife

Wild Birds Protection Act, 1887: This early legislation aimed to restrict the possession and sale of certain wild birds, specifically those that were captured or killed during their breeding season.

Wild Birds and Animals Protection Act, 1912: This law was introduced to extend protection to both wild birds and animals. It was further strengthened by the Wild Birds and Animals Protection (Amendment) Act, 1935, which updated and expanded its provisions.

Relevant Provisions under W.P. Act 1972

Under Chapter II, Section 5A of the Wildlife (Protection) Act, 1972, the Government of India established the National Board for Wildlife (NBWL) as a statutory authority[5]. This apex advisory body is tasked with guiding and supporting the conservation and sustainable development of the country's wildlife and forests. It plays a key role in advising the Central Government on matters related to wildlife protection and is responsible for reviewing and approving projects that impact national parks, wildlife sanctuaries, and other protected areas.

Hunting Regulations for Wild Animals

As per the Act, hunting any wild animal listed in Schedules I to IV is strictly prohibited. However, exceptions are allowed under certain conditions:

Hunting in the Interest of Public Safety or Welfare

If a wild animal poses a threat to human life or property (including standing crops), or if it is seriously injured, disabled, or suffering from a disease, the Chief Wildlife Warden may authorize hunting within a designated area[6].

Protection of Specified Plants

The Wildlife (Protection) Act lays down strict regulations for the protection of certain plant species listed as "specified plants." These provisions cover the prohibition of collection and trade, rules for obtaining permits, and restrictions on cultivation and ownership.

Ban on Picking or Damaging Specified Plants

Within forest areas and other locations notified by the Central Government, the following actions are prohibited

Intentionally picking, uprooting, damaging, destroying, acquiring, or collecting any specified plant. Possessing, selling, offering for sale, gifting, transferring, or transporting any part or derivative of a specified plant, whether alive or dead.

Exception: Members of Scheduled Tribes are allowed to collect and possess specified plants or their derivatives within their district of residence, provided it is for personal and genuine use.

Permit for Special Purposes: The Chief Wildlife Warden, with prior approval from the State Government, can grant permits for the collection, transportation, or acquisition of specified plants from forest areas for specific purposes such as educational use, scientific research etc.

Protected Areas

Protected Areas such as Wildlife Sanctuaries, National Parks, Conservation and Community Reserves, and Tiger Reserves are established under Chapter IV of the Wildlife (Protection) Act, 1972. These areas aim to conserve biodiversity, protect ecosystems, and preserve critical wildlife habitats[7].

Trade or Commerce in Wild Animals, Animal Articles, and Trophies

Chapter V of the Wildlife (Protection) Act, 1972, governs the trade and commerce related to wild animals, animal articles, and trophies. According to the Act, any animal, animal article, trophy, uncured trophy, meat, vehicle, weapon, trap, or tool that has been seized as part of an offence under the Act automatically becomes the property of the Central Government. If a person comes into possession of such government property, they are required to report this possession within 48 hours to the nearest police station or authorized officer. Upon request, the person must hand over the property to the officer in charge. Additionally, no individual is allowed to acquire, keep, transfer, destroy, or damage any government property without prior written permission from the Chief Wildlife Warden or an authorized officer. The Act also places strict restrictions on the transportation of wildlife and specified plants. No one is permitted to transport wild animals, specified plants, or their derivatives without obtaining prior authorization from the Chief Wildlife Warden or other designated officers authorised by the State Government. This ensures that all movement of wildlife and related materials is properly monitored and regulated. Furthermore, the purchase or acquisition of captive animals, wild animals (excluding vermin), animal articles, trophies, uncured trophies, or meat derived from these animals is prohibited unless the transaction occurs through a licensed dealer or an authorized person under the Act. This provision helps prevent illegal wildlife trade and ensures that all such commerce is carried out legally and transparently. Chapter VI of the Act addresses the prevention and detection of offences relating to wildlife protection. It grants powers to officials to enter premises, conduct searches, make arrests, and detain suspects involved in violations of the Act. It also outlines penalties for offences such as illegal hunting, possession, or trade of wildlife products, as well as for habitat destruction. India, with its immense biodiversity and status as a British colony, was a natural destination for European explorers and hunters. The country's expansive tropical forests, home to countless endemic species, drew scientists and trophy seekers alike. Even after independence, newly emerging elites adopted similar practices, continuing the legacy of wildlife exploitation under the guise of status and sport. By the late 1960s, the detrimental effects of widespread hunting and wildlife trade in India had become alarmingly clear. The Asiatic cheetah, once common, had already vanished by the early 1950s. Tigers, highly prized for their skins, were being hunted extensively, while reptiles like crocodiles, snakes, and monitor lizards were being killed for their hides and body parts. Globally desired luxury items such as ivory and shahtoosh wool were in high demand. Meanwhile, primates, birds, and frogs were being trapped in large numbers for scientific testing, meat, or the pet trade. This rampant exploitation caused significant declines in many animal populations, raising concerns among conservationists and policymakers. Still, putting a stop to this trade was far from simple. Wildlife product exports brought in much-needed foreign revenue and supported thousands of livelihoods, including those of trappers, exporters, and craftsmen. Despite the economic trade-offs, India opted to put conservation first. Reflecting its cultural values that emphasized non-violence and respect for life, the country began introducing stringent protective measures. The export of wild animal skins was officially banned in 1968. A year later, at the IUCN conference in New Delhi, Prime Minister Indira Gandhi publicly stated that India would not pursue foreign exchange earnings at the cost of its natural heritage. This stance led to a total ban on tiger hunting, the introduction of the Wild Life (Protection) Act (WLPA) in 1972, and the inception of Project Tiger. The WLPA, passed in 1972, became the cornerstone of India's legal framework for wildlife protection. Triggered by momentum from both the Stockholm Conference and the IUCN meeting, the law aimed to provide wide-ranging protection for India's flora and fauna. However, constitutional limits initially prevented the central government from directly legislating on the subject, as forests and wildlife were state matters[8]. This was resolved through Article 252, which allows Parliament to make laws on state subjects when at least two states request it. Nevertheless, bolstered by strong

political resolve and widespread cultural support, the Act was rolled out across the nation without major compromises. Over the years, it proved effective in bringing commercial wildlife exploitation nearly to a halt. The surge in forest and wildlife-related crimes has become a pressing global issue. These illicit activities threaten not just the rich diversity of life on Earth, but also the environmental equilibrium essential for sustaining life. Illegal wildlife trade and habitat destruction are often motivated by profit, endangering biodiversity[9] and destabilize ecosystems. Additionally, these crimes hinder economic progress, particularly in biodiversity-rich nations like India. India's wildlife faces serious challenges due to rampant poaching, trafficking, and environmental degradation, which are driven by urban expansion, deforestation, human encroachment, and illegal trade. Although the country has robust environmental laws and is a participant in global treaties such as CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora), it still ranks among the top 20 countries affected by wildlife trafficking and is one of the top ten nations for wildlife smuggling by air. With the demand for wildlife-based products used in traditional medicine, luxury goods, and cultural practice continuing to grow, illegal wildlife trade has become the fourth most lucrative criminal activity worldwide, trailing only drug, arms, and human trafficking, to name a few.

Global Wildlife Crime Networks

Wildlife trafficking is a highly organized, transnational crime that operates through extensive and often clandestine networks. Countries across the globe play different roles; some act as suppliers, others as transit points, and some as final destinations for the illegally traded species and their derivatives. Animals and plants are trafficked for various purposes, including food, medicine, religious rituals, exotic pets, and fashion. From rare orchids to reptiles and birds, nearly every element of biodiversity is at risk. The primary driver behind these crimes is demand; where there is a market, the supply chain continues to flourish. Despite India's active involvement in international conservation efforts, its enforcement efforts are impeded by weak border control, poor coordination between agencies, corruption, and increasing use of digital platforms, including the dark web, for illicit transactions[10].

India's Involvement in Wildlife Trafficking

India's status as one of the world's "mega-diverse" countries, home to about 8% of the planet's known wildlife ironically places it at the heart of the wildlife trafficking network. It functions as a source, conduit, and destination for illegal trade. Long and porous borders with countries like Myanmar, China, Bangladesh, and Nepal, where wildlife demand is high, make enforcement particularly difficult. Combined with the rise of e-commerce and social media, along with India's growing aviation connectivity, traffickers find many avenues to exploit.

Most Trafficked Species in India

Reports from agencies like the Directorate of Revenue Intelligence and studies such as TRAFFIC and "Smuggling in India 2020–21" reveal that ivory, pangolins, red sandalwood, Indian star tortoises, and ornamental fish are among the most frequently trafficked wildlife products in India.For instance, ornamental species like the zebra loach and barca snakehead (Channa barca) are heavily exploited for the aquarium trade. Pangolins are poached for their meat and scales, often smuggled to East Asian markets. Body parts of predators like leopards and tigers are trafficked for use in folk medicine and ritualistic practices. Additionally, there has been a rise in the smuggling of exotic animals such as kangaroos, red-eared slider turtles, parrots, tamarins, marmosets, lorises, and iguanas. A widely viewed 2022 video showing the rescue of a dehydrated kangaroo in West Bengal starkly illustrates the gravity of the issue.

Trafficking Routes and Use of Air Transport

India's smuggling routes are influenced by both its geography and infrastructure. The Northeastern corridor, particularly cities like Guwahati, Imphal, and Dimapur, serves as a primary route for trafficking to Southeast Asia and China. Other significant overland routes include the Indo-Nepal and Indo-Myanmar borders. Additionally, the Dooars region in West Bengal is a major transit zone for smuggled reptiles and birds.

RESULT AND DISCUSSION

A profound sense of love, compassion and duty towards plants and animals permeates the conscience of this nation since ancient times, and this is adequately reflected in our religious scriptures, mythological renditions, and social treatises, all of which embody the ancient jurisprudence of India. But with the gradual passage of time and with the dawn of modernity, materialism, Hedonism, along with shrewd individualistic pursuits, have somehow blunted the vigil of humanity and left it to seethe on the back burner, making way for indulgences and aggrandizement to lead our proclivities. The gradual decadence of humanity and the sharp ascendancy of ruthless materialism were alarming and an impending threat to the existentiality of the human race and the environment. Under these challenging circumstances, the global intellectuals as researchers, scientists, jurists, philosophers, lawyers and environmentalists felt it highly imperative to build a robust scientific and legal framework to protect the environment and the plants and animals as its indivisible biotic components. So far as the legal protection is concerned, it does assure legal enforceability, and this was thought to be the most potent form of deterrence to obviate the potential and penalise the actual offenders of wildlife crimes.

Wildlife Protection (Amendment) Act, 2022: A Brief Analysis

A close and purposive interpretation of the Wildlife Protection (Amendment) Act, 2022, in juxtaposition with the fundamental duties under the Constitution of India, unmistakably points to awareness and sensitivity of India and its citizens to the moral and legal duty towards the flora and fauna of this subcontinent. The Wildlife Protection (Amendment) Act, 2022, portrays a revised and elevated statutory assurance for wildlife protection in India in concordance with the global environmental obligations[11]. It is a structural and organic revamping of the entire Indian wildlife and environmental statutory framework in tandem with the international standards and expectations for wildlife conservation and preservation of species. The amended version focuses more on the best global conservation practices aimed at protecting the wildlife within the Indian jurisdiction. The authors will briefly look into the core provisions, positive reforms, and key concerns associated with the amendment in this segment of the paper. The Act indubitably introduces a host of commendable reforms, underscoring the growing need and efficacy of conservation and preservation of certain species of living organisms in order to ensure accomplishment of the broader goal of environmental sustainability, intergenerational equity and proliferation of species diversity. However, there are certain areas of concern as well in terms of its implementation, the inclusion of all the relevant stakeholders and potential societal and ecological implications. The most foundationally transforming factor in the amended Act is the clear and distinct rationalization of the schedules of species. The erstwhile version of the Act contemplated six schedules, which have been reduced to four post-amendments. Schedule I (species requiring the highest protection), Schedule II (lesser protection), Schedule III (protected plant species), and Schedule IV (CITES-listed specimens). This re-contouring is intended to streamline species categorization and to ensure a more clear and definitive level of protection[12]. In conformity with the CITES, the amended Act creates dual authorities: Management and Scientific to oversee and regulate the trade of listed species and guide them expertly[13]. The Act further introduces new statutory provisions to effectively deal with contemporary biodiversity and ecological challenges. The amended Act empowers the Central government to control and prohibit the import of Invasive Alien Species (IAS). These species are destructive in nature, and they can proliferate in a new habitat so fast that they can outgrow the native species and can disturb the natural ecosystem. Therefore, it is highly imperative to regulate their import, possession or trade[14]. Another positive development is the increased penalties for wildlife crimes, which aim to deter illegal trade and poaching[15]. Apart from the positives of the amended wildlife protection statute, there are some notable areas of deep concern that need to a mentioned. To begin with, the inclusion of 600 plus count of species in the CITES schedule without clear criteria and ecological justification have all the potential to overwhelm and confuse the enforcement agencies and may divert their attention and focus from more endangered species which are almost on the brink of extinction. Moreover, the amendment to section 43 of the Wildlife Protection Act is highly debatable for its vagueness as it allows transportation of captive elephants for "religious or any other purpose" opening the floodgates for exploitative practices in the name of religion or under the subterfuge of customs and traditions, all of which run counter to the essential tenets of animal welfare[16]. Another critical concern has been the

distorted federal balance owing to centralization of authority under the amended statute. The establishment of a Standing Committee within State Boards for Wildlife substantially attenuates the power of consultation of state governments and thwarts the voices of the local communities[17]. Moreover, the case of the forest-dwelling communities like the Van Gujjars, who fear increased surveillance and restricted access to traditional lands, is a major setback under the amended wildlife protection statute. The Van Gujjars are asemi-nomadic pastoral community which undergo seasonal migration across the forests of Uttarakhand, UP and HP in search of pasture. But the Wildlife Protection (Amendment) Act has brought the free grazing grounds within the purview of protected areas, rendering their natural and spontaneous mode of lifestyle almost impossible[18]. The amendment casts new obligations upon the enforcement authorities and the judiciary[19] because the legislative intent that the amendment exemplifies can best be achieved only through simultaneous investment in broadening and deepening the institutional capacity. The expanded list of protected species may also aggravate human-wildlife conflict, especially in the agrarian regions where animals like wild boars or nilgai destroy crops but now enjoy stronger legal safeguards[20]. The brief critical review of the Wildlife Protection (Amendment) Act 2022 is mixed, holding both promises and disappointments owing to some critical systemic failures that call for immediate overhaul. Fulfilling international commitments and modernizing the Indian wildlife statutory framework are no doubt laudable initiatives, but the amended law must engage more comprehensively and organically to respond to the socio-cultural and ecological complexities. The future legal and administrative machinery should aim towards efficacious community engagement, more transparency in species classification, and a better enforcement ecosystem. A nuanced approach that integrates ecological priorities with socio-economic realities will be essential for the Act's successful implementation.

Role of Technology in Combating Wildlife Crimes

Technological sophistication and efficacy are proven strategies to reduce and eliminate the risks and possibilities of wildlife crimes. A diverse varieties and modalities of crimes against animals have been historically perpetrated by unscrupulous, money-minded, selfish people for ages. Poaching, illegal trade and habitat destruction are some of the gravest forms of cruelty and injustice meted out to the wild animals. However, our gradual shift from an analogue to the digital stage has indubitably accelerated the precision of detection, impact of prevention and reduction of wildlife crimes globally. From real-time monitoring systems[21] to data analytics, technology plays a multi-pronged role in the prevention, detection, and prosecution of wildlife-related offences. Modern innovative concepts like satellite imagery and remote sensing are highly capable of real-time tracking of unauthorised deforestation and habitat encroachments,[22] rendering it possible for appropriate authorities to act promptly. The use of drones has substantially enhanced aerial surveillance, especially of those strips of forested regions which are usually inaccessible to humans. This has reduced the risks associated with physical patrolling by humans and has improved the coverage of such deeply forested terrains[23]. Moreover, the use of camera traps and GPS collars is are highly effective aid in tracking animals and monitoring their behaviour. This kind of study helps in analysing and understanding the patterns of poaching and taking suitable preventive measures[24]. The role of Artificial Intelligence (AI) and big data analytics does not need separate emphasis, as these algorithms and innovative technological inputs play a very crucial role in predicting poaching hotspots through analysis of historical data, climate variables, and ranger patrol routes. Predictive policing is a highly sophisticated technique that uses data analysis and algorithms to anticipate crimes and is highly effective in combating crimes related to the flora and fauna[25]. To add to this, even blockchain technology can be used as a potent technological instrument to ensure traceability of legit wildlife trade, making it quite difficult to launder illicit products through licit routes[26]. Further, DNA barcoding and isotopic analysis facilitate identification of species and tracing of origins, thereby strengthening prosecutorial strength by offering scientific evidence[27]. The mobile technology has recently introduced SMART (Spatial Monitoring and Reporting Tool), which, if implemented effectively, can trace and document real-time patrol efforts, creating an integrative, robust enforcement network and mechanism. Thus, SMART technology unlocks the potential and value of ranger-based monitoring for biodiversity conservation[28]. These technological aids no doubt both complement and supplement the process of regulation, but even they are not without constraints in the form of high investment costs, infrastructural bottlenecks and the mounting urgency for inter-agency coordination. It is quite pertinent to assess and appreciate the efficacy of technology when it supplements the traditional conservation strategies, rendering the

possibility of enhanced wildlife conservation through a more scalable, inclusive, and adaptive technological advancement.

Strengthening Inter-Agency Collaboration: Role of Customs, Police, CBI and the Interpol

Transnational crimes like cybercrime, terrorism, human and wildlife trafficking, and money laundering call for a robust and seamlessly integrated inter-agency collaboration. The role of customs, CBI, police and Interpol cannot afford to be fragmented anymore amid such horrendous international crimes having deep transnational implications. Intelligence sharing is the need of the hour. The customs being the watchdog and the first line of defence of the porous international coastal borders can quite effectively intercept contraband/smuggled goods, which may include even endangered wildlife products, among other things. But failure to promptly share such information with the police and the CBI often makes attempts for wider crackdowns on syndicates unsuccessful. For example, Customs data on import/export anomalies can inform CBI's investigations into money laundering and trade-based financial crimes, but only if information flows are institutionalized and digitalized[29]. Again, so far as local intelligence gathering is concerned, police play the most crucial role in maintaining law and order and executing warrants. However, the effectiveness of the local police forces is remarkably undermined owing to their lack of access to the central database system created and maintained by the CBI or Interpol. CBI, on the other hand, may need to rely on state police forces to solve cases involving multi-jurisdictional crimes but such reliance hardly works due to want of standard protocols, delays and duplication. Interpol's role has gained renewed significance on the face of growing cyber and transnational threats. India's National Central Bureau (NCB)[30] under the CBI coordinates with Interpol to issue notices, share intelligence, and extradite fugitives. Yet, real-time access to Interpol databases and timely responses to "Red Notice" alerts remain problematic due to procedural bottlenecks and limited technical integration with local agencies. The need of the hour is to construct a comprehensive interoperability framework having legal validity, requiring timely data sharing and joint investigations across agencies. Creation of an integrated crime database—accessible to the customs authorities, police, CBI, and Interpol (NCB) can facilitate real-time collaboration. Institutionally, regular joint training programs, inter-agency task forces, and designated liaison officers can bridge trust and capacity gaps. Latest initiatives like National Intelligence Grid (NATGRID), Crime and Criminal Tracking Network System (CCTNS), and the Inter-Operable Criminal Justice System (ICJS) are no doubt positive angles, but they suffer from incomplete implementation and limited interlinking which need to be set right on a war-footing[31].

Capacity Building of Enforcement Agencies and the Judiciary

Capacity building of the enforcement agencies and the judiciary is central to the effective administration of justice and the rule of law. It subsumes no merely infrastructural development but also institutional reforms that promote liability, adaptability and professionalism. Lack of domain expertise, technology constraint and paucity of manpower are some of the vital concerns that plague the enforcement agencies like police, customs, and specialized bodies like the Wildlife Crime Control Bureau, etc. Regular, domain-specific training in forensic science, cybercrime, financial investigations, and digital evidence handling is essential to modernize enforcement capabilities. Moreover, the integration of AI-driven surveillance, predictive policing tools, and interoperable databases requires officers trained in both technology and law. The judiciary must also address vital concerns like evolving complexities and the phenomenal volume of cases. Judicial academies imparting training to judicial officers should play a crucial role in ensuring continuous training and development of the bright judicial minds. Contemporary issues like data protection, privacy rights, IP crimes, environmental jurisprudence and biodiversity protection must be deliberated upon on a continual basis. The judicial delays are often the result of procedural clogs and lapses, and awareness regarding the use of technology-driven mechanisms for file and register maintenance, case management systems and alternative dispute resolution mechanisms may substantially expedite the justice dispensation system[32]. Moreover, inter-agency co-ordination, sensitisation about victimological jurisprudential insights are necessary. Institutionalizing performance audits, incentivizing specialization, and fostering a culture of ethical integrity are equally critical. Finally, sustained investment in the capacity building of enforcement and judicial institutions is not a peripheral reform—it is central to democratic governance. Without it, even the best laws remain ineffective when it comes to implementation.

Community Participation and Wildlife Conservation

If we observe and analyse our traditional forest conservation practices, it was more like an insular system. It was predominantly a State-led scaffold marked with regimented and authoritarian demeanour and reinforcing a State-centric command and control mechanism. However, with the growing consciousness for a welfarist economy in which the essential tenets of participatory governance run deep, an attempt is being made to dismantle exclusionary tendencies and instead embrace inclusionary approaches in the form of community participation of the local resources like the forest-dwelling indigenous population and their traditional knowledge systems for the effective conservation of biodiversity. The traditional model of forest conservation relied heavily on the top-down command and control strategy, resulting in alienation of the local people, but the community participation model acknowledges the ecological knowledge, resource stewardship, and custodial rights of local populations. Community-based forest conservation strategy aims to optimize the utilization of human resources residing sufficiently proximate to the forest and wildlife, and are in the best strategic locations to protect the biodiversity[33]. The Annapurna Conservation Area, Nepal and the CAMPFIRE program in Zimbabwe are illustrative of how the implementation of the idea of community ownership and benefit sharing enhances overall protection of the biodiversity[34]. Various modern initiatives like Joint Forest Management (JFM) and Eco-Development Committees (EDCs) under the Wildlife Protection Act have sought to integrate local communities into forest governance[35]. But this gesture seems pure tokenism as it is mired in half-hearted implementation and feeble legal entitlements. However, the Forest Rights Act, 2006, offers a more robust legal platform for community/participatory conservation initiatives. Community participation needs to be institutionalised through democratic models of decision-making, which will lead to empowerment of the local men and women. To harmonise forest and wildlife conservation with community rights can be an excellent step, which is not only desirable but ethically justified[36].

International Cooperation: CITES and Bilateral Treaties in Wildlife Conservation

International cooperation is critical in addressing transboundary wildlife crimes, which often involve complex networks spanning source, transit, and consumer countries. The Convention on International Trade in Endangered Species of Wild Fauna and Flora (CITES) and bilateral treaties have emerged as essential legal instruments in harmonizing efforts to regulate wildlife trade and protect biodiversity. CITES, adopted in 1973 and ratified by 184 parties, provides a legal framework for controlling international trade in endangered species through a permit-based system[37]. It classifies species into three Appendices based on the degree of protection required, thereby balancing trade with conservation. India, a party since 1976, has integrated CITES provisions into domestic law via the Wildlife (Protection) Act, 1972, and the Customs Act, 1962[38]. CITES has helped curb trade in species like the Indian Star Tortoise and Red Sanders through trade embargoes and compliance mechanisms. However, CITES alone cannot address the full spectrum of enforcement and capacity-building needs. Bilateral treaties supplement CITES by enabling intelligence sharing, joint operations, and targeted cooperation. India has signed MoUs on wildlife protection with countries like Nepal, Bangladesh, Myanmar, and Bhutan, which facilitate coordinated patrolling and extradition of wildlife offenders[39]. The Indo-Nepal and Indo-Bhutan agreements are particularly significant given the porous borders and shared ecosystems. Challenges remain in terms of harmonizing legal standards, overcoming jurisdictional hurdles, and addressing disparities in enforcement capacities among partner countries. Moreover, the effectiveness of CITES is contingent upon national implementation, which varies significantly across jurisdictions[40]. In conclusion, CITES provides the normative backbone for global wildlife trade regulation, while bilateral treaties serve as operational arms to enforce those norms. Their synergy is vital for dismantling international trafficking networks and promoting ecological sustainability.

Challenges and Opportunities

Wildlife crime control in India faces several structural and operational challenges. These include inadequate coordination among enforcement agencies, insufficient manpower and forensic infrastructure, porous borders facilitating cross-border trafficking, and weak prosecution leading to low conviction rates[41]. Additionally, outdated legal frameworks struggle to keep pace with the sophistication of wildlife crime syndicates. However, significant opportunities exist in the form of advanced technologies, international cooperation through platforms like CITES and

INTERPOL, and growing community awareness and participation. The increasing recognition of the link between biodiversity loss and public health—especially post-COVID-19—has further emphasized the urgency for reform[42].

Strategic Recommendations for Legal and Policy Reforms

Legal Modernization: Amend the Wildlife (Protection) Act, 1972, to introduce stricter penalties for organized wildlife crime and provide clearer definitions for offences involving digital and cross-border components.

Integrated Enforcement Framework: Create an inter-agency wildlife crime coordination authority comprising the Wildlife Crime Control Bureau (WCCB), Customs, Forest Departments, and Police.

Capacity Building: Institutionalize training programs for enforcement personnel, prosecutors, and judges on wildlife law and forensic techniques.

Use of Technology: Expand use of AI-based surveillance, e-DNA tracking, blockchain for legal trade authentication, and mobile reporting tools like e-Eye and M-STrIPES[43].

Community-Centric Policies: Strengthen implementation of the Forest Rights Act and incentivize local conservation through Payments for Ecosystem Services (PES) models.

CONCLUSION

India's wildlife crime control architecture is poised for transformation if systemic reforms are enacted. With increased international collaboration, integration of digital technologies, and community involvement, India can move from reactive enforcement to proactive conservation governance. The future depends on embedding ecological justice into law and policy—where biodiversity protection is seen not just as conservation, but as national security and social equity.

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RESEARCH ARTICLE

A Comprehensive Survey on Negative Influence of Social Networks using Deep Learning Methodologies

R. Hemnath[1]* and C. R. Sakthivel[2]

[1]Research Scholar, Department of Computer Science, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Periyanaickenpalayam, (Affiliated to Bharathiar University) Coimbatore, Tamilnadu, India.
[2]Associate Professor, Department of Computer Science, Sri Ramakrishna Mission Vidyalaya College of Arts and Science, Periyanaickenpalayam, (Affiliated to Bharathiar University) Coimbatore, Tamilnadu, India.

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***Address for Correspondence**
R. Hemnath
Research Scholar,
Department of Computer Science,
Sri Ramakrishna Mission Vidyalaya College of Arts and Science,
Periyanaickenpalayam, (Affiliated to Bharathiar University)
Coimbatore, Tamilnadu, India.
E.Mail: hemnathmca@gmail.com

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ABSTRACT

Social network users encounter four specific detrimental effects including anonymous communication combined with cyber bullying plus privacy violations and dangerous media dissemination. Social media users adopt the preventive measures through peer relationship building as well as content verification steps before submitting the dangerous information to authorities. Social networks are indeed of synchronized approaches to enhance the privacy. The research between 2018 and 2025 provides insight into addressing the negative impacts that occur in social networks. These analyzed survey data examine the Deep Learning (DL) solutions to manage social media platform disadvantages in research studies. Deep Learning systems implement identification and regulatory actions to manage misinformation along with the destructive content during social network operations. Research reports indicate that DL methods decrease dangerous content found in social networking environments effectively. The traditiotinal study depended on Parallel Social Behavior-Based Algorithm along with Non-Monotone DR-Submodular Optimization to perform its core investigations. Better network authenticity, enhanced user security and trust emerge through the social network implementation of this technology. This research gives research prospects to minimize the adverse social media characteristics in its final part.

Keywords: Deep Learning (DL), Diminish, Harmful content Misinformation Negative influences, Social networks

INTRODUCTION

Social networking websites have become the part of the communication to link human beings and organizations around the world. Social networking websites have also been breeding grounds for disinformation, cyber bullying, privacy violation, and violence that have caused enormously serious society-level threats, while an eye blink transmit disinformation, which impacts the popular perception, contribute to violence, and deplete e-trust [1]. Similarly, cyberbullying induces severe psychological trauma, particularly to susceptible users. Therefore, it is addressed in order to institute adequate mitigation controls [2]. Deep Learning techniques have been proven to be effective tools with the potential of detecting, analyzing, and mitigating the negative impacts on social networks. Some of the recent research works have proposed few DL methods addressing the challenges. Graph Attention Networks (GATs) have been promising in identifying mental illness from social media behaviors for enabling timely intervention to target users [3]. Long Short-Term Memory (LSTM) models identify the sentiments of social media posts efficiently, and real-time identification of toxic discourse and emotional distress is enabled [4]. In addition, blockchain-enabled DL offers decentralized frameworks for the detection of cyberbullying, ensuring data integrity and recommending appropriate action [5]. Another critical application of DL on social media is prediction and analysis of user behavior. Temporal link prediction models use reasoning mechanisms to make predictions about interactions, perhaps detecting and eliminating the risky behaviors in early phases [6]. Influence maximization techniques through submodular optimization also deter information diffusion and mute the effects of risky content [7]. Deep Learning assists in network structure analysis, allowing the sites to forecast and predict the user characteristics from patterns of connections. The knowledge aid in offering guided interventions for countering the trend towards disinformation and spamming campaigns [8], [9]. Besides, noise-corrected sampling methods make data more trustworthy for online social networks, decreases the content moderation bias [10]. With the technological advancements in social networks, the applications of DL techniques present an active solution to safeguard users and promote reliance on platforms. Utilizing complex neural networks, behavioral prediction models, and blockchain architectures, developers and researchers effectively remove the entire detrimental effects on social networks. In addition to making the web space more secure, these developments also encourage user engagement and confidence as well as the accountability and robustness of social media against potential destruction. Figure 1 shows the reason for social network negative effects resulted through misinformation, cyberbullying, intrusion on privacy, and algorithmic discrimination. Misinformation propagates extremely fast and misinforms the users, while harassment and data exploitation invade security and bliss. Echo chambers, psychological manipulation, over contentment, and commercial exploitation also contribute to the negative effect of social media.

LITERATURE REVIEW

Recent research explores DL methods to combat the ill effects of social media through identifying misinformation, cyberbullying, and hate speech. Adversarial activities are categorized using GAN and LSTMs, privacy and security are increased with blockchain-protected Artificial Intelligence (AI). Temporal link prediction and influence maximization algorithms manage sharing content. All of these measures improve online security, trust, and moral digital participation. Gu, S., *et al.* (2023) examined profit maximization methods in social networks, including non-monotone Diminishing Returns (DR)-submodular maximization. The study revealed the use of user action by firms to maximize the profits while being respectful of ethical concerns. Deep Learning methods for ad campaign maximization and sponsored content were analyzed in the study. Understanding the network structure enabled control over commercial exploitation and promoted user trust. The study was able to balance profitability with ethical online advertising. Hamdi, S. A. (2024) examined the way in which social media disinformation was disseminated under the patronage of the ideological square. The study questioned the process through which polarized narratives shaped public opinion and consolidated social cleavages. The study emphasized the importance of discourse analysis to ascertain ideological trends in disinformation. The study emphasized the challenge of containing biased content and necessitating AI-based verification processes. The results reiterated the need for advanced detection instruments that stood up to disinformation and strengthen evidence-based argument. Hosni, A.

I. E., *et al.* (2024) examined hybrid rumor prevention in dynamic multilayered social networks. The authors researched disinformation diffusion at high speeds and its impact on public opinions. Through the application of deep models and multilayer analysis, rumor detection and control were improved. It showed the efficacy of real-time observation in disinformation prevention. This research assisted in improving the misinformation countermeasures in developing online systems. Table 1 is a comparison table of different research studies based on the subject, methods used, results, and usefulness for social networks. It reflects the new technologies such as deep learning, big data, ethical AI, and adversarial analysis enable the questions on online platforms to be answered. Comparison offered insight into security improvement, trust, and user interaction improvement for social media. Mnasri, W., *et al.* (2021) also performed the same social behavior-based opinion leader identification experiment on social networks. The authors emphasized user behavior and interaction patterns that actively engaged in information diffusion. By examining network structure, an algorithm is started in identifying the influential users that made valuable contributions to discussions in online discussions. The study optimized marketing campaigns and information dissemination. This experiment enriched the influence discovery models to make digital communities lively. Panagopoulos, G., *et al.* (2024) examined the learning graph representations for influence maximization in social networks. The authors studied the impact of graph-based methods on influence prediction and diffusion. The methods of influence maximization were improved using DL models to choose the influential users effectively.

The research provided insights into the role of network structure for maximum information dissemination. This research helped to develop AI-based strategies for social media marketing and information campaigns. Table 2 compares the various studies in terms of objectives, methodologies, findings, and implications in digital networks and AI. It illustrates the application of various methods to manage misinformation, sentiment, disaster forecasting, and network optimization. The comparison provides insights that are used to improve online security, communication, and decision-making in social media and digital networks. Park, J. H., *et al*. (2025) described the application of machine learning for predicting the symptoms of anxiety in social anxiety disorder patients. Multimodal virtual reality session data were utilized in this research to enhance the prediction accuracy. By integrating physiological, behavioral, and text data, the authors achieved enhanced prediction accuracy in detecting early symptoms of anxiety. The authors used the example of the potential use of AI diagnosis of mental illness in virtual reality. The findings helped in altering the intervention for social anxiety patients. Table 3 contrasts the various studies in areas of interest, methodology, primary findings, social network and AI use. It shows the leading of various methodologies to energy efficiency, social media, revenue maximization, and behavior monitoring. Comparison is made based on improving decision-making, online participation, tracking data online. George, J., *et al.* (2023) examined the diffusion of misinformation using a multidisciplinary study methodology. Trends and patterns behind the diffusion of misinformation and those that justified its validity and diffusion were examined. Patterns of social media usage and algorithms were the determining drivers of the pace of the spread of false information. This research highlighted the need for sophisticated detection tools in a bid to adequately fight online disinformation. The research brought into the perspective of expanding public opinion and content verification against disinformation. Gruner, R. L., *et al.* (2019) conducted a study to analyze the impact of internet marketing and social media communication on new product launches. The authors analyzed the impact of web promotions on profit and sales quantity. The authors discovered that social media engagement with customers had considerable impacts on customers' behavior and purchasing activity. This research suggested the importance of internet advertising to promote product innovation in an effort to stay competitive in the market. These findings have implications for the extent to which consumer demand is web-based platform-driven.

Problem Identification

The pandemic, cyberbullying, and hate speech on social media have led to several problems of internet security and trust. Traditional moderation policies have failed to stem the fleeting spread of disinformation and bad manners. Social media algorithms are correctness-agnostic and popularity-driven. Therefore, spread the disinformation and polarize people. Privacy invasions and unethical applications of information fuel ill-influences in internet communication even more. Businesses utilizing social media for advertising are confronted with the challenge of

walking the tightrope between money-making schemes and ethics. The need for sophisticated AI-based methods of detecting, avoiding, and countermanding such issues was now a necessity to ethical online communication.

Existing Methodologies

Several methods have been constructed to address the social network problems in collaboration with sophisticated computational methods. Cyberbullying detection based on blockchain and neural networks use DL to enable real-time monitoring and store data securely. Fake news prevention models apply behavior analysis, machine learning, and NLP to detect misinformation and enhance credibility of content. Optimization methods like non-monotone DR-submodular maximization maximize profit-maximizing policies with ethics-based user interaction. Big data-driven forecast models use customer behavior and marketing-mix inputs in guiding business strategy selection. Algorithm models of fairness managing online ad delivery to prevent manipulative targeting and transparency in personal content delivery. Social behavior-driven algorithms use parallel processing to determine influencer users and optimize information sharing and engagement tactics. Also, AI-based text analysis tools utilize NLP and sentiment analysis to recognize social media markers of intimate partner violence through which online safety intervention and early intervention take place. This methods work complementary toward enhancing security, ethical decision-making, and user trust in online environments.

Blockchain-Based **Deep Learning Cyberbullying Detection and Prevention System**

The system uses DL techniques for real-time cyberbullying identification from vast volumes of social media interactions. Leaning on the present-day neural networks like Convolutional Neural Networks (CNNs) and LSTMs, the system detects hate text, image, and video patterns and accurately identifies offending material, and threats. Decentralized and unalterable records are maintained through blockchain technology and in reported cases the goal is to achieve transparency, security, and authenticity. Where cyberbullying is detected, the system provides suggestions wisely. For example, automatic removal of the material, instant warning to the user, and intervention such as providing counseling resources or notifying parents are suggested. Further, the system allows secure information sharing between agents such as platform moderators, mental health professionals, and police, in a way where the cyberbullying is managed effectively and coordination is established. Through the use of AI-powered identification and blockchain's authenticity, this process alters the pace of cyberbullying prevention to safer and more ethical online communication. Alabdali, A. M., and Mashat, A. (2024) Figure 2 illustrates a Blockchain-Based Deep Learning Cyberbullying Detection and Prevention System with key steps ranging from Data Collection, Preprocessing, and DL Model Training to Cyberbullying Detection and Blockchain Integration for secure record-keeping. On top of that, Intelligent Recommendations, Stakeholder Alerts, Real-Time Monitoring and Adaptation, and User Awareness and Reporting have been incorporated for better intervention and system adaptability. Finally, Evaluation and Improvement ensures continuous improvement so that the framework is increasingly effective in preventing cyberbullying attacks.

Multi-Disciplinary Fake News Detection and Mitigation Model

This model is end-to-end based on the combined application of different analytical approaches for discovering, analyzing, and fighting social network disinformation. This approach combines NLP for text content analysis, machine learning methods for detecting fake patterns, and social network analysis for following the information disinformation paths among users. Behavioral and psychological studies are combined in an attempt to understand the individuals accept and transmit false information by considering cognitive shortcuts, emotional signals, and social influence. Blockchain technology makes transparency possible through fact-checking processes that are verifiable so that the credible sources are input in a manner that has not been tampered with and false information generated. The model is also complemented with user awareness campaigns and media literacy training to inform the people to critically evaluate digital content. The model is also founded on real-time intervention tactics such as credibility scoring using machine learning, fact-checking of content using AI, and misinformation warnings that lead the users to the appropriate decision-making before access to fake content. This is done, through the integration of technology knowledge, psychology, and social science with precision and efficiency in detecting deception news, ultimately resulting in a responsible, informed, and credible online persona. George, J., *et al*. (2023).

Non-Monotone Dr-Submodular Optimization for Profit Maximization in Social Networks

Non-Monotone DR-Submodular Optimization for social network profit maximization uses the sophisticated optimization techniques for revenue maximization in terms of strategic and ethical equilibrium on the web. The approach uses non-monotone DR-submodular maximization for maximization in ad decision-making, content promotion, and user engagement models. Based on network structure, user behavior pattern and interaction dynamics analysis, the model identifies influential nodes with high influence that maximize information diffusion and influence propagation. It uses machine learning algorithms to maximize profit-maximization strategies under fairness, budget, and user experience constraints. It uses adaptive pricing and personalization recommendation systems to render advertisement spending effective on best-fit groups. It not only maximizes the monetization efficacy on social media but also balances revenue increase with user experience, responsible ads, and sustainable engagement, making the world of internet ethical and lucrative. Gu, S., *et al*. (2023)

Big Data-Driven Demand Forecasting Framework with Marketing-Mix Optimization

Big Data-Driven Demand Forecasting with Marketing-Mix Optimization solution uses the latest data analytics and machine learning algorithms to forecast demand for customers and combine significant marketing-mix drivers. It delves into vast structured and unstructured data on social media activity, consumer behavior, marketplace activity, and promotions. Based on predictive models, it determines volatility in demand and fine-tunes prices, advertisement, distribution, and product assortment to achieve the maximum responsiveness in the marketplace. The system employs real-time data analysis to modify marketing strategies in real time so that business entities can respond to evolving consumer behavior with maximum effect. It also features AI-powered decision-making tools that track historical trends of sales and outside economic factors to enhance forecasting strength. By combining the demand forecasting with marketing-mix drivers, the model make business more profitable, wastage-free supply chain and develop more customer-focused marketing strategies within a more competitive online marketplace. Kumar, A., *et al.* (2020). Figure 3 is a big data-driven demand forecasting Framework to Market-Mix Optimization. Data gathering is the initial step and proceeded by preprocessing for quality achievement. Feature selection and engineering push significant variables for predictive model construction. Dynamic real-time analysis and calibration enhance demand forecast, and marketing optimization pushes optimal business performance improvement. Model polishing iterative, which is provided real-time backed by improvement, pushes strategic planning decision support for best form.

Fair Algorithmic Targeting Framework for Online Advertising

The Fair Algorithmic Targeting Framework for internet advertising is in an effort to strike a balance between outsize commercial practice in the behaviorally targeted online advertising with accountability, transparency, and consumer protection. It uses ethical AI models to regulate algorithmic targeting to avoid the users' personal information being used to exploit and manipulative biased management of consumers' behavior. It uses privacy-preserving techniques such as differential privacy and federated learning to decrease the intrusive data gathering without compromising ad relevance. Apart from this, it puts rulebooks to manage ethical advertisement methods such as consent-targeting, discovery of ad distribution bias, and disclosure for automated decision-making. Hiding in between consumer safety and company advantage, the model restrains internet behavior advertisement deleterious effects and develops a more wholesome and the moralized internet advert environment. Laux, J., *et al*. (2021)

Parallel Social Behavior-Based Algorithm

Parallel Social Behavior-Based Algorithm for influential user identification is an approach that is designed for the efficient identification of influential users from social networks after observing interaction behavior and behavior trends. Parallel computational techniques are applied in the above approach to handle vast volumes of social network data. Hence, it is made efficient and accurate to identify the influential nodes. This algorithm selects the most likely users to spread information and shape the network by considering user connectivity, sharing behavior for content, and scores for connectivity. Predictions are enhanced with machine learning algorithms by means of continuous learning against dynamic social interactions. Influence propagation analysis is also dealt within the framework of marketing campaign optimization, information diffusion strategy, and community activation. The

technique improves web influence measurement, social media research, and business marketing by pinpointing the consumers who drive conversations, trends, and engagement on the web. Mnasri, W., *et al.* (2023)

Detection of Intimate Partner Violence on Social Media using AI-Powered Text Analysis Framework

The social media text analysis platform for the detection of intimate partner violence Using AI is based on natural language processing (NLP) and machine learning that identify signs of abuse from social media. The system scans colloquially written texts published on social networking sites, identifying linguistic markers, emotional markers, and contextual markers of intimate partner violence. Deep learning models tag and categorize documented cases with risk indicators to allow early intervention and support for victims. Sentiment analysis and entity recognition techniques enhance the detection of distress signals with greater accuracy, enabling the social service agencies to react accordingly. This system also integrates the ethical AI practices to protect user privacy and utilization of information for abuse. By AI computerized detection and real-time alarm, the practice is expanding the deployment to prevent intimate partner violence and providing an interactive platform for online safety and activism. Trinh Ha, *et al*. (2022**)**

DISCUSSION

Deep Learning technology deployment is important in avoiding the undesirable impacts in social networks by the factors such as cyberbullying, misinformation, and inappropriateness. Blockchain-Based Deep Learning Cyberbullying Detection and Prevention System enables improved real-time objectionable content identification and guaranteed transparency of data and secure processing. The Multi-Disciplinary Fake News Detection and Mitigation Model combine machine learning and social analysis to dampen the propagation of misinformation and construct an information-centric online environment. The Non-Monotone DR-Submodular Optimization framework attains moral maximum revenue by maximizing the user interaction plans without permitting manipulative behavior. The Big Data-Driven Demand Forecasting Framework applies the predictive analytics in re-designing marketing plans without sacrificing user preference violation and minimization of manipulative targeting. Fair Algorithmic Targeting Framework solves the privacy problems in an ethical and fair way of online advertising. Parallel Social Behavior-Based Algorithm supports opinion leader identification and promotes healthy information diffusion and suppression of negative information diffusion. Finally, AI-Powered Text Analysis Framework detects the intimate partner violence by conducting text analysis on social media as well as supports early intervention and victim rescue. All these methods show the neglect of sophisticated DL computers on adverse consequences and develop better strategic and secure cyberspaces.

CONCLUSION

This research analyzed 35 articles on minimizing the ill effects in social networks through DL methodologies. The examined researches were previously published between 2018 and 2025. The paper offers enhanced internet security through the application of deep learning in identification and reversal of cyberbullying, misinformation, and discriminatory activity on social media. The research identifies new DL methods of identification, evasions, and elimination of undesirable social network impacts on internet security. Its application ensures online security through deep detection, evasion, and neutralization of unwanted content in social networks. This research forms the basis in developing next-generation deep learning interventions for protection against cyber attack, scam, and unwanted interaction in social networks. Future research also focuses on improving DL algorithms for real-time detection, improving accuracy, and the application of ethical AI in ensuring fairness in content moderation. It aims at establishing the adaptive algorithms for combating future threats and securing social network interactions.

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Table 1: Comparison of Research Studies on Social Networks and AI Applications

Reference	Focus Area	Methodology Used	Key Findings	Application in Social Networks
Jackson *et al.* (2023)	Cultural evolution of morality in social networks	Adaptive heuristics in large digital communities	Morality evolved through social interactions and cultural adaptation	Helped in understanding ethical behaviors in online interactions
Jung *et al.* (2022)	Image-to-image translation and contrastive learning	Patch-wise semantic relation analysis	Improved AI-based content synthesis in media generation	Contributed to detecting AI-manipulated visual misinformation
Kumar *et al.* (2020)	Demand-driven forecasting using big data	Data-driven framework with marketing-mix variables	Optimized predictive analytics for business decisions	Enhanced targeted advertising and user engagement strategies
Laux *et al.* (2021)	Algorithmic targeting in online advertising	Market power analysis of digital advertising practices	Identified unfair commercial practices in AI-driven ad targeting	Helped regulate AI-driven behavioral advertising to prevent exploitation

Lewis and Moorkens (2020)	Ethical AI and trust in social media	Rights-based approach to AI governance	Advocated for ethical AI deployment and transparency	Supported the development of fair AI-based moderation systems
Ma *et al.* (2020)	Security risks in graph neural networks	Adversarial attacks on GNNs	Identified vulnerabilities in AI models for misinformation detection	Strengthened AI defenses in social network moderation systems
Maddodi and Kunte (2024)	Market resilience during geopolitical crises	Predictive analysis of stock market trends	Provided insights into market stability under economic stress	Assisted in understanding financial misinformation in social media

Table 2: Comparison of Research Studies on Digital Networks, AI, and Social Impact

Reference	Focus Area	Methodology Used	Key Findings	Application in Social Networks
Sewpersadh (2023)	Disruptive business value models in the digital era	Innovation-driven analysis of digital business trends	Identified emerging digital business models shaping modern economies	Contributed to understanding evolving financial trends in digital platforms
Sharevski *et al.* (2021)	Misinformation discourse on social media	Experimental design using the Voxpop platform	Demonstrated how misinformation spreads and how calibrated discourse can control it	Provided insights into misinformation control and discussion frameworks
Sun, Huang, and Zhao (2024)	Predicting hurricane evacuation decisions	Machine learning-based risk prediction models	Improved interpretability of AI models in disaster response decision-making	Highlighted AI's role in analyzing emergency responses in social media data
Tan *et al.* (2023)	Reinforcement learning in perception layer networks	Optimization strategies for network routing	Enhanced efficiency in network communication and data transmission	Improved data flow management in digital communication networks
Trinh *Ha et al.* (2022)	Identification of intimate partner violence in social media	Natural Language Processiong (NLP)-based analysis of free text descriptions	Successfully detected signs of abuse using computational linguistics	Contributed to social media monitoring for abuse prevention and support
Wang *et al.* (2025)	Public opinion evolution in digital platforms	AI-based emotion and text analysis using Top2Vec-RoBERTa	Modeled how emotions influence public discussions and topic shifts	Helped track sentiment trends and misinformation evolution in online discourse

Table 3: Comparison of Research Studies on Digital Networks, AI, and Social Impact

Reference	Focus Area	Methodology Used	Key Findings	Application in Social Networks
Wenninger *et al.* (2022)	AI in residential building retrofitting	Explainable AI with socio-demographic data analysis	Identified optimal retrofitting practices based on data-driven insights	Showed how AI can enhance decision-making in energy-efficient housing

Whelan, Golden, and Tarafdar (2022)	Technostress and social media self-control	Behavioral analysis of academic performance and well-being	Found that excessive social media use negatively impacted academic success	Highlighted the psychological effects of social media overuse on students
White (2020)	Social media's role in nationalism and civic education	Globalism vs. nationalism discourse analysis	Showed how social media shaped civic engagement and political ideologies	Demonstrated the influence of social platforms on political awareness and activism
Xiong *et al.* (2019)	Revenue maximization in mobile social data markets	Dynamic pricing models in network effects	Developed pricing strategies to optimize revenue in social data sharing	Provided insights into social network monetization and data economy
Zhang *et al.* (2017)	Social connections in online gaming markets	Nonlinear interaction analysis in goal attainment	Found that social interactions influenced gaming spending and goal achievements	Contributed to understanding user engagement in digital entertainment platforms
Zhao *et al.* (2018)	Social network monitoring and data aggregation	Temporal aggregation analysis in network surveillance	Showed how different time scales affected social network trend analysis	Improved social media monitoring and early detection of online trends

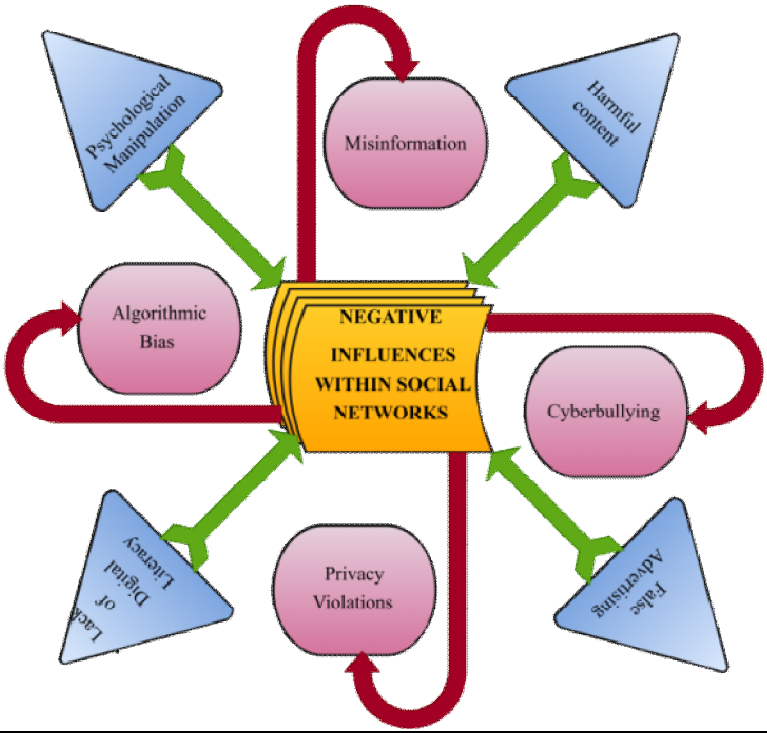


Figure 1: Causes for the negative influences in social networks

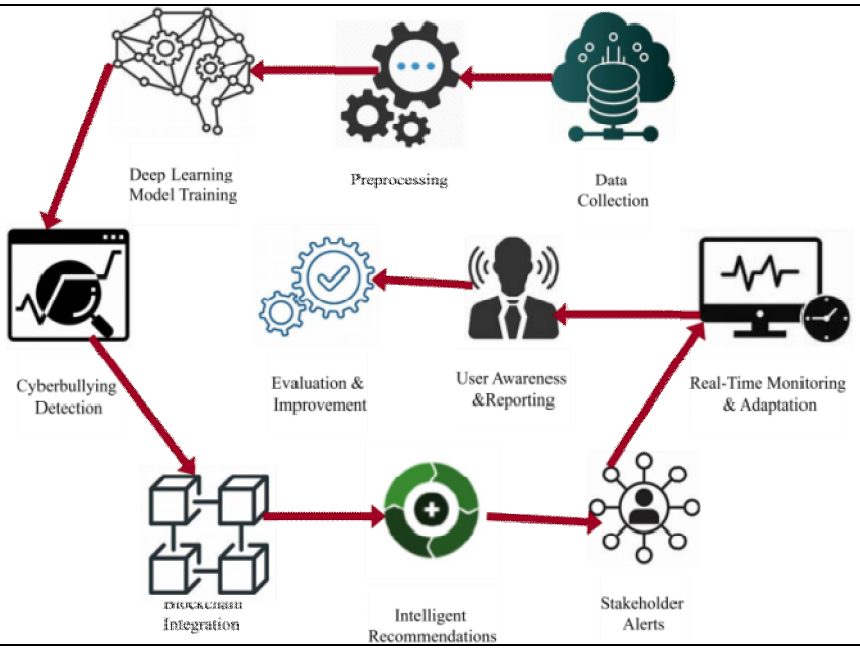


Figure 2: Architecture of Blockchain-Based Deep Learning

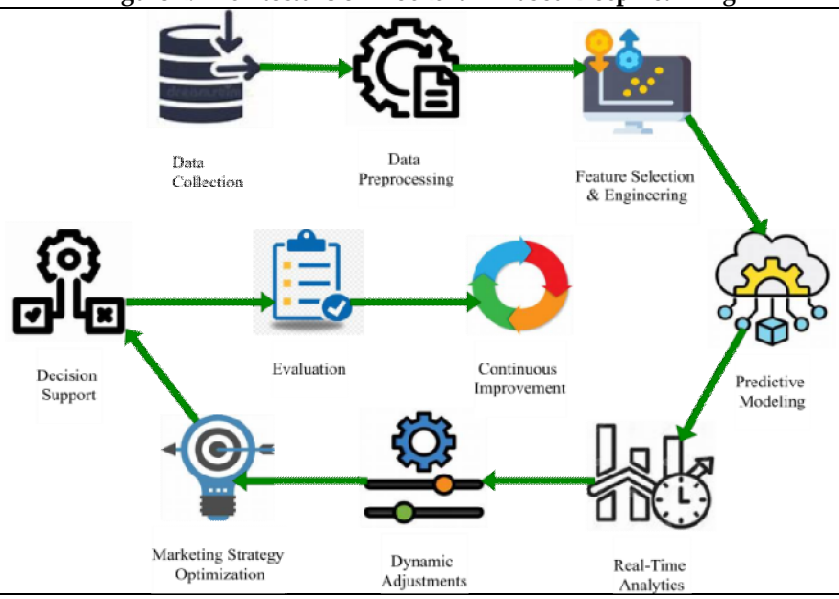


Figure 3: Architecture of Big Data-Driven Demand Forecasting Framework

RESEARCH ARTICLE

A Comparative Study of Fear of Missing Out and Anxiety on Instagram and LinkedIn in Digital Age

Aditi Gupta[1]* and Anju Dangwal[2]

[1]Student, Department of Humanities and Liberal Arts, DIT University, Mussoorie, Makkawala, Dehradun, Uttarakhand, India.
[2]Assistant Professor, Department of Humanities and Liberal Arts, DIT University, Mussoorie, Makkawala, Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Aditi Gupta
Student,
Department of Humanities and Liberal Arts,
DIT University, Mussoorie, Makkawala,
Dehradun, Uttarakhand, India.
E.Mail: aditikansalgupta@gmail.com

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ABSTRACT

This study examined the levels of Fear of Missing Out and anxiety among adult users of Instagram and LinkedIn, focusing on how these experiences manifest across social and professional digital environments. A total of 119 participants aged 21 to 40 years completed standardized self-report measures assessing both Fear of Missing Out and generalized anxiety in relation to their engagement with each platform. Descriptive analyses indicated that the mean levels of Fear of Missing Out were comparable between Instagram and LinkedIn, with only minimal differences observed. Inferential statistical tests revealed no significant difference in Fear of Missing Out scores between users of the two platforms, suggesting that the phenomenon is similarly prevalent regardless of whether the context is social or professional. The results suggest that the tendency to experience Fear of Missing Out is not exclusive to visually appealing, socially oriented platforms like Instagram, but is a widespread phenomenon influenced by digital connectivity across both social and professional online spaces. The findings underscore the importance of considering a range of contextual and individual factors in understanding Fear of Missing Out and point to the need for future research to explore how specific platform features interact with user characteristics to shape emotional experiences online.

Keywords: Fear of Missing Out, Anxiety, Instagram, LinkedIn, Social Media, Digital Well-Being

INTRODUCTION

In today's interconnected world, social media has become the heartbeat of human connection, fulfilling our deep-rooted need to engage with others. What once was celebrated as a breakthrough in communication now reveals a more complicated reality of these platforms, while connecting us can also take a toll on our mental well-being. Research has made it clear that spending excessive time on social media is linked to rising levels of depression and anxiety highlighting the hidden costs of our screen-filled lives. As we scroll through endless streams of carefully curated images and stories, we often find ourselves caught in a web of comparison. The perfect lives displayed online can spark feelings of loneliness, inadequacy and dissatisfaction. This experience is closely tied to a modern psychological phenomenon known as the Fear of Missing Out or FOMO. Defined as the anxious feeling that others are enjoying rewarding experiences without us, FOMO drives a relentless urge to stay connected and in the loop (Przybylski *et al*., 2013). It's a feeling so universal that different cultures have their own words for it-like "kiasu" in the Hokkien dialect, which captures the fear of losing out to others. Though FOMO has long been a topic in popular media, only recently has it attracted serious academic attention. Scholars have begun to explore how unmet psychological needs fuel this fear, often linking it to problematic social media use and its impact on motivation, especially among students. Alongside FOMO, anxiety itself-a condition marked by worry, tension and physical symptoms-has been part of human experience for centuries from ancient times to Freud's early descriptions in the 19th century. Today anxiety disorders affect millions worldwide in India alone, nearly 45 million people grapple with anxiety, underscoring the urgency of understanding how digital life influences mental health(The Lancet, 2017). Among many social platforms, Instagram and LinkedIn stand out for their contrasting roles in our digital lives. Instagram, launched in 2010 and now home to over a billion users worldwide, enables individuals to share moments from their lives through photos and short videos, frequently enhanced with filters and hash tags for creative expression and broader reach. It's a space where lifestyle and social identity take center stage and its reach continues to grow rapidly especially in countries like India. LinkedIn, on the other hand serves a different purpose. Since 2003, it has been the go-to platform for professional networking, career advancement and business connections. With its focus on resumes, skills and job opportunities, LinkedIn creates a unique environment where users compare themselves in terms of career success rather than social life. The rapid flow of information and constant connectivity characterizing our modern digital landscape has transformed how FOMO and anxiety are experienced. Social media's omnipresence amplifies these feelings, particularly among young adults navigating the pressures of both social and professional worlds. Despite growing concerns, few studies have directly compared how platforms like Instagram and LinkedIn differently shape these psychological experiences. Instagram's vibrant social atmosphere may trigger social FOMO, while LinkedIn's professional focus might evoke career-related anxieties a distinction that remains underexplored. This research aims to fill that gap by examining how the unique content and culture of these two platforms influence FOMO and anxiety. By understanding how social and professional comparisons operate differently across Instagram and LinkedIn, this study hopes to offer valuable insights for users, mental health professionals and policymakers alike. Developing a unified framework to define and analyze FOMO is essential for advancing this field, enabling more targeted strategies to mitigate the negative psychological impacts of social media and promote healthier digital engagement.

REVIEW OF LITERATURE

A substantial body of research has examined the psychological dynamics of Fear of Missing Out (FOMO), especially in the context of social media use and its wider psychosocial implications. Collectively, this literature highlights the multifaceted nature of FOMO, identifying it as both a catalyst for and a result of problematic digital behaviors, mental health issues, and evolving patterns of social interaction in the digital era. Most recently Kovan *et al*. (2024) studied Turkish university students and found that FOMO plays a significant role in driving social media addiction. Their research demonstrated that this relationship is mediated by reduced self-control and personal responsibility indicating that interventions aimed at strengthening these traits could help curb maladaptive digital behaviors. This finding provides a crucial foundation for understanding how individual psychological characteristics interact with

digital environments to intensify addictive tendencies. Shifting the focus to younger users, Kothari (2023) explored FOMO among adolescents, revealing that it is not only associated with compulsive social media usage but also linked to risky behaviors such as substance abuse. These results underscore the pervasive and potentially hazardous nature of FOMO, especially during developmental stages marked by heightened social sensitivity and identity formation. Continuing along this developmental trajectory, Putri, Hurriyati and Hassanbasry (2023) examined early adults and demonstrated a strong positive correlation between FOMO and Instagram addiction. The authors attributed this vulnerability to emotional instability and the self-exploratory tendencies characteristic of this life stage. These findings echo Kothari's conclusions and further highlight the role of life transitions and identity-seeking behaviors in intensifying FOMO. Turning to platform-specific effects, Faelens *et al*. (2021) conducted a systematic review that illuminated the mental health risks associated with Instagram use. Their analysis identified increased social comparison, negative body image, and disordered eating as key outcomes, highlighting the nuanced ways in which FOMO manifests depending on the design and content of specific platforms. In the same year Tandon *et al*. (2021), synthesized findings from 58 empirical studies in an effort to bring coherence to the field. They called for greater conceptual clarity in the definition of FOMO and identified psychological (e.g., low self-esteem), social (e.g., peer influence) and technological (e.g., notification systems) drivers of the phenomenon. Their review also reinforced prior findings by linking FOMO to adverse well-being outcomes such as anxiety and poor sleep, highlighting the interplay between internal dispositions and external triggers. At a more conceptual level, Gupta and Sharma (2021) provided a comprehensive overview of the psychological origins of FOMO. They linked the phenomenon to the basic human need for social belonging, arguing that social media amplifies this need through constant exposure to curated social experiences. Their theoretical insights resonate with the findings of Dhir *et al*. (2019), who examined social media fatigue and demonstrated how constant connectivity and information overload contribute to reduced life satisfaction and increased stress. Together, these studies point to a cyclical relationship between FOMO and digital exhaustion, where each reinforces the other. Adding an experimental perspective, Rozgonjuk *et al*. (2020) explored the mediating role of FOMO in impairing daily functioning and work productivity, emphasizing its impact on focus and efficiency through problematic engagement with platforms like WhatsApp, Facebook and Instagram. Hunt *et al*. (2018) provided robust evidence of a causal relationship between reduced social media use and improvements in mental health outcomes, including decreased depression, loneliness and FOMO. Offering a nuanced perspective, Roberts and David (2019) found that while FOMO may increase social media engagement and foster a sense of social connection, it simultaneously exerts a direct negative effect on subjective well-being. This duality emphasizes the complex and multifaceted nature of FOMO's psychological influence, which can oscillate between perceived social gains and genuine emotional costs. Foundational work by Abel *et al*. (2016) and Przybylski *et al*. (2013) laid the groundwork for contemporary research on FOMO. Abel *et al*.(2016), developed a reliable measurement scale, reinforcing the validity of FOMO as a distinct psychological construct. Przybylski *et al*..(2013), further operationalized the construct and established its association with lower life satisfaction and higher social media engagement, illustrating its behavioral and emotional correlates. Taken together, these studies present a rich and multidimensional understanding of FOMO as a construct deeply embedded in contemporary digital culture. They collectively highlight its significant implications for mental health, social behavior, productivity and well-being across different age groups and socio-cultural contexts. This integrated body of literature underscores the urgent need for preventive strategies, digital literacy programs and psychological interventions to address the escalating effects of FOMO in the digital era.

MATERIALS AND METHODS

This study employed a comparative cross-sectional research design to examine levels of Fear of Missing Out (FOMO) and anxiety among users of two social media platforms, Instagram and LinkedIn. The sample consisted of 119 active social media users (Female: N=50, Male: N=69) aged between 21 to 40 years, recruited via convenience sampling. The majority of participants were in the 21–25 age groups, with fewer individuals represented in the 36–40 age range. Inclusion criteria required participants to be active users of both Instagram and LinkedIn. Participants completed a structured socio-demographic questionnaire, followed by standardized self-report measures assessing FOMO and generalized anxiety. The Fear of Missing Out Scale developed by Przybylski *et al*. (2013), is a 10-item instrument

designed to assess individuals distress related to missing out on rewarding social experiences. Each item is rated on a 5-point Likert scale ranging from 1 ("not at all true of me") to 5 ("extremely true of me") with higher scores indicating greater FOMO. The FOMO Scale demonstrates strong psychometric properties, including high internal consistency (α = .82–.90) and established validity across diverse populations. Anxiety was measured using the Generalized Anxiety Disorder 7-item scale, a widely validated instrument developed by Spitzer *et al*. The GAD-7 assesses the frequency of anxiety symptoms experienced over the past two weeks, with items rated on a 4-point Likert scale from 0 (not at all) to 3 (nearly every day). Total scores range from 0 to 21, with higher scores reflecting greater anxiety severity. The GAD-7 has demonstrated high internal consistency Cronbach's alpha .89. All measures were administered via a secure online platform through Google Forms. Prior to participation, respondents reviewed a comprehensive digital informed consent form outlining the study's purpose, voluntary nature, confidentiality assurances and the right to withdraw at any time without consequence. The average completion time for the survey was approximately 10–15 minutes, minimizing participant fatigue and promoting response quality. This methodology enabled a systematic comparison of FOMO and anxiety across two distinct social media contexts, providing insights into the psychological effects of platform-specific engagement. The results of the study are presented through both descriptive and inferential statistical analyses offering a nuanced understanding of the levels of Fear of Missing Out (FOMO) experienced by users of Instagram and LinkedIn.

Descriptive Statistics

Table 1 presents the descriptive statistics for FOMO levels among Instagram and LinkedIn users in the sample (N = 119). The analysis reveals that Instagram users (n = 44) reported a mean FOMO score of 21.52 with a standard deviation of 7.28, whereas LinkedIn users (n = 75) exhibited a slightly higher mean FOMO score of 22.21, with a standard deviation of 8.31. The standard deviations observed indicate considerable variability in FOMO experiences across both groups. LinkedIn users demonstrated a slightly greater spread in scores, which may reflect broader individual differences in emotional responses and patterns of engagement with the platform's content. A graphical representation of this data is provided in Figure 1, illustrating the comparative distribution of FOMO levels across the two platforms. The bar graph above presents the descriptive statistics for Fear of Missing Out (FOMO) levels among Instagram and LinkedIn users. As shown, the mean FOMO score for Instagram users is 21.52, while LinkedIn users have a slightly higher mean score of 22.21. The standard deviation for Instagram is 7.28, indicating moderate variability in FOMO levels within this group. LinkedIn users exhibit a standard deviation of 8.31, suggesting slightly greater variability in their FOMO experiences. Overall, the results indicate that FOMO levels are comparable across both platforms, with only minimal differences observed in both the mean scores and the variability of responses. The study examined the levels of Fear of Missing Out (FOMO) among users of two distinct social media platforms: Instagram and LinkedIn. Descriptive statistics showed that Instagram users reported a mean FOMO score of 21.52 (SD = 7.28, n = 44), while LinkedIn users had a slightly higher mean score of 22.21 (SD = 8.32, n = 75). The standard deviations indicate considerable variability in FOMO experiences within both groups, with LinkedIn users showing slightly more variation. This variability may reflect diverse patterns of engagement and emotional responses to each platform's content and social dynamics. These findings suggest that users of Instagram and LinkedIn experience comparable levels of FOMO, despite the platforms' differing social and professional orientations. This challenges the common assumption that Instagram's visually rich and socially driven environment would necessarily elicit higher FOMO than LinkedIn's professional networking context. Instead, the similar FOMO levels may indicate that both platforms foster social comparison and awareness of missed opportunities, albeit within different domains.

Gender Distribution

The gender composition of the sample is outlined in Table 3. Of the 119 participants, 69 identified as male and 50 as female, indicating a modest predominance of male participants in the study. To assess whether the observed differences in FOMO scores between Instagram and LinkedIn users were statistically significant an independent t-test was conducted. The results are summarized in Table 2. The results of the independent samples t-test, assuming equal variances, indicate that there is no statistically significant difference in Fear of Missing Out (FOMO) levels between Instagram and LinkedIn users. The mean FOMO score for Instagram users (M = 21.52, SD = 54.26) was slightly lower than for LinkedIn users (M = 23.33, SD = 58.90). The t-test yielded a t statistic of -1.26 with 117 degrees

of freedom and a two-tailed p-value of 0.21, which is greater than the conventional significance threshold of 0.05. Consequently, the null hypothesis stating that there is no difference in FOMO scores between the two groups cannot be rejected. These findings suggest that the platform of social media use may not be a significant factor influencing FOMO among users in this sample.

Interpretation of Findings

The findings challenge prevalent assumptions about the emotional impact of different social media platforms. Despite Instagram's socially rich and visually immersive environment, which is often linked to elevated FOMO, users did not report significantly higher FOMO levels than those using LinkedIn a platform with a primarily professional orientation. The slightly higher mean FOMO score observed among LinkedIn users suggests that feelings of missing out may also be salient in professional contexts, potentially driven by exposure to others achievements, career updates and professional opportunities. Importantly, the substantial standard deviations in both groups highlight the heterogeneity of FOMO experiences. This variability underscores the influence of individual-level factors such as personality traits, usage patterns and social comparison tendencies on the psychological outcomes of social media engagement. These results emphasize the importance of adopting a broader conceptual lens when analyzing FOMO, one that moves beyond platform-centric explanations. The experience of FOMO appears to be shaped not merely by the social or professional context of the platform, but by a complex interplay of user motivations, psychological dispositions, and the dynamic nature of online interactions. Thus, rather than being confined to a specific social media environment, FOMO emerges as a multifaceted construct reflective of the broader socio-digital ecosystem in which individuals are embedded.

DISCUSSION

This study challenges simplistic assumptions about the influence of specific social media platforms on users' psychological experiences by demonstrating that Fear of Missing Out (FOMO) is not confined to either social or professional digital environments. The comparable FOMO levels observed across Instagram and LinkedIn underscore the importance of looking beyond platform-centric explanations and considering the broader, multifaceted factors that shape digital well-being. Although the differences in FOMO scores were minimal and not statistically significant, the substantial variability within each group highlights the diverse ways individuals engage with and are affected by online communities. These findings point to the evolving nature of digital socialization, where personal motivations, interaction patterns, and cultural contexts intersect to influence emotional experiences. By reframing FOMO as a dynamic outcome of complex, ongoing interactions rather than as a product of isolated platform features, this research advocates for more nuanced theoretical models and interventions. Ultimately it positions digital emotional experiences as adaptable and context-dependent offering promising directions for supporting psychological well-being across a variety of online settings and life stages. The implications of these findings suggest that interventions aimed at reducing FOMO should be tailored to the specific social media context. For Instagram users, strategies might focus on managing social comparison and emotional regulation related to social interactions, such as setting timers to limit app usage to scheduled periods. For LinkedIn users, addressing professional-related FOMO and its impact on career anxiety may be more pertinent, incorporating strategies like limiting participation to meaningful interactions and connections, and establishing clear goals and priorities. Further research should undertake comprehensive comparative analyses of Instagram and LinkedIn to better understand how platform-specific content contributes to the experience of FOMO. Such studies can illuminate the distinct ways in which each platform's focus whether on social lifestyles or professional achievements shapes users' perceptions and anxieties. There is also a need to develop and evaluate effective strategies to mitigate and manage the negative consequences associated with FOMO on these social media platforms, promoting healthier and more intentional engagement for users. A key limitation of this study is its focus solely on young adults a choice driven largely by constraints related to time and resources. Future studies should consider including a broader age range and more diverse socio-demographic groups to improve the generalizability of the results. Such an expanded sample would offer deeper insights into how FOMO and anxiety present across different populations. Overall, these findings

reinforce the idea that FOMO is a widespread phenomenon influenced by a complex interplay of individual, contextual, and platform-specific factors, rather than being tied to any single type of social media environment. This underscores the need for holistic, adaptable approaches to digital well-being that address the unique challenges and opportunities presented by both social and professional online platforms.

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Table 1: Descriptive Statistics of FOMO Levels on Instagram and LinkedIn (N = 119)

Platform	Mean	Standard Deviation
Instagram	21.52	7.28
LinkedIn	22.21	8.31

Table 2: Inferential Statistics Comparing FOMO Scores Between Instagram and LinkedIn Users Independent Samples T-Test

	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference
FOMO	-1.26	117	0.21	-4.49	1.261

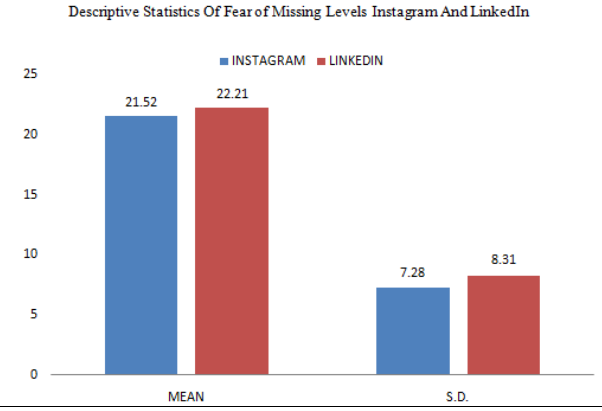


Figure 1: Graphical Representation of Descriptive Statistics Comparing FOMO Levels Between Instagram and LinkedIn

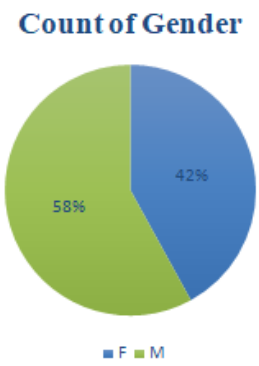


Figure 2: Gender-Based Distribution of Participants (N = 119)

RESEARCH ARTICLE

Effects of Mobilizations and Combination Therapy in Frozen Shoulder (Diabetic vs Non-Diabetic Cases) A Comparative Case Study

Imran Hussain[1]* and Miss Ritika Kumari[2]

[1]Physiotherapist, Viklang Bhawan Hospital, Kankarbagh, Patna, Bihar, India.
[2]Intern (Batch 2020), Department of Physiotherapy, Bihar College of Physiotherapy and Occupational Therapy, Kankarbagh, (Affiliated to Aryabhatta Knowledge University, Mithapur) Patna, Bihar, India.

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***Address for Correspondence**
Imran Hussain
Physiotherapist,
Viklang Bhawan Hospital,
Kankarbagh, Patna, Bihar, India.
E.Mail: imranhssn74@gmail.com

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ABSTRACT

Physiotherapists in a variety of settings treat individuals with frozen shoulder. Frozen shoulder syndrome is a condition in which a soft tissue Glenohumeral capsular lesion is accompanied by painful and restricted active and passive shoulder motion. Frozen shoulder in individuals with Diabetes significantly affects ADLs and QOL. Traditional Physiotherapy methods often provide slow recovery over extended period of time. Advanced treatment protocol along with Maitland's method of Mobilizations and Combination therapy (UST and IFT) provide better results in Diabetic Frozen shoulder as compared to traditional methods of treatment.

Keywords: Physiotherapists, Frozen shoulder, Glenohumeral, Diabetes.

INTRODUCTION

Adhesive capsulitis, Periarthritis shoulder and frozen shoulder are all terms used to describe a painful stiffness of Glenohumeral joint. This inflammatory condition causes fibrosis of the Glenohumeral joint causes gradual progressive stiffness and significant restriction in the joint Range of Motion. Joint mobilization is a form of passive movement in a broad spectrum of exercise used to treat painful and stiff synovial joints. For the purpose of this study, mobilization will refer to passive oscillatory movement of one articular surface in relation to its counterpart. The oscillatory movements will be in the direction of the joints accessory motions (Concave-Convex rule[14]) which are small spinning, gliding, rolling or distractive motions that occur between joint surfaces and are essential for normal mobility. As for an example of accessory motion at Glenohumeral joint would be motion of head of humerus inferiorly as it moves on the glenoid fossa during normal abduction. The gliding motion is necessary for greater tuberosity of humerus to pass under the coracoacromial arch and thereby allow full elevation of the arm. Accessory

motions are like in normal synovial joints when one articular surface is stabilized and the other articular surface is moved passively by the examiner. Several textbooks on the examinations and techniques of joint mobilization are available by Cyriax[9], Kaltenborn[10], Maitland[2] and Mennell[11]. For the purpose of this study, mobilization will refer to techniques of joint mobilization by Maitland[2].

According to Maitland the Grades of Mobilization[22]-
Grade I – small amplitude movement at the beginning of the available range of movement.
Grade II – large amplitude movement at within the available range of movement.
Grade III – large amplitude movement that moves into stiffness or muscle spasm.
Grade IV – small amplitude movement stretching into stiffness or muscle spasm.
The purpose of this investigation was to determine the effects of joint mobilization along with Combination therapy (UST + IFT) in combination with an active exercise program, on pain and hypomobility associated with frozen shoulder. The results were compared with the patients having Diabetic history over the Non-diabetic cases.
The importance of careful examination and selection of a specific course of treatment for each patient obviates the use of an identical method of treatment for all patients. Thus, the intent of this study was to assess the therapeutic value of joint mobilization along with combination therapy as an adjunct to other aspects of a treatment program between Diabetic and Non-diabetic cases.

Study Design: - Comparative Study
Case: - Two groups of 20 individual inclusion criteria.
Group **A** having 10 patients with history of Diabetes.
Group **B** having 10 patients with no history of diabetes.
Treatment differed between the groups. Group Areceived 12 sessions over 2 week of SWD and passive mobilization and an active exercise program along with Combination therapy (UST + IFT) whereas Group B received 12 sessions over 2 week of SWD and passive mobilization with Combination therapy (UST + IFT) along with an active exercise program.

Age Group: - Both male and female above 40 (40-60) years with diagnosed frozen shoulder.

Exclusion criteria
- Any form of malignancy.
- Known history of fractures of shoulder, clavicle, and humerus.
- Diagnosed cases of Shoulder infection.
- History of dislocation of shoulder joint, Subluxation.

Tools and Parameters used
- NPRS[15]
- Goniometer[1]
- Reflex hammer
- Pain Questionnaire[15]
- Special Tests[15]
- UST [18,19,20,21]
- SWD[18,19,20,21]
- IFT[18,19,20,21]

METHODS

This comparative study was conducted in the department of Physiotherapy, Viklang Bhawan Hospital, Bihar college of Physiotherapy and Occupational therapy, Patna, Bihar for 12 sessions over 2 week and the subjects were

considered for this study as soon as they were referred from a physician. The presence of shoulder pain and limited passive motion at Glenohumeral joint were criteria for eligibility with following exclusions-

- Unstable fracture of Humerus, scapula and clavicle.
- Recurrent shoulder dislocation and subluxation.
- Rheumatic disease
- Advanced Osteoporosis.
- Malignancy
- History of extensive steroid therapy.
- History of any trauma to the shoulder joint.
- Severe pain unrelieved by resting the joint.
- Peripheral neurological involvement of upper extremity.

If the eligibility criteria were met and informed consent was obtained, the subjects were assigned to the predetermined two groups on the basis of diabetes. During the initial session after group assignment, each patient completed a pain questionnaire to assess the degree and nature of the symptoms. Also at the initial session, a history, subjective examination and objective examination related to the involved shoulder were performed. The cervical spine, acromioclavicular, sternoclavicular, scapulothoracic and elbow joints were also examined to determine if these areas were the source of the patient's signs and symptoms. Joint ROM was measured using the traditional goniometer method as described by Cynthia C. Norkin and D. Joyce White[1]. Assessment of pain and mobility was done initially before treatment started and then at weekly intervals for a period of 2 weeks and then final measurements were taken following at 2 weeks treatment period. Subjects in both groups reported for treatment 6 times per week during the 2 week period. In addition to the weekly measurements of pain and mobility, passive motion was examined prior to each treatment to assess the degree of stiffness and irritability. Special tests were performed to assess the cause of underlying disease. Tightness, contracture and deformities were checked for proper joint and muscle function. Before the first treatment and after the last treatment, each subject marked NPRS[15] for pain assessment.

Interventions: The targeted capsule was preheated by the use of SWD[18, 19, 20, 21]and by use of thermal Ultrasound effects. The intention was to alter the Viscoelastic properties of the connective tissue and maximize the effectiveness of the stretch mobilizations to follow. The UST[18, 19, 20, 21]was applied at 1.5 W/cm^2 continuously for 10 minutes along with IFT[18, 19, 20, 21]at the same time as Combination therapy for 12 sessions over 2 week. Following the electrotherapy sessions the two groups were assigned to exercise therapy sessions which included shoulder AROM exercises, Codman's pendular exercise, stretching exercises, shoulder wheel and pulley exercise, shoulder ladder exercise, shoulder isometric exercises, beads counting, overhead sanding activities and T-bar exercises. Following this exercise sessions progressive manual mobilization was done on shoulder joints as described by the Maitland. The subjects were advised for active home exercise program which was followed by moist heat or hot fomentation. They were also advised for postural correction techniques and preventive measures were also explained thoroughly.

RESULTS

We did a comparison between diabetic and non-diabetic. After the treatment sessions among both the groups, Group B showed better outcomes in relation to pain, ROM and return to ADL and QOL. The outcomes were assessed after the final treatment sessions.

DISCUSSION

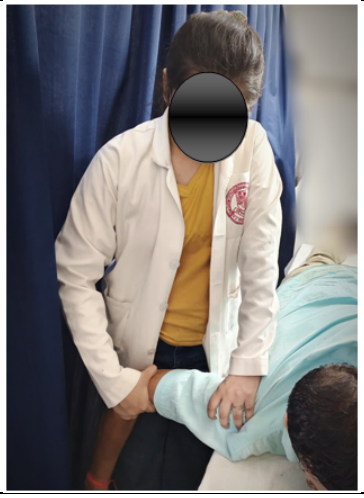
The same treatment protocol was compared for both the diabetic and non-diabetic groups – GROUP A and GROUP B and it was observed that better outcomes in terms of recovery for group A came significantly lower as compared to the group B.

CONCLUSION

The result of this comparative study indicates that SWD[18,19,20,21], combination therapy (UST + IFT) [18,19,20,21] along with Maitland joint mobilization and Exercise program were more effective in treating Frozen Shoulder without Diabetic history over the Diabetic cases.

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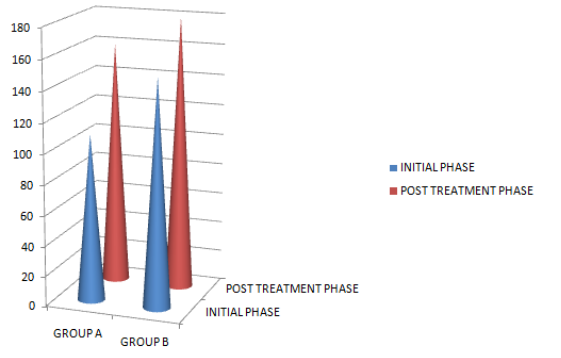


Fig.:5- Flexion Rom Comparison Pre And Post Treatment Phase

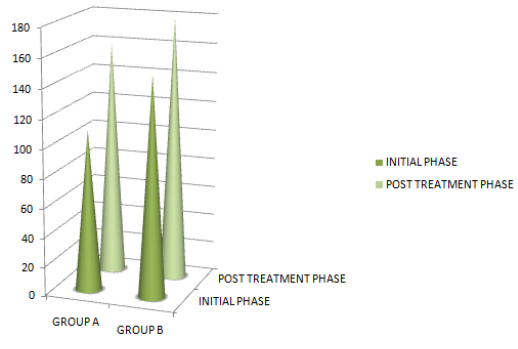


Fig.6:- Abduction Rom Comparison Pre And Post Treatment Phase

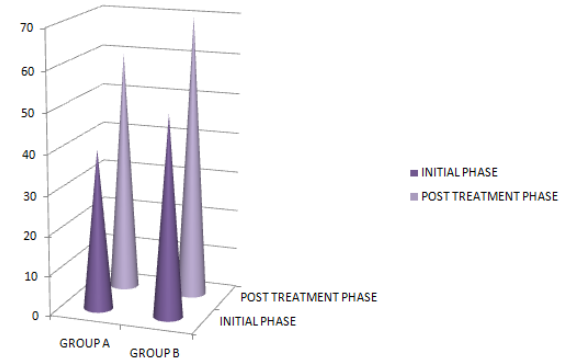


Fig.7:- Internal Rotation ROM Comparison Pre And Post Treatment Phase

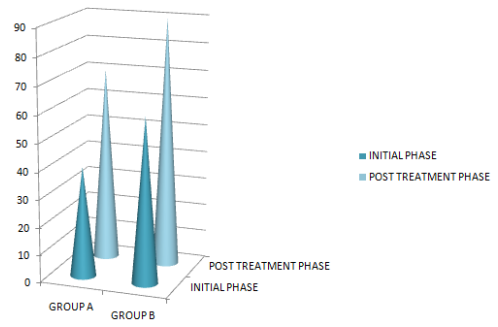


Fig.8.:- External Rotation ROM Comparison Pre And Post Treatment Phase

REVIEW ARTICLE

Digital Agriculture Revolution: A Comprehensive Review of e-Commerce, Mobile and Internet

Shreya Palani[1], Ashok Kumar[2]*, Shri Prakash Dwivedi[2] and Vicky Kumar[1]

[1]M.Tech Scholar, Department of IT, Govind Ballabh Pant University of Agriculture and Technology, Uttarakhand, India.
[2]Assistant Professor, Department of IT, Govind Ballabh Pant University of Agriculture and Technology, Uttarakhand, India.

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***Address for Correspondence**
Ashok Kumar
Assistant Professor,
Department of IT,
Govind Ballabh Pant University of Agriculture and Technology,
Uttarakhand, India.
E.Mail: ashu.gbpec@gmail.com

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ABSTRACT

This review paper gives detailed and comprehensive overview of researches that how digital technology is restructuring agriculture. The work done is emphasized by peer review and developments including e-commerce, mobile applications, marketing in social media, digital productivity solutions and internet connection. It highlights the capacity of technological advancements, through which they can revolutionizes the agriculture sector by acknowledging challenges that are there since a long time such as low productivity, inefficiency, limited market access facility, especially for small scale farmers. The paper also draws attentions towards the integration of digital agriculture with United Nations Sustainable Development Goals (SDGs) which include zero hunger (SDG 2), innovation in industry (SDG 9), and eradication of poverty (SDG 1). It shows how social media marketing has changed the market access and internet connectivity allows farmers to get engaged with consumers which also helps in building awareness. Despite of having these advancements, there are barriers that still exist. This paper also talks about the successful adoption on localized partnership, user centric designs, robust policy support and public private partnership. This also conveys the critical policy initiatives and gives emphasis on the need of interoperability of data privacy and ensuring inclusivity.

Keywords: Digital agriculture, e-commerce platform, mobile applications, sustainable development, small scale farmers and policy frameworks.

INTRODUCTION

Traditionally, farmers in so called Third World are referred as a subject of inefficiency, bad productivity, and restricted access to real time information. With the widely evolving digitization of global economy and the wide spread of the deployment of information and technologies of communication that is ICT, there has also been wide expansion of advanced tools which are performing revolution in agriculture. The change is seen in small scale farmer systems through which producers till now are able to access the unexpected markets, information and services due to the digital services, websites and mobile usage like mobile apps. Agricultural digital solutions share a very intimate connection with the United Nations' Sustainable Development Goals (SDGs), perhaps more so for food security (SDG 2), innovation (SDG 9), and poverty eradication (SDG 1) [1]. In addition to the revolution in farming methodologies, these technologies are helping rural development through enhanced transparency, new business ventures, and climate adversity resilience. There is a small window to augment solutions for farmer empowerment and food security with the coming of 4G and now 5G internet connectivity, AI-based decision tools, satellite imagery, and data platforms [10]. Challenges such as lack of funding, bad infrastructure, and digital literacy continue despite these advances. Thorough studies that evaluate what works, why, and where more efforts are necessary are the imperative with governments, non-governmental organizations, and private sector partners collaborating to transform digital agriculture. This article provides an in-depth explanation of existing work in five high-priority themes: e-commerce platforms, mobile apps, social media advertising, digital productivity tools, and rural internet penetration.

REVIEW OF KEY LITERATURE

Integration of E-commerce in Agriculture

Findings: It was found that farmers using E-Commerce platforms reported higher profit margins because they were less dependent on brokers or third party or middleman.

Challenges: Digital divide poor transportation in rural areas and trust in online transactions were seen as major problem. In developing nations, e-commerce has emerged as a new trend where the agriculture market is inefficient and segmented. The area of attention is direct link between producer and consumer without any third-party interruption who compressed the profit margin of farmers. Now, farmers can set the price access market and expose product by using online platform and online transaction system. The minimum necessity to incorporate E-Commerce systems in agriculture, according to studies by [3] and [4] are electronic market places, distribution coordination, transaction securit, transparency in prices and consumer education.

Digital marketplaces: The e-agriculture markets or online agriculture markets are platform, where client, producers, wholesaler and consumers can shop and purchase the goods directly from the farmer that have been put on offer. These include sites such as DeHaat, AgriBazaar, and Mandi Trades [6] and [4]. This allows different products to be put on the system, support a bilingual feature and have information that is seasonally offered, such as the production of demand as well as prices trends. For [4], platform saved time to market by 40% and enable farmers to sell above Mandi prices up to 20 to 30%.

Logistics and Distribution: The success to e-commerce is the efficient logistics in agriculture. In comparison to unsustainable goods in typical e-commerce, agricultural inputs are sustainable and require immediate controlled temperature in transportation. E-commerce websites include logistics modules which manage delivery, cold storage, and pickup with providing real time monitoring. Research represents that end-to-end integrated e-logistics systems have the ability to reduce post-harvest losses up to 25%.

Security and financial transaction: Safe and track able transactions that is transparent transactions are to be developed among users for trust. UPI (Unified payment interface), digital wallets and mobile banking are integrated into various E-Commerce platform in their payment processes [1].The use of cash is reduced using the services which

provide better financial inclusion and speed the payment reconciliation. The farmers using these integrated facilities in rural areas shows are 63% increase in transactions that are made digitally and a corresponding decline in fraud complaints [3].

Price discovery and transparency: Presence of symmetry in information where farmers are not aware of real time prices in distant markets are factors which are suffered in traditional agriculture. E-Commerce platform solve this by providing daily updates on prices which enables farmers to make decisions dependent on data. The dynamic algorithm helps to match the regional supply demand which stabilizes the prices overtime.

Case Studies and its impact

[3]Analyses e-commerce platform in Bihar, India where small scale farmer selling vegetables sold their produced vegetables to various consumers in urban areas by using app directly. Income of average farmer rose by 22% in 6 months with the customer retention of 75% through direct sourcing and assured quality. And [5] concluded that Telangana's e-commerce platform which were based co- operatively on network, enhanced market credit availability and decreased the reliance on informal lenders.

Challenges: Despite of benefits, there are challenges which are still to overcome. In hilly and tribal location, connectivity limits the real time action. Many farmers have not yet embraced digital media fully because of the trusted issues generated. Technical malfunction, delay in payments and fake goods may undermine the trust of farmer sometimes. Furthermore, more small producers will be forced to be out by the prevalence of large agriculture companies, if the strong anti-monopoly regulations are not in the place.

Mobile Applications for small scale farmers

Significance: Marginal farmers get benefit by using mobile apps by promoting participation in ecosystems that is created digitally and allow farmers to make data driven decisions.

Limitations: Digital divide faced by farmers which restricts them from utilizing facilities fully and unequal distribution of access to smartphones. Using mobile application enhances the agriculture practices of small-scale farmers, decision making ability and access to market that the gap of information is filled by mobile technology, also enables real-time communication and provides simple tools that are easy to understand and adapt well for the use of marginal farmers. The features of mobile applications are weather forecast, recommendation for the crop selection, pest and disease management, inspection of soil and market price monitoring. The app discussed in the paper [6] was provided to different farmers in different regions and the results were always appreciated. It increases the confidence of users in their farms, reduced the input cost and increases the yields of the crops. Voice enabling navigation technology was appreciated by farmers which made the more efficient use of app. To overcome the issues many trainings, demonstrations and workshop were conducted. The study notified the importance of current user support and regular updated of the app to address the seasonal and feedback needs. It concluded with the deployment of mobile applications to small scale farmers providing the immense potential to transform their livelihood by ensuring user friendly and multi lingual support with localized content. With the growth of infrastructure and corresponding legal frameworks, mobile applications might soon become a foundation of digital farming practices.

Social Media Marketing in Agricultural Commerce

Insight: By establishing connection between rural producers and urban area consumers, social media helps farm based enterprises in increasing awareness of their brand.

Key Limitation: There is a considerable risk of inaccurate information and digital fraud, and platform algorithms don't necessarily favour small-scale sellers. [8]Paper talks about the farmers, cooperatives, and Agri-startups employ social media in offering their produce, communicate directly with customers and exam information that are related to farm. One of the central findings of the study is democratization of market communication. Now the farmers were

able to use images in videos for the promotion of their product, direct price negotiation with retailers or consumers and supply data about how they produce it.The establishment of direct communication channel between consumer and producer not only boosts the income of farmers but also reduced their dependency on the middle men who tends to acquire significant portion of their profit margin. In addition to this, social media also provided the opportunity for learning and providing feedbacks from the consumer to the producer. Inspired these advantages the same challenge of digital divide, digital literacy and lack of knowledge to use the resource app up to the mark remains a major hurdle. To mitigate these issues, the recommended solution is to provide training program on social media and other platforms. Based on the research finding to become an active participant in the market, social media can empower farmers especially women and youth when integrated with more holistic digital agriculture programs. This facilitates the innovation across Agri-value change increase transparency in prices and grows the establishment of hyper local food system.

Advanced digital technology for Agriculture Productivity

Strengths: There is strong proof that ICR increases crop yields, save water wastage and alleviate climate change anxiety.

Gaps: Lack of rural infrastructure restricts wide adoption and large initial outlay of the infrastructure. The paper [10]provides description of how various digital tools a transforming the farming landscape. It analyses various he digital interventions like remote sensing, precision agriculture, Geographic Information Systems(GIS), Global Positioning Systems(GPS) and artificial intelligence-based analytics [10]. Using these technologies, farmers can make the optimum use of land, manage resources effectively and monitor the well-being of crops in real time. The concept of site-specific crop management where farmers used digital mapping and data analysis to treat different areas of field differently based on topography, soil condition and past production is emphasized. Precision based method with minimum use of pesticides shows the increase in yield significantly which makes it more sustainable. Another important aspect is the decision support system of the mobile systems. These integrate the real time weather forecasts, market intelligence, pest alerts and advices given by the services. The challenges are faced in the scaling of these technologies, affordability majorly for small scale farmers. The lack of training and technical support often acts as a setback for effective usage of the resources along with the interoperability issues between tools and protocols. The recommended solution by the author is to use private partnerships to subsidize the technology to safe access and build capacity programs. They also talk about the open data ecosystems and integrate different platforms.

Internet Embedding and Rural Entrepreneurship

Methodology: Comprehensive survey data and economic modeling combined with empirical research.

Conclusion: internet embedding boosts entrepreneurial intent and reduces rural-urban movement if there is institutional support for digital upskilling. This section emphasizes on the ways in which wireless network enhances the innovation, entrepreneurship and productivity in agriculture which are evident from the study [5]. Agriculture is influenced by internet in different ways such as bringing production up to date, promoting financial inclusivity and bringing market together by sharing information. The influence of internet in agriculture works using multiple pathways which are modernisation of production, farmers adopting precision agriculture practices, accessing guidance to online services and automating task with the help of internet connectivity. For bypassing traditional supply chains, market integration and e-commerce platforms are used, which sell produce directly to consumers. Income of farmers is enhanced by the growth of online farming practices and marketplace. Knowledge dissemination and learning, depicts that internet facilities extends agriculture practices using digital content. The idea of group learning promotes the adoption of breaks practices in farming and expands the resilience to various vulnerabilities such as climate change and pest outbreaks. The study used Henan Province as a case study [1], where E-Commerce in transportation services have brought 10% rising ruler employment and 15% in agriculture output after using internet connectivity. Digital divide remains a persistent challenge which was also there in low-income regions. Limitation on digital literacy, lack of infrastructure and disparities in accessing market based on gender restrict equitable and sustainable benefits. Also, the new risk in digital environment of agriculture are cyber security and

misinformation. To handle these challenges, the report suggests policies are included in support of development of broadband connectivity, incorporating ICT studies into agriculture and offering incentive to rural IT entrepreneurs. Using inter-sector strategy which involves government investment, private sector innovation and community participant is seen as a necessity to implement sustainable changes into ruler areas. In short, internet embedding is most significant for rural enterprises in quality agriculture developments. As the ruler communities are becoming more digitally connected, they are creating new growth opportunities that are sustainable, innovation driven, resilient and inclusive

Embedding internet and entrepreneurship in rural areas
Methodology: Detailed survey of data and economic modelling combined with empirical research.

Conclusion: internet embedding boosts entrepreneurial intent and reduces rural-urban movement if there is institutional support for digital upskilling. This part talks about the ways in which wireless networking enhances the innovation, entrepreneurship and productivity in agriculture which is evident from the study [5]. The agriculture is influenced by internet in various ways such as up-to-date production practices, promotes financial inclusiveness, brings market all together, and share information. The internet influence use multiple pathways such as modernization of production, in which farmers about precision agriculture techniques, access guidance through online services and automate tasks with the help of connectivity. The integration of market and e-commerce allow farmers to bypass traditional supply chain mechanism and sell produce directly to consumers without interaction of third party or middlemen.

Thematic Synthesis
Digital literacy and access: It is observed that the easier to access the digital agriculture the more effective it is. Though social media and mobile apps have improved the connectivity for accessing information, but they are only effective when the individual using them are literate digitally. Hence it is very important to have training programs that close the digital divide and more inclusive practices.

User centric design and localization: User input and localized data showed greater influence by working on mobile apps and decision support systems. The case study of Andhra Pradesh, mobile app experiment was showed to produce greater farmer usage and easier user interface with participatory design [6]. Another great practice is to design farm tools with best user interaction and end user support.

Market empowerment and integration: E-commerce also known as selling market online, and marketing through internal using social media, are the sections which has scope of expanding individual access to markets and limits power of middlemen. Bank and internet facility plays a important role in enhanced entrepreneurships among youth and women in Agri group.

Policy facilitator and infrastructure: The two constraints in all the studies which remain persistent were physical that is transport and logistics and internet access, affordability of phones. Key feature of policy facilitator includes digital subsidy programs, rural broadband initiatives, and public private initiatives.

Data governance and interoperability: Even if some solutions work well, integrating systems is a difficult task. Interoperability between mobile apps, e-commerce websites, decision support systems is still unexplored. In short, although each research yields valuable data on one facet of digital agriculture, combined, they suggest that there has to be a systems-thinking mind. It is not technology in isolation that will drive digital agriculture; people, technology, and policy need to conspire to make it happen with maximum impact in total. The next horizon is one of convergence, of the accumulation of platforms, applications, infrastructure, and governance into useful architectures which are specific to the requirements of rural farm communities.

Policy and Practical Implications

The digital revolution in agriculture has the huge potential to foster rapid rural sustainable development, enhance food security, and catalyse economic development of agricultural communities. But the gains have to be achieved through enabling policies and pragmatically attuned interventions sensitive to the unique challenges facing smallholder farmers. The research papers presented for consideration in this paper agree on some general areas where policy attention and field-level action are necessary.

Closing the Digital Divide: While the increased usage of mobile phone and various technologies by rural communities, women, old farmers, and poor people are far behind in accessing the digital tools and techniques. They are restricted to this world of advancements. In order to end this digital divide, government must design simple and use local languages for apps, implement study focused on digital literacy, and provide different subsidies for buying smart phones and to connect to network. Voice operated systems and navigation based on icons have been used to enhance usability among the people with low literacy. Another step is to integrate digital skills into agricultural extension for digital inclusion.

Enhanced Infrastructure and Connectivity: Electrical and internet connectivity dependency is crucial for the effective functioning of digital agricultural tools. Government must increase the broadband usage in rural areas initiative such India's BharatNet project and invest in alternative connectivity measures such as satellite internet or community hotspots. Public-private partnerships can improve the final stage of digital infrastructure, especially in areas with challenges of topologies.

Innovation Support Ecosystems: Agri tech innovation can be triggered using investment and facilitative regularities. Startups creating mobile application, online marketplaces, AI- driven software needs grant for research and development, incubation space, and expedited tracking of regulatory process. Creating Agritech innovation groups at the state or district level will guide in localizing solutions and also facilitate farmer, researcher, technological interaction.

Data Interoperability and Privacy: As more reliance on digital technology in agriculture, there needs to be openness in data collection and application. Farmer's rights need to be safeguard over data and informed consent by policy regulations. Data formats and APIs need to be standardized so that data can be made interoperable across many platforms so that there can be effective communication between different digital advisory tools, mobile apps, and e-commerce channels.

Farmer Group and Cooperative Organization: The effective way to introduce digital tools is by collective structures. Cooperatives, SHGs, FPOs can guide individuals in adopting digital technologies by providing digital kiosks, services of agritech and group accessibility. The goal of central policy must be to enhance the institution's ability to act as a digital aggregators and mediators.

Inclusive and Context-Sensitive Policy Making: Policy interventions have to be context-based; if local institutional capacity, sociocultural context, or local agroclimatic conditions are not considered, digitalization initiatives do not succeed. Participatory policy making with farmer feedback loops can enhance uptake and appropriateness, and institutionalization of the impact assessment of digital interventions has to inform adaptive policymaking.

Integration with Farm Finance: With data on farm size, yield, and transaction history, digital platforms can open access to finance, insurance, and subsidies. Governments need to collaborate with financial institutions to enable fintech innovation in agriculture, for example, crop insurance apps operating on real-time data, digital wallets for subsidy payments, and credit rating platforms for smallholder farmers.

Monitoring and evaluation of Digital Interventions: Strong M and E systems are needed for digital agriculture programs. Along with the downloads of app, important parameters to monitor tangible impacts like yield growth,

reduction of costs and improved access to market. Replicability evidence for successful practices can be generated through the use of independent evaluation and academic collaborations. At last, it can be concluded that the performance of the digital technologies will be depending on the environment provided by the legislation and the organizations, a environment which is inclusive, strong and farmer centric and which must be cooperated by the government, private sector and the civil society. By balancing the technical innovation, it can enhance the wellbeing and dignity or human beings.

Way Forward

Following the examination of digital interventions in agriculture, additional development must be guided by inclusive policies that keep digital change in a range of rural settings in combination with technical solutions. Five interrelated trajectories are highlighted by the synthesis study as underpinning an innovative agenda for digital agriculture.

Development of Digital Hubs across Multiple Platforms: Following that, development must be aimed at comprehensive digital platforms that combine various features like weather forecasts, pest warning, digital payments, input value chains, and access to markets as opposed to one single mobile application or individual e-commerce sites. Adoption is inhibited by fragmented platforms [4] and [3] argue, due to the fact that they are redundant and complex. For technophobe farmers, the creation of ecosystem-based systems that integrate services into one interface would be simpler to use and build trust.

Increased Customization and Localization: Effective digital technologies are founded upon local language, customs, and crop seasons, the researched works have proven to assess. In order to provide advisories in the context of local seasonal calendars and customary knowledge systems, future content must transcend simple translation and embrace cultural adaptation. AI and machine learning algorithms trained on local data can develop scalable context-specific solutions.

Gender and Youth Inclusion: Women and youth are identified as future drivers of digital change in different rural entrepreneurship studies and mobile app usability. Socioeconomic factors, however, discourage them from participating. Future agritech platforms must institutionalize design elements that are gender- and youth-friendly, including training sessions, anonymous entry options, and flexible dashboards.

Institutional collab and cross-sector integration: The future of digital agriculture depends on collaborative governance. Government agencies, NGOs, private firms, institutions must co-implement digital projects. Like, agricultural universities can function as field testing sites for new tools and telecommunication firms can provide subsidized data packs for app usage.

Scaling and Feedback loops derived from evidence: Future digital projects need to be evidence-based for scaling, and not scaling for the sake of scaling. Pilot testing, iterative design based on feedback, and effect surveys are all part of this approach. Long-term sustainability and credibility are enhanced by iterative development through feedback loops. It ought to be business as usual to track farmer happiness and performance in real time through digital dashboards. The future is not isolated form of innovation but building an ecosystem that is user centric, data driven, sustainable and equity based. The recommendation is to integrate technological advancements with inclusivity of the institutions to ensure digital agriculture becomes transformative for each one of us.

CONCLUSION

The future of digital agriculture not also involves innovation but also creating the robust ecosystems that are based on equity, data driven and user friendly. The path is to consolidate technological innovation with the inclusivity of institutions to help and ensure digital agriculture benefits to all the stakeholders. It a transformation that employee

small farmers, promotes environmental well-being and guarantees food security to various sections of society which are rapidly evolving in society. Agri-digital is not only a temporary trend but also a powerful force which reshape farm practices, market access and rural socio-economic settings. Internet platforms have been demonstrated to cut down on transactional inefficiencies and enhance access to markets, especially for smallholder farmers, and mobile applications are increasingly valuable tools for providing real-time weather information and agronomic recommendations. The comparative study shows that while technology advancements is necessary, effectiveness is highly dependent on social, cultural and institutional context. In digital space, providing legislative suggestions towards improving accessibility, diversity, and sustainability must be tailored to meet local circumstances. The priority in future is to build a better digital ecosystem that fulfills the needs of various stakeholders, that is women, youth and small-scale farmers. The new approach should be collective which should be backed up by infrastructure development and capabilities of institutions should be optimum. Digital agriculture offers a evolving opportunity to handle different challenges which can be handled using traditional methods, provided to pursued with sensitivity to equity, local relevance and sustainability. This review desires various sectors of society to create empowered agriculture using digital tools and technologies which will ensure dignity, prosperity to all the people involved in agriculture. Also, the information and knowledge available globally with respect to agriculture will enhance the innovation, particularly in global South, as it has to respond with then ongoing population growth, volatility in market and change in climate. Other parameters that have to be included are balanced measurements of user experience, livelihood impacts, inclusivity and environmental impacts in the metrics of success such as yield increase. The main thing to address is the moral dimension of the agriculture. With huge collection of digital data, issues of ownership of data, permission, bias of algorithm and tracking are centers for gain. In the long run, trust and adopting will be about creating farmer driven data governance systems that value accountability and transparency. The potential of digital agriculture is to build a robust environment, secure and equitable food systems that are sensitive to human limits. Hence, the future of digital agriculture is demanding as well as rewarding.

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Comparative Analysis

Table 1: Comparison based on several papers

Aspect	Source	Key points	Technology	Region	Features/Challenges
Internet Entrenching and Entrepreneurship	[9]	It tells how access to internet in rural areas promote e-commerce, entrepreneurship; The choice of entrepreneurial channel is influenced by social capital.	E-commerce platforms, ICT infrastructure	China	It encourages independent startups; Social capital influences success; There is a need for digital skill development
Mobile App usage	[6]	It investigates mobile app use in small agribusinesses for keeping records and smooth flow of information.	Mobile Apps like WhatsApp, KisanSuvidha	India	It enhances communication and reduces information asymmetry; Adopted is halted by tech technology literacy gaps.
Role of E-commerce in Agriculture	[5]	It explains how e-commerce empowers farmers through better pricing and market access.	General e-commerce	India	It reduces exploitation by middlemen and lack of awareness.
Supply Chain Optimization through E-commerce	[2]	It tells how e-commerce restructures supply chains with consolidated logistics, finance and information system.	E-commerce Platform	China	It creases the efficiency of supply chain, reduce wastage and improve trace ability.
Technology trends in Agriculture E-commerce	[1]	It presents the study of research trends; identifies key technologies and provides a conceptual framework.	Blockchain, IoT, AI, systems for traceability.	Global	It focuses on interoperability, traceability, data standardization; Hurdle include costs and fragmentation.
General Review on Agri E-commerce	[4]	It reviews on opportunities and constraints in Indian Agri e-commerce; Also emphasizes policy and awareness.	E-commerce	India	There is a need for better digital policies and infrastructure for scale-up.
Digital Tools for productivity	[10]	It reviews digital agricultural tools for improving yields and reducing costs.	Digital agriculture tools, sensors, automation.	Southeast Asia	Productivity improved using data driven decision making techniques: complexity and costre main the challenge.
Social Media Marketing and	[8]	It investigates adoption of social media	Social media	China	High value perishable good are fit for

product suitability		marketing			marketing: Grain crops are less suitable for digital marketing.
M-commerce App for farmers	[7]	It develops and tests e-fresh apps and evaluated the usability and potential to improve income.	M-commerce (E-fresh mobile apps)	Malysia	It improvedease of selling, good feedback from uses; itis effective for young entrepreneurs.
Mobile Advisory apps and impact of yield	[3]	It compares knowledge gain and yield improvement among users of mobile apps.	Kisan Suvidha App, e-Nam	India	Kisan Suvidha users had highest yield and adoption; local language barrier.

REVIEW ARTICLE

Medicinal Marvels: Mangroves of Coringa - A Review

Rasheed Shaik[1]*, Balakrishna Talamanchi[1], Kundana Terli[2], Lavanya Devabattula[3]

[1]Assistant Professor, Department of Pharmaceutics, V. V. Institute of Pharmaceutical Sciences, (Affiliated to Jawaharlal Nehru Technological University, Kakinada) Andhra Pradesh, India.
[2]Professor, Department of Pharmaceutics, V. V. Institute of Pharmaceutical Sciences, (Affiliated to Jawaharlal Nehru Technological University, Kakinada) Andhra Pradesh, India.
[3]Assistant Professor, Pharmaceutical Analysis, V. V. Institute of Pharmaceutical Sciences, (Affiliated to Jawaharlal Nehru Technological University, Kakinada) Andhra Pradesh, India.

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***Address for Correspondence**
Rasheed Shaik
Assistant Professor,
Department of Pharmaceutics,
V. V. Institute of Pharmaceutical Sciences,
(Affiliated to Jawaharlal Nehru Technological University, Kakinada)
Andhra Pradesh, India.
E.Mail: Rasheedsk1798@gmail.com

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ABSTRACT

Mangroves are not only saline-tolerant but they also have a high medicinal value. Mangroves have a wide spectrum of medicinal uses, and they have been used in local medicine for centuries. Mangroves are generally found in coastal areas and they host wide variety of flora and fauna apart from their role in medicine and natural conservation these mangroves protect the coastal areas by stabilizing the shore and preventing erosion by trapping the sediment by their roots Coringa estuary located in Andhra Pradesh, India is one such place where 24 species of mangroves are found this biodiversity hotspot holds remarkable interest in researchers.

Keywords: Mangroves, Coringa, Anti-Inflammatory, Antioxidant.

INTRODUCTION

Located in Andhra Pradesh, India, the Coringa estuary is a large mangrove forest of high ecological and medicinal importance. Stretching over 235.7 km2 it is the third-largest mangrove ecosystem in India it exists between latitudes 6°30' to 17°00' N and longitudes 82°14' to 82°23' E. It was established in the year 1978 and is under the management of Andhra Pradesh Forest Department. This unique habitat supports 24 species of mangroves and 120 bird species. Bird species also include the critically endangered vulture species such as a white-backed vulture and the long-billed vulture. Though the Management and protection of Coringa estuary entails various challenges, its importance, with

regards to biodiversity as well as being a source for new medicines, gives rise to urgent need for conservation along with sustainable management[1].

Mangroves: Mangroves are amazing salttolerating trees and shrubs thriving along the intertidal coasts of Tropical and Subtropical regions. Adapted to survive in salty, poorly oxygenated soil, they exhibit very complex root systems for stability and respiration above the ground. Mangroves serve as important nurseries for marine life, protect coastlines from erosion, and absorb carbon Beyond its ecological role in shoreline protection and maintaining ecosystem balance, mangroves have been treasured for their medicinal properties for over a thousand years[2]. These mangroves also play very crucial role in local Eco-tourism.

Mangroves of Coringa: The Coringa region boasts several species, including *Avicennia officinalis* and *Rhizophora apiculata,* traditionally used by locals to treat ailments ranging from common fevers to skin diseases. These plants contain a treasure trove of compounds like alkaloids and terpenoids, which have shown promise in modern pharmacological research Several Mangrove Trees and shrubs found in Coringa creek are Mentioned below

Table1: List of Some true Mangroves found in the Coringa Region[3]

The current review focuses on the Mangroves that are found in the Coringa Region and their medicinal uses.

Avicennia officinalis: belonging to Acanthaceae family is an important mangrove, known commonly as Indian mangrove it attains a maximum length of 8-18 meters, and a diameter of 1 meter the leaves are oval-shaped of about 10 centimetres in length and 5 centimetres width. The leaf's upper side is a shiny green, with the underside possessing a golden-brown color These leaves are oppositely pair-growing.

Its largest flowers in Avicennia species are showy with orange-yellow to lemon-yellow petals that reach a diameter of 6 to 10 millimetres when fully expanded[4]. The bark of the tree is smooth, from dirty green to dark Gray in color, and does not flake. It is characterized by its fruit, which is heart-shaped and tapered to a point, with at least 2.5 centimetres coloured in length, and green or brown widely distributed across the Indian subcontinent and Southeast Asia. The tree-like plant is a bank of bioactive compounds such as alkaloids, glycosides, Terpenoids flavonoids, steroids, tannins, saponins, phenols, etc, with proven analgesic, antioxidant, and antimicrobial activity, diuretic and can also be utilized in the treatment of rheumatism, paralysis, asthma[5,6].

Avicennia marina: The Avicennia marina of Acanthaceae family is a significant mangrove species that found across the Indo-Pacific area[7,8]. This robust plant, spread from Australia to Africa, including Southeast Asia and the Middle East, is extremely salt tolerant, withstanding seawater concentrations double those of normal levels. Its propensity to thrive in adverse situations, such as high tides, temperatures, strong winds, and saturated soils, makes it an exceptional survivor in coastal environments. This plant is considered a natural reservoir of phytopharmaceuticals like aliphatic alcohols and acids, amino acids, alkaloids, carbohydrates, carotenoids, hydrocarbons, free fatty acids, lipids, pheromone, phorbol esters, phenolics and related compounds, steroids, triterpenes and their glycosides, tannins, and other terpenes and has numerous therapeutical benefits for humans like Anti-ulcer, Astringent, Anti-hemorrhagic, Anti-malaria, Anti-diarrheic, anti-tumor activities[9,10].

Avicennia alba: It is native to Southeast Asia , and has been valued for its medicinal value for centuries. Packed with phytochemicals such as steroids, saponins, and flavonoids, it has been found effective in relieving pain, fever, and inflammation. Although the initial studies are encouraging, additional studies, such as clinical trials, must be conducted to determine its overall therapeutic value. Investigating its traditional uses and sustainably cultivating *Avicennia alba* can help to conserve it and create new, culturally relevant healthcare practices. By further investigating its phytochemistry, pharmacology and its safety profile, we can unlock the full potential of this important medicinal resource[11,12].

Lumnitzera racemosa: Combretaceae family mangrove species, thrives in tropical and subtropical coastal areas[13]. This species possesses great scientific value because of its potential medicinal uses. Rich phytochemical content in the form of flavonoids, tannins, saponins, and terpenoids in *Lumnitzera racemosa* showed promising antibacterial,

antifungal, and anti-malarial activity in initial investigations. These bioactive substances hold promise for combating the world problems of infectious diseases and antibiotic resistance. Of particular interest is the plant's potential to develop compounds that kill malaria, an ongoing threat to health in most tropical countries. But to best determine the drug potential of *Lumnitzera racemosa*, extensive studies such as clinical trials and toxicity testing are necessary[14,15].

Excoecaria agallocha: *Excoecaria agallocha*, the blind-your-eye mangrove or a shrub-like mangrove with a very distinctive appearance, belongs to the family Euphorbiaceae. Found commonly throughout the Indo-Pacific, *Excoecaria agallocha* has drawn scientists' interest with its possible medicinal uses[16].Its name notwithstanding, which refers to its irritating latex, the species has been of interest in early studies. A variety of phytochemicals like diterpenoids, triterpenoids, and flavonoids. These compounds have shown diverse biological activities within the laboratory. But caution need be taken in the medicinal utilization of this mangrove species. The toxic latex of the plant requires proper handling and processing to avoid any mishappenings. In addition to promising as preliminary results are, there is a need for thorough investigation to thoroughly comprehend the chemical composition of *Excoecaria agallocha* need to be evaluated its likely benefits and hazards. Clinical trials and long-term experiments are necessary before any therapeutic assertions to be made[17,18].

Rhizophora apiculata Bl: *Rhizophora apiculata* Bl is a species from Rhizophoraceae family that grows in mangrove forests of the Indo-pacific Region this plant is also called as red mangrove these plants are distributed along the coasts of Asia, Australia, and the Pacific Islands. Closely related to their Atlantic and Eastern Pacific counterparts, these mangroves typically inhabit intertidal wetlands, adapting to life between high and low tide levels.It has the ability to grow upto 5 to 8 meters. *Rhizophora apiculata* is source of phytochemicals such as tannins, flavonoids, saponins, and terpenoids, making it have broad medicinal uses. These phytochemicals are found to be promising in other pharmacological actions like antioxidant, anti-inflammatory, antimicrobial, and anti-diabetic activities. It has been demonstrated to exhibit hepatoprotective and cytotoxic activities in studies. Yet more research is required to completely elucidate the underlying mechanisms and clinical uses of this mangrove[19].

Sonneratia alba Sm: a member of family Lythraceae, *Sonneratia alba* Sm. is a mangrove with robust morphology. With a robust, erect trunk and a dense, spreading crown, it thrives in coastal habitats. Its leaves, adapted to the saline environment, are simple, opposite, and leathery. This mangrove species possesses vast coverage within the Indo-Pacific region and occurs well under intertidal environments[20]. Phytochemical analysis has proved the existence of tannins, flavonoids, saponins, and terpenoids in *S. alba*. The secondary metabolites of this species are responsible for the traditional medicinal use of this species, e.g., curing specific diseases such as diarrhoea, dysentery, and skin disease. Although ethnomedicinal information are abundant, extensive scientific investigation is the mainstay to establish the veracity of the reports and explain the mode of action of the bioactive constituents of this mangrove species[21].

Sonneratia apetala Buch.-Ham: *Sonneratia apetala* or the Bengal apple is a mangrove found with a medium height and characteristic growth habit. Tending to have a crooked trunk and grey-brown, fleshy bark, it grows in estuarine and coastal areas. Its simple opposite leaves are leathery. The plant has solitary, axillary flowers that grow into big, fleshy fruits filled with many seeds. *Sonneratia apetala*, native to the Indo-Pacific region, has learned to overcome the adversity of saline conditions. Its ability to thrive in waterlogged soils has made it ecologically important in coastal ecosystems. Aside from its role in the ecosystem, the plant has been given attention for what could be used as medicinal values. Phytochemical studies have shown a broad range of chemicals in *Sonneratia apetala* that include tannins, flavonoids, saponins, and terpenoids[22]. These chemicals were associated with the plant's attributed pharmacological activity. Traditional uses have involved all parts of the tree to treat various diseases like gastrointestinal disorders, skin infections, and inflammatory conditions. Yet, scientific studies are necessary to confirm these age-old assertions and to identify the exact compounds that are responsible for the reported therapeutic activities. Although initial studies have been promising, more studies are needed to fully appreciate the medicinal value of *Sonneratia apetala*[23].

Rhizophora mucronata Lam being a member of Rhizophoraceae is a unique mangrove species, is distinguished by its robust structure, large prop roots, and abundance in the Indo-Pacific area[24]. It is a good source of phytochemicals such as tannins, flavonoids, and terpenoids, which have been utilized traditionally for their medicinal properties. Traditionally, different plant parts have been used to cure conditions like diarrhoea, dysentery, and skin diseases. Modern research validates its use for anti-inflammatory, antioxidant, and antimicrobial purposes. Being a foundation species in coastal communities, *Rhizophora mucronata* is a key species that helps protect coastlines and maintain biodiversity[25,26].

Scyphiphora hydrophyllacea C.F. Gaertn A woody mangrove species with shrub or small tree habit. It grows into dense thickets in the intertidal area. The species has a distinctive leaf pattern with leaves aggregated towards the tips of the branches and forming a tufted look. Leaves are simple, opposite, and leathery with entire margins. The flowers are small, greenish-white and in dense globose heads. The fruit is small, drupe-like in structure. Native to the Indo-Pacific, *Scyphiphora hydrophyllacea* has a wide distribution in coastal and estuarine habitats. It grows well under saline conditions and has a high tolerance to soil flooding. India, Bangladesh, Myanmar, Thailand, Malaysia, Indonesia, and Australia are among the countries where this mangrove species is found Phytochemical studies on *Scyphiphora hydrophyllacea* are comparatively less when compared with other mangrove species. The initial studies have reported the occurrence of compounds like tannins, flavonoids, and saponins. More research is required to fully describe the phytochemical content of this species. Traditional medicine systems have linked some medicinal activities *to Scyphip horahydrophyllacea*. The plant has been employed in some communities to treat several diseases, including skin infections and gastrointestinal disorders. The phytochemical screening of this plant exhibited cytotoxic activity, anti-oxidant activity[27].

Xylocarpus moluccensis: *Xylocarpus moluccensis* is a large, evergreen Meliaceae species. The tree grows up to 30 meters tall with a trunk diameter of around 70 centimeters. The tree has a thick, grey-brown bark with longitudinal fissures. The leaves are alternate and compound and consist of 2-4 pairs of elliptic to oblong leaflets[28]. Its flowers are creamy-white and occur in groups that are large in size. The fruit is a big, round, woody capsule containing many seeds in a fibrous matrix. *Xylocarpus moluccensis* is widely distributed in tropical coastal areas and grows in mangrove swamps and backwaters. Its distribution varies from Sundarbans in India and Bangladesh to Southeast Asia, Malesia, to tropical Australia. Even though extensive studies on its chemical composition are scarce, initial research has established the occurrence of triterpenoids, steroids, flavonoids, and fatty acids in the plant. In traditional medicine, different parts of *Xylocarpus moluccensis* have been used for medicinal purposes in dealing with inflammation, while it has also found applications in antioxidant and wound-healing activity. But strong scientific evidence for these folk uses is still limited[29,30].

Aegicera scorniculatum is a highly adaptable plant also referred to as black mangrove or river mangrove. his shrub/small tree from the Primulaceae family is well known for its tolerance of saline conditions. Having leathery, obovate leaves and sweet-scented, white flowers in clusters, it's a familiar figure on coastlines[31].Its geographical area is quite extensive, ranging across India, Southeast Asia, China, Australia, and the Pacific. This mangrove plant has drawn attention due to its reported medicinal potential. Investigations have revealed the occurrence of phytochemicals such as saponins, flavonoids, and tannins in its structure. Such compounds are thought to be responsible for the antioxidant, anti-inflammatory, and antimicrobial properties of the plant. Various parts of *Aegiceras corniculatum* have been used in traditional medicine for curing various diseases, but further scientific investigation is needed to define its full therapeutic potential[32].

Bruguiera gymnorrhi also referred to as the black mangrove, is a major mangrove tree with broad ecological and economic significance. It belongs to the family Rhizophoraceae and is a medium-sized tree that occurs naturally in coastal and estuarine settings. The tree's distinguishing feature among mangrove trees is the development of short prop roots. It has smooth reddish-brown bark and elliptically shaped leaves. Its solitary flowers have white or cream-colored petals that later become brown. It has unique fruit resembling a top and at maturity, falls into the mud where it forms roots promptly[33]. This viviparous mode of germination is an impressive adaptation to the extreme

mangrove habitat. *B. gymnorrhiza* is an important species in coastal environments, serving as a habitat for numerous flora and fauna, safeguarding shorelines against erosion, and acting as a carbon sink. Aside from its ecological significance, the species has been utilized traditionally for a number of uses, such as timber, fuelwood, and medicine. Phytochemical research has shown that *B. gymnorrhiza* contains compounds with potential pharmacological activity. These are tannins, flavonoids, and other bioactive compounds. *Bruguiera gymnorrhiza* as an anti-oxidant, anti-inflammatory, anti diabetic, and with microbial properties and is known to enhance digestive health[34].

Bruguiera cylindrical: It is a medium-sized Bruguiera species that is also highly adaptable in different coastal areas. It features a unique cylindrical shaped propagules, which sets it apart from other Bruguiera species[35]. It has a bushy appearance with many aerial roots, locally referred to as pneumatophores, to facilitate oxygen supply in water-saturated soils. The leaves are simple, oppositely paired, and leathery in texture with glossy green upper side. Its flowers are borne in clusters and are succeeded by the typical cylindrical propagules. The propagules mature attached to the parent tree, a type of reproductive strategy called vivipary, which improves seedling survival in the harsh mangrove environment[36]. *B. cylindrica* is an important species in coastal environments, serving as habitat for numerous organisms, shore stabilization, and carbon sequestration. It is also an important resource to local communities, and its wood is utilized for timber and fuelwood. As with other mangrove species, *B. cylindrica* possesses potential medicinal uses. Compounds containing antioxidant, anti-inflammatory, and antimicrobial activity have been detected in the plant in preliminary investigations[37,38].

***Ceriops decandra* (Griff.) W. Theob.:** *Ceriops decandra* (Griff.) W. Theob.is a mangrove of the family Rhizophoraceae. Indigenous to the Indo-Pacific, it is distributed commonly in brackish coastal regions[39]. It has a tall, narrow trunk and leathery leaves. This mangrove possesses aerial roots known as pneumatophores to ensure gaseous exchange in low-oxygen soils. Its small, greenish-white flowers are followed by oblong fruits carrying a solitary seed. Rich in flavonoids, tannins, terpenoids, and alkaloids which are bioactive compounds, *Ceriops decandra* has found use in traditional medicine for their numerous health-related benefits. Diarrhea, inflammation, and wounds have traditionally been treated by the leaves, bark, and roots. *Ceriops decandra's* antioxidant activity also contributes to the ability of cells to resist damage resulting from free radicals[40]. Pharmacological Activities of Various Mangroves (Table 2)

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This work did not involve human participants, animal subjects, or any material that requires ethical approval.

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Table1: List of Some true Mangroves found in the Coringa Region[3]

S.no	Plant species	Family	Vernacular Name
1.	*Avicennia officinalis* L	Acanthaceae	Nallamada
2.	*Avicennia marina* (Forsk.) Vierh.	Acanthaceae	Tellamada
3.	*Avicennia alba* Bl	Acanthaceae	vilavamada
4.	*Lumnitzera racemosa* Willd	Combretaceae	Thanduga
5.	*Excoecaria agallocha* L	Euphorbiaceae	Tilla
6.	*Rhizophora apiculata* Bl	Rhizophoraceae	Uppu Ponna, Kakkiponna
7.	*Sonneratia alba* Sm	Lythraceae	Peddakalinga
8.	*Sonneratia apetala* Buch. - Ham	Lythraceae	Kalinga
9.	*R. mucronata* Lam	Rhizophoraceae	Ponna

10.	*Scyphiphora hydrophyllacea* C.F. Garetn	Rubiaceae	Nara thanduga
11.	*Xylocarpus moluccensis* (Lam.) M. Roem.	Meliaceae	Senuga
12.	*Aegicera scorniculatum* (L.) Blanco	Myrsinaceae	Guggilam
13.	*B. gymnorrhiza* (L.) Savigny	Rhizophoraceae	Kandriga
14.	*Bruguiera cylindrical* (L.) Bl	Rhizophoraceae	Urudu
15.	*Ceriops decandra* (Griff.) W. Theob.	Rhizophoraceae	Togara

Table 2 : Pharmacological Activities of Various Mangroves

S.No	Plant Species	Pharmacological Activity	Reference
1.	*Avicennia officinalis L*	Antioxidant(L and B) Antimicrobial(L and B) Carbohydrate Metabolizing (L and B), Analgesic (L), Cytotoxic (L)	4,5,6
2.	*Avicennia marina (Forsk.) Vierh.*	Anti-ulcer, Astringent, Anti-hemorrhagic, Anti-malaria, Anti-diarrheic, anti-tumor	7,8,9,10
3.	*Avicennia alba Bl*	Anti-inflammatory, Antipyretic, Analgesic	11,12
4.	*Lumnitzera racemosa Willd*	antibacterial, antifungal, and anti-malarial	13,14,15
5.	*Excoecaria agallocha L*	Anti-inflammatory, Analgesic	16,17,18
6.	*Rhizophora apiculata Bl*	Antioxidant, Anti-Inflammatory, Antimicrobial, and Anti Diabetic	19
7.	*Sonneratia alba Sm*	Diarrhoea, Dysentery	20,21
8.	*Sonneratia apetala Buch. - Ham*	Promotes Gut Health	22,23
9.	*R. mucronata Lam*	Diarrhoea, Dysentery	24,25,26
10.	*Scyphiphora hydrophyllacea* C.F. Garetn	Cytotoxic, anti-oxidant	27
11.	*Xylocarpus moluccensis* (Lam.) M. Roem.	anti-inflammatory, antioxidant, Wound healing	28,29,30
12.	*Aegicera scorniculatum* (L.) Blanco	antioxidant, anti-inflammatory, and antimicrobial	31,32
13.	*B. gymnorrhiza* (L.) Savigny	anti-oxidant, anti-inflammatory, anti diabetic ,	34
14.	*Bruguiera cylindrical* (L.) Bl	antioxidant, anti-inflammatory, and antimicrobial	35,36,37,38
15.	*Ceriops decandra* (Griff.) W. Theob.	Anti-diarrheic, Anti-inflammatory	39,40

RESEARCH ARTICLE

The Power of NLP: Understanding its Techniques, Tools and Real - World Applications

Vaishali Gupta[1]*, Suhani Nimkar[2], Sahil Sharma[2], Kalyani Tiwari[3] and Angeeta Hirwe[1]

[1]Associate Professor, Department of CSE, IPS Academy, Institute of Engineering and Science, Indore, (Affiliated to Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal) Madhya Pradesh, India.
[2]Student, Department of CSE, IPS Academy, Institute of Engineering and Science, Indore, (Affiliated to Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal) Madhya Pradesh, India.
[3]Assistant Professor, Department of CSE, IPS Academy, Institute of Engineering and Science, Indore, (Affiliated to Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal) Madhya Pradesh, India.

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***Address for Correspondence**
Vaishali Gupta
Associate Professor,
Department of CSE, IPS Academy,
Institute of Engineering and Science, Indore,
(Affiliated to Rajiv Gandhi Proudyogiki Vishwavidyalaya, Bhopal)
Madhya Pradesh, India.
E.Mail: vaishali.gupta77@gmail.com

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ABSTRACT

Machines' ability to understand, generate, and respond to human language has been radically altered by natural language processing (NLP), a key enabler of human-computer interaction. Natural language processing (NLP) is covered extensively in this review paper, which covers its basic principles, resources, and several practical applications. From simple rule-based systems to complex deep learning and transformer-based structures, we examine the evolution of natural language processing (NLP). The study delves into the various stages of natural language processing (NLP), including lexical, syntactic, semantic, discourse, and pragmatic. It also examines how NLP is used to support applications such as chatbots, machine translation, sentiment analysis, text summarization, and information extraction in sectors such as healthcare, education, and finance. By surveying the existing literature, we trace the evolution of NLP, examine current trends and challenges including explainability, bias, and low-resource language processing, and identify critical knowledge gaps. Lastly, we summarize potential future paths, including multimodal natural language processing, responsible and ethical artificial intelligence, and progress towards real-time, low-resource, and explainable systems. Researchers and practitioners interested in current NLP's power and potential can find a basic resource in this review.

Keywords: Natural Language Processing (NLP), Sentiment Analysis, Information Extraction, Text Mining, Speech Recognition, Automated Summarization, Morphological Analysis.

INTRODUCTION

As the world increasingly becomes a space of digital interactions and data overloading, it becomes increasingly vital to know how to process and analyze language effectively. To truly understand the relevance of Natural Language Processing (NLP) and its application, we must first understand 'what' NLP is. Through here, we will look at the concepts behind NLP, its historical development, and investigate its relevance, application and use in practice.

What is NLP?

Natural Language Processing (NLP) is a subfield of AI that aims to mimic human communication by understanding and responding to human language. It involves managing and analyzing large volumes of linguistic data, including spoken language meaning interpretation, written language translation, speech recognition, and written language generation. NLP aims to bridge the gap between language as a human communication medium and the text or symbols that machines work with, enabling computers to grasp meaning and react appropriately to various situations. The goal is for computer and digital system interactions to be as natural and successful as human-to-human communication. The Power of NLP within the AI Framework (Figure: 1)

Why is NLP required?

The demand for Natural Language Processing (NLP) stems from one core challenge: determining how to better facilitate communication between a human and a machine. The digital sphere continues to expand rapidly, and the amount of unstructured language data (text, speech, social media content, etc.) has grown enormously. Most traditional computational methods are and will continue to be inadequate for processing and understanding unstructured language data; therefore, more advanced methods are required. There are several critical reasons for requiring NLP

Data Interpretation and Analysis: Today, most new information created and made available is in natural language. Businesses, researchers, and governments all need advanced tools to help them analyze this data. NLP allows humans to search for meaning and draw insights from larger datasets, which ultimately leads to more accurate decision-making, the collection of comprehensive trend information, and increased awareness of user behavior.

Task Automation: Many tasks that depend on language, such as customer service, translation of documentation, and writing of content, require many hours of work when performed by humans, resulting in tasks that are tedious and time-consuming. Human labor can be largely replaced by NLP, improving overall efficiency, and allowing for instant responses in all types of applications including chatbots, virtual assistants, and automated translation services.

Improved Human-Machine Interaction: We live in a time when technology is evolving rapidly. The expectation for more natural or informal interactions between people and computers continues to rise. NLP can allow machines to understand and interpret human language, making interfaces more user-friendly and accessible to many people. This is particularly important with devices that could be voice operated or apps designed to give users a seamless experience.

Global Communication: In an increasingly globalized world, the ability to communicate across languages holds great value. NLP can help to provide greater access to various tools, like machine translation, which can enhance communication and collaboration across cultures by translating text and documents.

Facilitating Accessibility: NLP is instrumental in developing inclusive technologies - especially for people with disabilities. Speech recognition and text-to-speech technologies provide support for individuals with hearing disabilities or speech impairments to engage with digital content. It highlights the unique ways that NLP can assist in building technologies that provide equitable access to all users.

Where NLP can be used?

Natural Language Processing is useful for a wide spectrum of applications, which indicates its versatility and relevance in contemporary technology. Below are important domains where NLP is successfully utilized:

Chatbots and Virtual Assistants: Smart chatbots and virtual assistants that can understand and answer to user inquiries in natural language are built using natural language processing (NLP). Users can enjoy a natural and engaging experience with these systems in a variety of settings, including customer service, personal assistant, and information retrieval. Virtual assistants like Siri and Alexa on Apple devices employ natural language processing to understand and fulfill user requests for things like reminders, weather reports, and answers to frequently asked questions. These technologies give users instantaneous conversational support and help to improve their experiences by leveraging natural language processing.

Machine translation: With the use of natural language processing, people are no longer limited by language boundaries while communicating via text or audio. Services that translate text, provide content in multiple languages, and facilitate global communication all make extensive use of this technology. One popular tool that can rapidly translate text or voice into another language is Google Translate. Natural language processing and machine translation make it easier for users to communicate in situations when more than one language is spoken, such as at international business meetings or while traveling.

Sentiment Analysis: In multiple domains such as marketing and social media monitoring, NLP is used to determine the sentiment expressed in text data. This approach attempts to determine the opinions, feelings, or attitudes expressed in textual reviews, social media, or feedback forms. Sentiment analysis tools, such as those employed by Brandwatch, detect consumer sentiment in their feedback in order to gain an understanding of public perception, sentiment, feelings, etc. associated with their products or services. For example, by analyzing social media sentiment related to a product at the point of launch, organizations may gain invaluable insight into consumer sentiment, reactions, and overall preferences.

Speech Recognition and Generation: NLP is responsible for programs that identify spoken words and transcribe (convert spoken words to written text) them into text (speech-to-text), as well as generating speech through text (text-to-speech) and converting written text to spoken audio. Software such as Dragon Naturally Speaking allows a person to dictate text directly into their computer with the use of speech recognition. Voice recognition in applications has a wide range of uses, one being Google's Text to Speech technology that converts written text to auditory natural-sounding spoken words (text-to-speech), which is popular in applications around audiobooks and voice responses in customer service systems. These technologies form the backbone for voice activated applications, as well as transcription services, and many accessibility tools.

Information Retrieval and Search Engines: NLP contributes to the capabilities of search engines by improving understanding of the user's queries, thereby providing better search answers. Google Search utilizes NLP techniques for interpreting and indexing large amounts of content on the web, which assists in providing accurate and relevant search responses, this means monitoring, analyzing and indexing a lot of text to give efficient information retrieval.

Text Summarization: NLP is capable of automatically summarizing large amounts of documents or articles into shorter summaries. Summarization tools, like the ones provided in news aggregators or in research paper databases, provide summaries for long articles that condense the materials into overviews that allow users to gather info quickly without reviewing the entire document. This is especially valuable for organizing content, digesting information, and providing short overviews of longer documents.

Content Generation: NLP is used in content generation that includes written content including reports, news articles, and marketing language. Automated content generation software makes extensive use of NLP to generate written content that includes not only news articles but also marketing copy. OpenAI's GPT-3 is an NLP model that can

generate coherently written text that is contextually accurate in relation to a draft article, marketing content, or simply generating text as part of an application.

Healthcare and medical records: Another sector where NLP is applied is in healthcare to process and analyze medical records and academic literature. For example, IBM Watson Health leverages NLP to identify and process relevant information from patient records and academic literature to assist healthcare professionals with clinical decision making and to help healthcare professionals identify trends in patient data.

Financial Analysis: The financial and trading sectors have begun to utilize NLP to assess summary reports, news articles, and social media to identify market and sector trends and sentiments of other investors. An example of this application is Bloomberg Terminal, which uses NLP to scan news articles and social media outlets to provide actionable insights to inform investment decisions and market forecasts, which is invaluable for decision-making and forecasting in finance/financials.

Educational Tools: NLP has opened up the floodgates in educational technology in the education field by allowing systems to provide intelligent tutoring systems (ITS), language learning applications, and tools for supporting complex tasks. An example of this is Duolingo which uses NLP as the underpinning foundation to help students learn a new language through gamified motives, providing contextually relevant and adaptive exercises and feedback to assist the user in learning a new language. These types of systems primarily rely on NLP for personalizing student, feedback, prompts, and academic support.

NLP TECHNIQUES AND IMPLEMENTATION

Integrating Natural Language Processing (NLP) solutions utilizes a blend of traditional linguistic approaches and modern computational methods. These approaches are likewise designed to capture the complexity of human language and optimize performance in multiple NLP tasks. NLP implementation Approaches (Figure 2)

Rule-based approaches: Rule-based approaches in nlp leverage explicit rules about language to analyze and process textual elements. The rules are coded into the system by domain experts that encode specific syntactic and semantic rules in the system. Rule-based methods are generally effective in applications that need exact parsing of language, such as grammar checking and named-entity recognition. However, one of the drawbacks of these systems is incapacity for new or ambiguous language input, and scale of use. For example, this could include a grammar checking tool with predefined rules to delineate and correct syntactical errors present in written text.

Statistical Approaches: Statistical models utilize a probability model to analyze language patterns, typically implicating usage of a language on a large corpus of text, assuming use of a model built on tokens, hence identifying the probability of particular structures of language and of words. Particularly, Hidden Markov Models (HMMs) and n-gram models are prevalent approaches. Also, statistical methods have been used prominently in machine translation where they predict the most probable translations of words and phrases in conjunction with the relevant context. A language model predicting the next word in a sentence based on the probability of sequence of words is an example of a statistical approach.

Machine learning Approaches: Natural Language Processing (NLP) that makes use of machine learning involves developing algorithms that learn from a large collection of labeled examples to perform a certain task. Some possible tasks include classifying text, conducting sentiment analysis, or named entity recognition, which involves determining names or dates from within a sentence. For these machine learning algorithms, the training step involves providing the algorithm with a large number of examples. Each example is labeled, which helps the algorithm learn the relationships between the labels and the input. Once the algorithm has been effectively trained to learn relationships in the data set, it can apply that knowledge to perform a prediction or some decisions about the new data, data that has been previously unseen. As an example, consider having a sentiment analysis tool that was made to label a customer review as positive, negative or neutral. In developing that tool, a machine learning

algorithm would be trained on a large number of customer reviews that had already been labeled with sentiment. The machine learning algorithm would learn from those labelled examples, and for example learn that words like "great" or "excellent" indicate a positive sentiment and words like "bad" or "terrible" indicate a negative sentiment. Once trained, the model could now take a new customer review as input, even without having seen the review before, and predict the sentiment.

Deep Learning Strategies: Deep learning has now brought a significant new approach to Natural Language Processing (NLP) with neural networks and the advanced ways they understand language. Picture the neural network as intricate patterns of connections designed to recognize and interpret language structure. Neural Networks- "Think of these as digital brains, capable of learning and interpreting decisions based on input data. They facilitate our understanding of language in terms of patterns and relationships." Consider BERT (Bidirectional Encoder Representations from Transformers): Imagine you, as the reader, are trying to interpret a word's meaning based on the other words surrounding it. BERT finds a word's meaning by interpreting the entire sentence two different ways (from beginning to end and end to beginning). For example, when reading, "The bank was full of fish," BERT determines that "bank" refers to the place you find fish, not the financial institution. Primary Models are:-

Recurrent Neural Networks (RNNs): These are good at dealing with sequences after all, a sentence is a sequence, and an RNN will remember everything that it has read before.

Long Short-Term Memory (LSTM) Networks: A specific variation of RNN capable of remembering longer sequences and keeping track of relevant information.

Transformer-based Models (like BERT and GPT): A very powerful and deep model that looks at all parts of a sentence in the same way at once, rather than just at the beginning or end of the sentence; this gives it a much better understanding of context.

Hybrid Approaches: Hybrid approaches consist of combining rule-based paradigms with machine-learning approaches in order to better handle non-trivial work in Natural Language Processing (NLP). Rule-based approaches look at the language through predefined rules and/or constructs for the purpose of interpretation and processing. The rules are assembled from linguistic and psycho-linguistic knowledge, and they work best for simple and unambiguous tasks. Machine-learning approaches rely on using various datasets that allow the model to learn and recognize patterns on its own and allow the model to make some predictions based on the data. When a hybrid system applies both techniques, it can handle trivial (simple) language tasks with any dimensions of the matter in question. For example, imagine a customer service chatbot. That chatbot could use rule-based approaches to respond to the constant customer-service-who-questioning like, "What time do you open?"- with one possible answer. Then the chatbot could use machine learning, for a question that has potentiality in context that could also include customer-service-complications, would be "I have a problem with my order-man. Can you help?" - which could be a longer or shorter answer. In that sense, the hybrid system could be precise for the brief and simple, yet also be more adaptable for the complicated.

Transfer learning: Transfer learning takes a pre-learned model and adapts it to a more specific task. As a method, it works well because it uses the immense information derived from very large and varied datasets to perform better on a smaller, domain-specific task. The two components of transfer learning are: -

Pre-training: extending out on a large and diverse dataset, as a certain number of books, articles, and webpages. For example, models like BERT and GPT are pre-trained on a lot of text from the internet to learn general language patterns.

Fine-tuning: Then extending out on a smaller dataset specific to the new task. For example, if you want the task to classify legal documents, you would fine-tune the model on a dataset of legal texts. This would allow the model to

adapt its general knowledge of language, in this case, to also comprehend legal language and its terminologies. This would increase accuracy for things like legal document classification. With this transfer learning approach the model could perform even if it had not had an extensive amount of data for the specific task on which it is being trained.

Reinforcement learning: In reinforcement learning, a model improves its decisions by learning from the feedback responses it receives. In essence, the model provides decisions or actions and then receives feedback about how good or bad the response was. In this situation, it produces rewards for nice actions and punishment for mistakes. The feedback guides whether the model should continue using that action in a different context. So, in reinforcement learning, the model has an idea of what works and what doesn't. In repetition, the model uses the feedback to refine its choices and learn from the mistakes. This is similar to how we learn from trial and error based on previous experiences, with the outcome being a more focused and refined resolution to fit the choice. For example, let's assume a virtual assistant is learning to provide conversation with users. At first, the model may provide some wrong or irrelevant answers. Over time, the virtual assistant begins to interact with more users. Each reply receives feedback, such as an immediate rating or correction. The one-to-many feedback responses allows the assistant to change the wrong or irrelevant answers to improve each one of their responses. The result is a growing baseline of which types of answers were more helpful. A useful model would seem to learn how to provide a better experience for the users along the memo of they questions they provided and the feedback received.

Phases of NLP (Figure 3)

Natural Language Processing, also referred to as NLP, is a subfield of artificial intelligence that allows computers to understand, analyze, and interact with human language in order to derive meaning. NLP can typically be decomposed into five areas of processing: Lexical Analysis, Syntactic Analysis, Semantic Analysis, Discourse Integration, and Pragmatics Analysis. Each of these areas contributes to the overall decomposition of natural language understanding and processing.

Lexical and Morphological Analysis

Tokenization: The lexical phase in Natural Language Processing (NLP) is where the initial parsing of text into smaller chunks (such as paragraphs, sentences, and words) occurs. If we break text down into small enough parts, a word maybe a token, a token or lexeme is a small amount of meaning to analyze further. Tokenization is probably one of the most important steps of NLP as it allows for word-level paper processing by understanding the text more easily. In addition to tokenization, the following are more techniques related to data cleaning and feature extraction for processing text quality, in general:

Lemmatization Lemmatization refers to the action of reducing a word to its root or base form. Lemmatization allows variations of the same word to be analyzed together.

Stopword Removal Stopwords are common words with no significant meaning, for example; "and", "the", "is", etc., removing them, allows us to think about the content with purpose and not the words.

Spelling Correction Correct spelling text is a step all to itself because in tasks during NLP it is important to follow a sequence that makes sense, thus correcting any spelling errors maintains accuracy and reliability.

Preprocessing techniques such as these are important for bettering the readability of the text and that text is clear for effective analysis and processing in the following phases of your NLP.

Morphological Analysis: Morphological Analysis:- Morphological analysis is another important step in NLP that focuses on identifying and analyzing morphemes. Morphemes are the smallest meaningful units in words, which cannot be broken down further. Understanding morphemes' structure and function is key to understanding words and their parts.

Syntactic Analysis

Syntactic analysis or parsing is a component of Natural Language Processing (NLP) that involves examining the structure of the syntax of a sentence. The objective of this phase is to analyze the formulation and relationship of words in the sentence. Using more plain language, it's more about understanding the grammar in order to see how the words all work together to form meaning. It is also known as the Second Phase of Natural Language Processing (NLP). During this phase, the sentence is deconstructed into its grammatical elements (nouns, verbs, adjectives, etc.) and then the relationships between the elements are analyzed. For example, in "The cat sits on the mat" syntactic analysis determines "the cat" is the subject, "sits" is the verb, and on the mat is the prepositional phrase which shows where the action occurs.

Examples of Syntax

Correct Syntax: "She enjoys reading books."

Explanation: This sentence follows the correct structure of the standard Sentence syntax: Subject (she) + Verb (enjoys) + Object (reading books). It is included in the proper sentence structure and is grammatically correct.

Incorrect Syntax: "Reading she enjoys books."

Explanation: The word order is incorrect, rendering the sentence confusing and incorrect grammar. The meaning is unclear because the sentence does not follow the standard Subject-Verb-Object structure.

These examples help show how important correct syntax is for clear communication.

Example: Consider the sentence "She loves ice cream." Syntactic analysis will break it down as follows:

- "She" (subject)
- "loves" (verb)
- "ice cream" (object)

This breakdown helps the NLP system understand who is performing the action and what the action is, which is critical for accurately processing language. This analysis aids in analyzing the structure of the sentence which is a critical part of more complex nature language processing (NLP) tasks including machine translation, where a sentence's meaning must be preserved in another language. By understanding the syntax, the NLP system can better understand and process how words relate to each other, allowing the nature of the text to be comprehended in a more efficient way.

Semantic Analysis

Semantic analysis is the third stage in Natural Language Processing (NLP) that seeks to uncover the meaning behind the words contained in a sentence. Whereas syntactic analysis incorporates the structure of a sentence, semantic analysis considers a broader approach that tries to determine the actual intention or meaning of text. In this stage, NLP systems are considering the relationships between words as well as their combination to create meaningful phrases or sentences. The system must identify concepts, like entities (people, places, things) and their relationships, to understand the context of how each word is used. Semantic analysis doesn't attempt to analyze the grammatical structure of the sentence like syntactic analysis, but rather attempts to determine what the sentence is saying. For instance, "John bought a new car." Semantic analysis allows the NLP system to identify that "John" is a person, "bought" implies a purchase action, and "car" is the object of the purchase. The system recognizes that "new" modifies the car and provides much more meaning. So, semantic analysis allows the NLP system to know that the sentence is about a person named John, who purchased an object--a brand-new car. This understanding is vital for the NLP system to analyze the text and know how to respond.

Example of Semantic Analysis: "The car drank the coffee."

Syntactically Correct: The sentence follows proper grammatical rules, with a subject ("The car"), a verb ("drank"), and an object ("the coffee").

Semantically Incorrect: The sentence is nonsensical because a car cannot drink coffee. Although the structure is correct, the meaning fails to convey a logical understanding. Through semantic analysis, the mismatch in the subjects and actions would be highlighted and the sentence would be labeled nonsensical, even though the structure is grammatically correct. Semantic analysis is a vital part of Natural Language Processing (NLP) because it helps machines and systems understand the meaning and context of the text. Semantic analysis goes deeper than grammar--it resolves ambiguities and captures word relationships to ensure sentences are interpreted properly. Knowing what different words mean ensures high levels of performance in machine translation, sentiment analysis, chatbots, and many other applications with heavy reliance on language. The result of effective semantic analysis is strong reliability and intelligence in NLP systems, foreshadowing simplicity in more complicated tasks that demand well informed and meaningful responses.

Discourse Integration

Discourse integration is a Fourth phase of Natural Language Processing (NLP) that considers the whole point of a conversation or text beyond single sentences. The branches of NLP work with sentence-level understanding of language, such as syntactic analysis and semantic analysis, but discourse integration works to connect phrases and sentences into an understanding of the overall meaning, or flow, of a text.

Contextual Understanding: Discourse integration allows the system to understand the interrelationship of sentences. For example, if a text says, “John went into the store. He bought some milk”, discourse integration helps with linking “He” to the previously mentioned “John” and understanding that to buy the milk, it logically follows that John went into the store first.

Maintaining Coherence: It allows the text to stay coherent and logical. For example, if a narrative moved from discussing a cat to talking about a dog, discourse integration would allow the discourse tracking system to recognize the movement and maintain discourse without shifting the content unnecessarily.

Managing Pronouns and References: This step involves recognizing what pronouns (such as "he," "she," or "it") are referring to across sentences. This can help resolve ambiguity and tell what is being talked about and who is being referenced. In conclusion, integrating discourse is critical for ensuring that NLP systems read longer texts correctly, by connecting sentences and maintaining a clear meaning through the conversation or text. Discourse integration is crucial for Natural Language Processing (NLP) as it helps computers understand sentence structure, connections, and work together. It helps provide cohesive interpretation, recognizing relationships between sentences and responding appropriately. This helps in summarizing content, providing relevant responses, and enabling precise, cohesive responses and better pragmatic interpretations.

Pragmatic Analysis

Pragmatic Analysis is the fifth and final phase of Natural Language Processing (NLP), and it focuses on finding out what a word means in relation to the context it is placed within and the background knowledge about the world. The stages of NLP that precede this dealt with grammar, structure, etc., and pragmatic analysis looks at how language is used in a specific context to convey rules, meaning, or comprehension in situations rather than simply searching for meaning based on semantics. Pragmatic analysis will go beyond the literal meaning considered in semantic analysis, as we are attempting to understand latent meaning or what the writer or speaker intends to convey. In natural language, it should not be surprising that we have all encountered language where a word or phrase will have no one meaning, and the expressed meaning can rely on context, tone, and many other situational-based references. Here’s a detailed explanation with examples:

Contextual Understanding: Pragmatic analysis allows a reader to understand author's intention of a sentence by considering context and other factors. For example, if someone uttered the phrase, "It’s getting late," the literary meaning would simply be correctly interpreted as time is elapsing. However, in pragmatic analysis, the speaker may be implying that it is time to leave or finish an activity.

Interpreting Implicit Information: This step involves interpreting what is meant as opposed to what is said. For example, in a conversation regarding grocery shopping, if someone says "I forgot to buy milk," while they could simply be reminding the other listener of their need to make the purchase, they may actually be hinting and requiring a light nudge or reminder to do so. A pragmatic analysis helps the system catch these implied hints.

Recognizing Social and Cultural Contexts: Pragmatic analysis considers social norms and cultural expectations. For example, if a person were to say, "Good job!" after someone made a mistake, pragmatic analysis would take this as sarcasm rather than sincere praise. To understand sarcasm, one must understand the social context and tone.

Understanding Speaker's Intent: This phase also determines what the speaker intends to achieve with their language. For example, if I said, "Could you pass the salt, please?" A pragmatic analysis would interpret that using language to make a polite request rather than a question about the listener's ability to pass the salt.
In conclusion, pragmatic analysis is important for NLP systems to process language in a way that represents how people use language in life. Pragmatic analysis allows interpreters to pay attention to context, implied meanings, socio-cultural factors, and intentions of speakers to inform how they use language. Pragmatic analysis leads to better communication in NLP systems across a number of applications.

LITERATURE SURVEY

There have been several paradigm changes in the field of Natural Language Processing (NLP) throughout its lengthy history. Natural language processing (NLP) originally depended on systems that followed predetermined norms of language. One such early natural language processing system was SHRDLU, developed by Winograd in 1972. It was able to carry out commands given in natural language within a simple, limited setting (Winograd, 1972). However, due to their reliance on rigid syntactic restrictions and difficulty in being modified, these systems had limited usage. The statistical tools that emerged in the late 80s and early 90s revolutionized the industry. Machine translation performance was enhanced when researchers, such as Brown *et al*. (1990), utilized big bilingual corpora to develop probabilistic models. Processing could be more probabilistic and flexible with a data-driven approach than with older systems. The deep learning revolution also made another jump in the capabilities of NLP. Mikolov *et al*. (2013) made Word2Vec possible by representing words in dense vector spaces and bringing relationships of meaning into play (Mikolov, Chen, Corrado, and Dean, 2013). Other models followed Word2Vec, like Recurrent Neural Networks (RNNs), as well as Long Short Term Memory (LSTM) networks (Hochreiter and Schmidhuber, 1997), which had established methods of modeling sequential data. Indeed, deep learning systems that emulate human cognition find artificial neural networks in their bag of tricks. Later models built on Word2Vec included those based on the Transformer architecture, which changed the landscape of language processing with their introduction (Vaswani *et al*. 2017). Since then, BERT (Bidirectional Encoder Representations from Transformers) and GPT (Generative Pre-trained Transformer) have established new performance benchmarks for many different NLP tasks (Radford, Wu, Child, *et al*. 2019). The real-world implications of NLP are enormous and consequential. The most salient applications include virtual assistants like Siri and Alexa, which rely on NLP to enable voice-based interactions. Bilingual communication is also made possible, in part, by NLP tools like Google Translate, which help humans to communicate despite language differences. Firms may also use sentiment analysis to heuristically define public opinion based on consumer reviews and social media remarks (Pang and Lee, 2008). NLP techniques also enhance the user interaction of speech recognition systems and search engines to retrieve information for users. Contemporary NLP is engaged with key challenges such as ethical multiplicities including bias in language models, how to explain model outputs, and how to scale to large amounts of data. Researchers in the field are narrowly and widely interested into developing multilingual systems as well as developing systems that can reason with multimodal data (Kiela *et al*., 2019), for example, learning to understand and produce language while also being able to reason about and respond to visual and auditory data. As NLP increasingly becomes a normalized area of inquiry for natural language researchers, future work will need to focus on ethical AI directives, low resource languages and developing systems that are contextually aware and intelligent. Recent developments in Natural Language

Processing (NLP) technologies have focused mainly on hybrid architectures, emotion analysis, and reasoning frameworks. In their review of hybrid and swarm-deep learning models that incorporate RNN, Transformer and ensemble approaches, Jia *et al*. (2023) demonstrated significant performance enhancement in natural language tasks (i.e. sentiment analysis, named entity recognition, summarization), but they also noted the increased resource use for these models. Emotion Analysis is still an evolving junior field. Plaza-del-Arco *et al*. (2024) reviewed the last 154 publications of the field to identify enduring saliences within it--demographic and cultural bias, ambiguously defined emotional categories, inconsistent use of terminology, and the lack of interdisciplinary presence. Moreover, Yu *et al*. (2023) reviewed common natural language reasoning studies including those related to logical inference, multi-hop question answering, commonsense reasoning, backward and defeasible reasoning, and encouraged the growth of multi-step reasoning frameworks in the field. Alongside these academic reviews, industry reviews have noted transformational approaches in the field, including multimodal learning, low-resource utilization efficient LLMs (i.e., QLoRA, adapter), retrieval-augmented generation (RAG), methods for hallucination detection, instruction tuning, and knowledge-grounded dialogue as important dimensions to operationalize across industries in 2024–2025, including with clinical decision support, risk assessment in finance, law applications, and within scientific literature processing.

Early Development: The historical context of Natural Language Processing (NLP) describes the evolution of the field from its initial beginnings. Initially, NLP relied on rule-based systems, where researchers created rules for computers to understand language. As an example, the SHRDLU system made by Winograd in 1972 could work with commands, but only in the simulated environment created for it. These systems had their limits as they came to be heavily reliant on previously established rules which limited the complexity of language they could work with.

Shift to Statistical Methods: In the late 1980s and early 1990s, NLP also experienced a tremendous shift to statistical techniques. Brown and others began to use large amounts of bilingual text data to develop models for machine translation. Rather than modeling the rules of language as prior researchers had done, this new data-driven approach leveraged the abundance of patterns available in large datasets especially taken in context with the increased processing capabilities of computers.

The Deep Learning Revolution: The Deep Learning Revolution was defined by advances in deep learning. In 2013, Mikolov gave us word embedding (Word2Vec), which took words and represented them as continuous vectors in a way that captured their meaning better, naturally. The development of advanced neural networks, in particular, neural networks that process sequences of data (sentences) in advanced contexts (Recurrent Neural Networks and Long Short-Term Memory), was also an evolution. The branches of natural language processed by transformer networks, such as BERT and GPT, effectively transformed NLP with high performance levels evaluated and via a broader set of tasks augmenting the language revolution implications.

Current Trends and Challenges in NLP

Natural Language Processing (NLP) has made great strides in the past few decades, and it has become a key piece of many modern AI applications. With these advancements bring new challenges and emerging trends that are influencing how we will do NLP in the future. In the next section we will take a look at some of the most up-to-date trends and challenges in NLP.

Ethical and social issues: As NLP technologies continue to evolve, they continue to come with ethical issues, particularly around biases in language models. Models can and do unintentionally mimic and perpetuate gender, racial, and cultural biases, which can lead to fairly non-non-disciminatory outcomes. It is a challenge to combat and alleviate biases in general, especially for fairness in NLP systems.

Explainability in NLP: The complexity of NLP models and systems continues to grow, especially with the introduction of deep learning for many tasks. For many domains and applications, an explainable model is important if not crucial, when a model is able to provide a result but also an account and explanation of the underlying reasons.

While a machine-generated result may be correct, without explanation, users may lack trust. This is especially important for certain domains and/or applications (e.g., healthcare, finance, etc.).

Scalability and Real-time Processing: Natural language processing (NLP) models have seen increasing use cases with the growth of sophisticated techniques for processing big data on the fly, including applications such as customer service chatbots and social media monitoring. While increasing the scale of NLP models to accommodate large data sets has become more possible, it is still a major challenge to scale these models responsibly and efficiently. Although some models can use negligible resources, the computational resources needed when training or deploying NLP models can be great, which raises questions about their sustainability with regard to potential coal-based power use in resource extraction practices to sustain our energy demands, and the environmental impact in general.

Multilingual NLP: We are also seeing a growing popularity and trend towards making NLP systems language-agnostic, meaning able to interpret multiple languages, particularly low-resource languages. While the general language processing has progressed with very popular languages/domains like English, there is still a need for more models that can be more inclusive of the full linguistic diversity of the world's population.

Integration of Multimodal Data: In the future, NLP will likely involve an interaction of text with other data types, such as including audio, images, and video file types. The multimodal approach presents much higher order and more robust applications of NLP models, however, it also presents new challenges regarding the incorporation and processing these data as components of their respective model designs and training.

Current Gaps and Future Directions in NLP

Natural Language Processing (NLP) has made substantial progress and continues to exhibit gaps that can be exciting areas for future research. For instance, one major gap relates to developing NLP models for low resource language contexts. While NLP has developed astounding models like BERT and GPT for specific languages that have substantial amounts of datasets available, it is important to highlight that there are over 3,500 languages in the world, many of which lack the corpus required to train robust NLP models. To emphasize this point, only around 20 languages have large-scale and well-annotated datasets available, which leaves thousands of languages underrepresented in NLP exploration. Another area for future exploration relates to the need to include multimodal data, or integrating text with other forms of data, such as images, audio, or video data. NLP models developed today generally process text alone, but as we know human communication occurs on multiple levels. Research by Kiela *et al*. (2019) suggests that models can significantly improve upon their performance when visual data are included, such as in tasks like image captioning and visual question answering, in turn, inviting more complex and robust multimodal NLP models that will aid in understanding the underlying processes to human language and in generating human language. In addition, it is worth noting ethical AI and human-AI collaboration as relevant directions for the future of NLP as well. As NLP models become more capable, it is increasingly important to ensure they are fair, transparent, trustworthy, and aligned with human values. Research has long documented the prevalence of gender and racial bias in NLP models and demonstrated that unfair or harmful outcomes can result from biased models. Efforts aimed at removing biases and creating ethical guidelines for NLP systems will be instrumental as the field continues to emerge. In summary, while the advances in NLP are certainly impressive, focusing on multilingual NLP, multimodal data, and ethical AI will be instrumental to filling gaps and summoning the future of this ever-changing field.

Application of Natural Language Processing

NLP has a wide variety of interesting and useful applications that are relevant to different parts of our everyday experiences. Through the application of our language technologies, NLP supports task automation, improvements in communication, and extraction of insights through large amounts of data in text form. Below are some areas where NLP is producing significant outcomes:

Automated Text Summarization with NLP

Automated text summarization is an attractive NLP application that offers the potential to speed up the identification of core/main points in large quantities of text, thereby saving time and needless effort in the reading through this weight of text. This area finds practical applicability in and across many areas including news aggregator applications or legal research efforts.

News Aggregation: News aggregators simply aggregate news articles from various sources and repurpose them into a single stream. NLP here will summarize the articles so readers know what is contained without having to traverse many sources. Example: think of something like a news aggregator-think of Flipboard. It is identifying and extracting important sentences in different articles from distinct news sites. This is based on term frequency, overall positioning of sentences, named entities, characters, organizations, locations, and results in a short summary. For example; there is a 1,500 word article on a recent economic summit, a summarization function may provide us with a 100 word summary that reads

- Participating countries
- General categories discussed
- Top outputs or decisions agreed upon during the economic summit.

Legal Briefs and Research Papers: When we consider legal and academic work, documents can pile up to hundreds of pages when printed, which is an overwhelming amount of information to assess and internalize. legal professionals can use NLP-based summarization technology to relieve that burden by allowing them to read shortened summaries, whilst still having the most important information captured. For example, consider a lawyer having to quickly read a 60-page hello saying on a court judgment about a case involving several legal precedents. The lawyer can use an NLP summarizing tool, putting that 60 pages into the tool which will synthesize it into a summary with key, thematically, reportable areas including

- Legal focus areas and issues
- Major points argument made by either side
- The final conclusions reached and the supporting reasons for them

NLP Techniques Used to Summarize

Extractive Summarization: The extractor considers the text and takes sentences from it, which the extractor has determined are important. Methods: The extractor relies on algorithmic ways to choose important sentences. For example, TextRank which is similar to Google's PageRank algorithm ranks sentences from the important list in order of importance.

Abstractive Summarization: It creates new sentences that describe the main ideas in place of copying sentences from the text. Methods: The extractor uses a deep learning model - specifically Transformer architecture, like BERT and GPT - to understand context with the given text and summarize accordingly.

Hybrid methods: It is a combination of extractive and abstractive to ensure better quality and coherency in the summaries.

Sentiment Analysis

Sentiment analysis applies NLP to process the sentiments behind written text. In fact, sentiment analysis uses text data to help organizations to comprehend the public's perceptions of experience, much like surveys that measure sentiments, along with identifying certain characteristics of comments/words used. This use of NLP in sentiment analysis is applicable for research and businesses that will aim to measure and respond to the sentiments created by their audience.

Sentiment Analysis and Market Research

A global electronics provider has released its new model smartphone. The organization is attempting to assess customer sentiment and use a sentiment analysis tool that collects and assesses thousands of reviews across multiple e-commerce platforms, social media comments, and online forums.

NLP Process

Data Collection: An NLP system collects data from various external platforms like Twitter, Facebook, and Reddit as well as customer reviews on Amazon or their own website.

Preprocessing: The collected data is cleaned and preprocessed by removing noise, HTML tags, special characters, and stopwords, followed by tokenization into words and/or phrases.

Sentiment Identification: Each piece of text has sentiment classification, either positive, negative or neutral, using pre-trained models or custom built using supervised learning.

Aggregation and Analysis: The outputs are aggregated to give an overall sentiment score for the new smartphone model. The NLP tools can also capture key features or aspects, like battery life or camera quality, elucidating more precisely what customers specifically like or do not like about a product.

Strategic Adjustment: The marketing team could adjust their campaigns based on the sentiment analysis. If many consumers are dissatisfied with the battery life, they might highlight improvements in future models in their advertisements. The advantage is the company can quickly assess public opinion without having to read through thousands of reviews manually while saving on costs and time.

Sentiment Analysis in Political Campaigns

For a political party, assessing the public's perception of both their candidate and the candidate's key issues is a critical part of the election campaign process. Sentiment surveillance and analysis helps to track the public's attitude towards the political party's candidate by monitoring social media activity, blog activity, and comments on the news.

NLP Process

Data Collection: The NLP system will gather data from Twitter feeds, Facebook posts, Instagram comments, and from political blogs.

Preprocessing: The NLP will preprocess the text for different styles, languages, and dialects that is prevalent on social media.

Sentiment and Topic Analysis: The system will determine the sentiment, whether it is positive, negative or neutral, and identify the topic, which may include health care, economy, education, etc., using topic modeling.

Geographical and Demographic Insights: Sentiment analysis will show how the geography or demographics of the sample feels about the candidate, or about policy issues, which will inform targeted campaigning.

Real-time Monitoring: The campaign will receive real-time sentiment analysis, which allows them to keep up with changing feelings from the public and address misinformation.

Message Optimization: Sentiment analysis can inform the campaign to optimize messaging to potentially highlight popular policies or address misconceptions.

Sentiment analysis enables the political party to stay attuned to voter sentiments, allowing for strategic adjustments that may enhance their chances of success in the election process.

Information Extraction in Biomedical Literature and Financial Reports

Information Extraction from Biomedical Literature: Because of the volume of literature being published every day, NLP has now evolved to be an essential component to the field of biomedical research. Here is how it works:

Data Handling: There are millions of articles published everyday in the biomedical databases like PubMed. The design of NLP systems allows them to parse these articles and, ultimately, information and data from it.

Named Entity Recognition (NER): NLP models can recognize and classify text entities - genes, proteins, diseases, drug names. In a research article examining Alzheimer's, for example, NLP tools can identify mentions of proteins such as "amyloid-beta" and genes, such as "APOE."

Relation Extraction: Additionally, NLP systems can extract relationships between identified entities. In this component, the NLP system may extract "a particular drug reduced levels of a particular protein." This type of relationship extraction helps "provide" prospects about the mechanism of action regarding the drug.

Use Case in Drug Discovery: Consider scientists at a pharmaceutical company that needs to identify all articles reporting on the efficacy of a new compound by citing studies. The researchers could use automatic extraction and run NLP to identify all studies upon multiple efficacy outcomes and determine whether or not to pursue additional research and/or clinical trial of the drug in a very timely, efficient manner.
Imagine a pharmaceutical company that is looking to investigate treatment options for a rare genetic disorder. They would use NLP to review all literature on that disorder, pulling out genes, mutations, and compounds that have been previously tested, which would speed up their R and D process.

Information Extraction from Financial Reports: Financial reporting is another area where NLP tools improve data extraction and analysis.

Parsing Financial Documents: Organizations issue quarterly and annual reports that include massive amounts of data. NLP tools can be used to parse financial documents and extract selected financial metrics including revenue, profit, expense categories, as well as key metrics like ratios.

Sentiment and Tone Analysis: NLP can also be used to analyze the tone of the management discussion and analysis (MD and A) portion of financial documents as a means of deducing management's attitude and finding red flags.

Automating Compliance and Auditing: NLP systems can automate the process of identifying and classifying transactions or financial activities will help Auditors review transactions for compliance and adherence to financial guidelines.

Use case in Investment Assessments: Investment companies use NLP to track news from various sources and categorize relevant financial information about companies they are tracking. This allows investment professionals to make timely and informed decisions quickly about shifts in their strategy.

Applications of NLP (Figure 4)

For example, an investment analyst in a hedge fund might use NLP techniques to analyze earnings call transcripts of a company to assess the sentiment of the CEO and extract specific financial metrics that may signal upcoming trends to show future performance. By automating the extractions of relevant information from large data sets, NLP technologies provide considerable efficiency and accuracy gains in both biomedical and financial research. When applied to analysis, NLP technologies provide a competitive advantage and allow for much richer insights.

Summary of NLP Techniques, use Cases, and Tools (Table 1)

Natural Language Processing (NLP) uses many different methods to solve particular language problems. Rule-based systems run on established grammatical and syntactical rules. The approach is useful in tasks like grammar checking, language recognition, and pattern matching. Statistical approaches use very large linguistic corpora and help assign probabilities to word usage and structure, allowing for enhanced tasks like machine translation, auto-correct, and spell check. Machine learning employs labeled data to construct a predictable model for text classification, sentiment analysis, and named entity recognition which provides an even greater flexibility to unknown language patterns. The development of deep learning, which consists of RNNs, LSTMs, and transformers, to provide systems with contextual understanding and the ability to generate coherent text, also brings the wave of hybrid systems that bridge the interpretability of rule-based systems and the learning-based perspective of machine learning or deep learning, producing solid platforms for chatbots and virtual assistants. Transfer learning and reinforcement learning are taking NLP capabilities to new levels through pre-trained models and learning on the fly to build performance in new areas. An increasing number of tools and platforms, such as NLTK, spaCy, TensorFlow, PyTorch, BERT, and GPT, will provide all of the approaches and support the capability to implement them in the real world.

CONCLUSION

Natural language processing (NLP) is one of the most promising domains of artificial intelligence and is critical for helping to bridge the gap between understanding on the machine side and communication on the human side. In this review, we characterized the journey from the early days of natural language processing (NLP), from rule-based systems to the current epoch of machine learning systems (including deep learning and transformer-based systems like BERT and GPT) and found that, as a field, natural language processing (NLP) has moved through multiple stages, from lexical/morphological analysis to pragmatic understanding, as it has allowed for the development of systems that can, and are using, natural language in analysis, generation, and interaction. As a result of our literature review, we highlighted the diversity of fields in which NLP techniques have made it feasible, including healthcare (e.g., medical record analysis), finance (e.g., sentiment-based market prediction), education (e.g., automated tutoring systems), and everyday technology (e.g., virtual assistants, chatbots). Like all emerging fields, and with the significant advancement that natural language processing (NLP) has made, there remain a variety of challenges. Models continue to struggle with linguistic ambiguity, contextual understanding, and fairness, especially in low-resource and multilingual settings. Concerns about bias, data privacy, and the societal and environmental impact of large-scale models also require urgent attention. In the years ahead, NLP will emphasize creating more accurate, effective and also ethical, accessible, sustainable systems. Significant opportunities will come from developments in multimodal natural language processing, few-shot and zero-shot learning, and real-time, on-device language processing. Moreover, in-building user trust and ensuring the ethical use of language technologies meant a larger focus would have to be placed on explainable NLP models. By incentivizing the NLP community to fill in obvious areas of vacancy and utilize language as a force for good, this review hopes to make a valuable contribution toward subsequent research and innovation.

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Table 1: Summary of NLP Techniques, Use Cases, and Tools

Approach	Description	Common Use Cases	Popular Tools and Models
Rule-Based	Uses predefined linguistic rules	Grammar checking, rule-based NER	NLTK, spaCy (rule-based components)
Statistical	Employs probability models and corpora	POS tagging, translation, spelling correction	SRILM, GIZA++, NLTK
Machine Learning	Learns from labeled data to classify or extract	Sentiment analysis, spam detection	Scikit-learn, spaCy, CRF++
Deep Learning	Uses neural networks to	Text generation,	TensorFlow, PyTorch,

	model language	summarization, Q and A	BERT, GPT
Hybrid	Combines rule-based and ML/DL techniques	Chatbots, intelligent assistants	Rasa, spaCy, Snips
Transfer Learning	Fine-tunes large pre-trained models	Domain-specific NLP (e.g., legal, medical)	BERT, RoBERTa, T5, GPT-3
Reinforcement Learning	Learns via feedback and rewards	Adaptive chatbots, dialogue systems	GPT-4 + RLHF, Deep Q-Networks

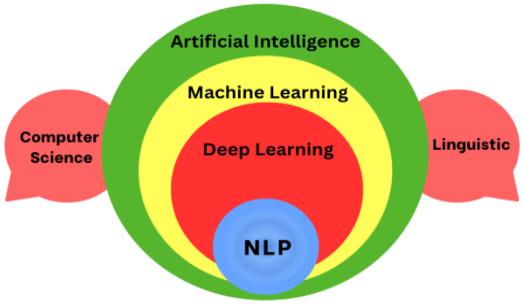


Figure 1: The Power of NLP within the AI Framework

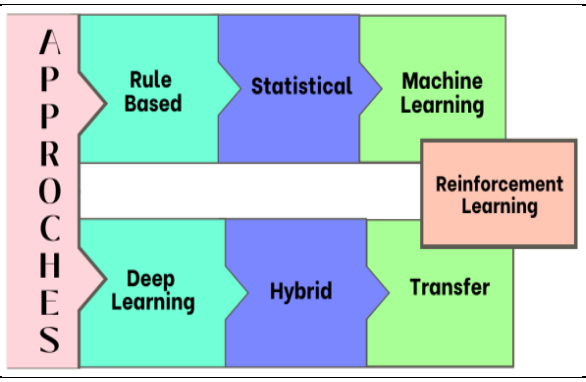


Figure 2: NLP implementation Approaches

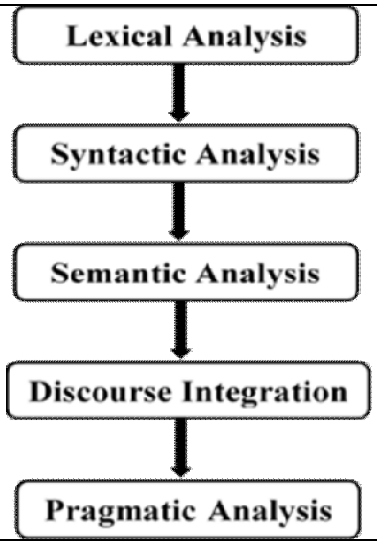


Figure 3: Phases of NLP

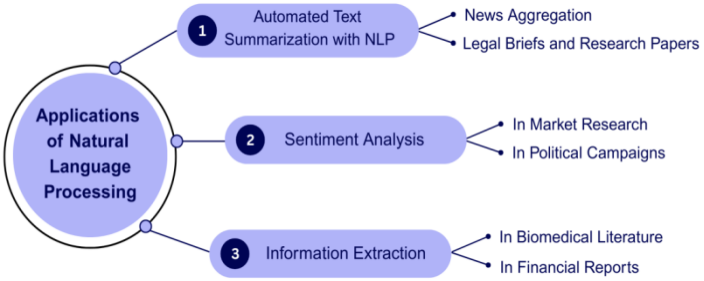


Figure 4: Applications of NLP

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RESEARCH ARTICLE

Fractional Lateral Transshipment Problem under Neutrosophic Fuzzy Environment

U. Johns Praveena[1] and J. Merline Vinotha[2]*

[1]Research Scholar (Reg. No.: BDU2110182779665), PG and Research Department of Mathematics, Holy Cross College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

[2]Assistant Professor, PG and Research Department of Mathematics, Holy Cross College, (Autonomous), (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
J. Merline Vinotha
Assistant Professor,
PG and Research Department of Mathematics,
Holy Cross College, (Autonomous),
(Affiliated to Bharathidasan University),
Tiruchirappalli, Tamil Nadu, India.
E.Mail: merlinevinotha@gmail.com

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ABSTRACT

The fractional lateral transshipment problem is crucial in logistics and supply chain management for minimizing total operational costs and enhancing service levels through an efficient distribution system. However, in real-world scenarios, the parameters in this model are often uncertain due to uncontrollable factors such as fuel prices, road conditions, weather, and traffic. This inherent uncertainty complicates the estimation of transportation costs and profits. To address these challenges, all parameters in the model are represented as trapezoidal neutrosophic fuzzy numbers. In this paper, the neutrosophic fuzzy fractional lateral transshipment problem is formulated to optimize an objective function based on the ratio of total cost to total income. The fuzzy model is then converted into a deterministic form using a ranking function. To handle the nonlinearity of the resulting model, the Charnes–Cooper variable transformation method is applied. Finally, a numerical example is provided to demonstrate the accuracy and effectiveness of the proposed approach.

Keywords: Fractional two stage transshipment problem, variable transformation method, trapezoidal neutrosophic fuzzy numbers, lateral transshipment.

INTRODUCTION

The transshipment problem is a linear programming problem in which goods are transported from sources to destinations through one are more intermediate points. Each of these points can act as either an origin or a destination. The concept of transshipment problem was first introduced by Orden (1956) [1]. The transshipment problem can also use to finding the shortest route between two network nodes. King and Logan developed a mathematical method for simultaneously determining the threads linking product processor market points, and Rhody proposed a methodology based on a reduced matrix [2,3]. A general linear model extension of the transshipment problem to a multi-plant, multi-product, and multiregional problem was proposed by Judges et al. [4]. Hurt and Tramel suggested the alternative formulation of transshipment problem based on transport model, which would provide solutions to King and Logan's common problems without the requirement to remove artificial variables [5]. Garg and Prakash investigated the time reducing transshipment problem [6]. After that, Herer and Tzur focused at the dynamic transshipment problem [7]. Transshipment problems with fractional objective functions are often used to evaluate performance in many real-world applications. When the objective function is expressed as a ratio of two objective functions in a transshipment problem, it is called a fractional transshipment problem.Jain. S proposed an algorithm to solve the fractional transshipment problem and determine the feasible solution where goods are transported directly from source to destination [8]. However, in the business world, goods are usually transported from production houses to retailers through intermediate points. This process involves two stages of transshipment and is referred to as a two-stage fractional transshipment problem. Garg, H., et.al developed a fractional two stage transshipment problem using extension principle approach [9]. On the other hand, shipments between entities at the same level, known as lateral transshipments, enhance the flexibility and robustness of a supply chain. Kumari, A. G. K. C., et.al [10] provided a comprehensive review of literature on various lateral transhipment inventory models. However, there is a need for extended studies focused on the fractional lateral transshipment problem. In many practical processes, decision experts (DEs) typically challenge to appropriately explain their concepts with precise numbers because of the uncertainty of the information and the lack of knowledge and experience of DEs. however, they can be represented as [0, 1] interval numbers.

Zadeh (1965) has introduced the concept of fuzzy sets [11]. As an extension of Zadeh's concept of a fuzzy set, Atanassov (1983) presented intuitionistic fuzzy sets [12]. In intuitionistic fuzzy sets, the degree of membership (belongingness) and the degree of non-membership (non-belongingness). Furthermore, the concept of IFSs has been extended to Pythagorean fuzzy sets (Yager, 2013[13], 2014 [14] where the sum of squares of membership and non-membership value of any object can be less than or equal to 1 which makes PFSs more flexible than IFSs. Later, to handle inconsistent and intermediate information Smarandache introduced a neutrosophic set in which truth, falsity, indeterminacy membership function belongs to [0,1] [15]. This study introduces a new version of the fractional lateral transshipment problem, where all parameters are represented as trapezoidal neutrosophic fuzzy numbers, and the objective function is a fractional term—the ratio of total shipment costs to benefits. This proposed model is called the neutrosophic fuzzy fractional lateral transshipment problem. In this model, the resulting solution will have a fuzzy cost. Compared to the standard fractional lateral transshipment problem, the fuzzy fractional version is more complex to solve. This increased complexity arises because fuzzy numbers must be compared to determine the minimal cost, and comparing fuzzy numbers is significantly more challenging than comparing exact values. A comprehensive review of generalized fractional mathematical models and their applications has been presented by Frenk and Schaible [16]. The proposed neutrosophic fuzzy lateral transshipment problem requires specific techniques for its solution. In this study, the concept ranking function isdiscussed to address the neutrosophic fuzziness of the problem. The well-known Charnes–Cooper variable transformation method is used to convert the ratios in the objective function into a linear form. The novelty of this proposed model is neutrosophic fuzzy fractional lateral transshipment problem is discussed to solve the complex problem which is not yet investigated in this literature. The rest of the paper is designed as follows. In section 2, the basic preliminaries in neutrosophic fuzzy numberare briefly summarized; Section 3 contains the mathematical model of the neutrosophic fuzzy fractional lateral transshipment

problem; In section 4, provides algorithm for proposed method; in section 5, the numerical example is solved to illustrate the algorithm; Section 6 contains the conclusion of the paper with the future studies.

PRELIMINARIES

The basic concepts of neutrosophic fuzzy set, trapezoidal neutrosophic numbers, ranking function for defuzzification are given in this section.

Definition 2.1[17] $\tilde{S}^F$ in a universal set E is called a single valued neutrosophic and it is defined by $\tilde{S}^N = \left\{\left\langle e, T_{\tilde{S}^N}(e), I_{\tilde{S}^N}(e), F_{\tilde{S}^N}(e)\right\rangle : e \in E\right\}$ where truth, Indeterminacy, and Falsity membership functions and satisfies the condition $0 \le T_{\tilde{S}^N}(e) + I_{\tilde{S}^N}(e) + F_{\tilde{S}^N}(e) \le 3 \forall e \in E$.

Definition 2.2[17]A single valued trapezoidal neutrosophic number $\tilde{S}$ (SVTrNN) on a real line set ℝ and is defined by $\tilde{S}^N = \left\{\left\langle s_1, s_2, s_3, s_4\right\rangle : w_{\tilde{S}^N}, u_{\tilde{S}}, v_{\tilde{S}}\right\} where\ w_{\tilde{S}}, u_{\tilde{S}}, v_{\tilde{S}} \in [0,1]$ with the condition $s_1 \le s_2 \le s_3 \le s_4$, whose truth, indeterminacy, falsity membership functions are follows

$$T_{\tilde{S}^F}(e) = \begin{cases} w_{\tilde{S}} \frac{e - s_1}{s_2 - s_1} & s_1 \le e \le s_2 \\ w_{\tilde{S}} & s_2 \le e \le s_3 \\ w_{\tilde{S}} \frac{s_4 - x}{s_4 - s_3} & s_3 \le e \le s_4 \\ 0 & \text{otherwise} \end{cases}$$

$$I_{\tilde{S}^F}(e) = \begin{cases} \frac{s_2 - e + u_{\tilde{S}}(e - s_1)}{s_2 - s_1} & s_1 \le e \le s_2 \\ u_{\tilde{S}} & s_2 \le e \le s_3 \\ \frac{s_3 - e + u_{\tilde{S}}(s_3 - e)}{s_4 - s_3} & s_3 \le e \le s_4 \\ 1 & \text{otherwise} \end{cases}$$

$$F_{\tilde{S}^F}(e) = \begin{cases} \frac{s_2 - e + v_{\tilde{S}}(e - s_1)}{s_2 - s_1} & s_1 \le e \le s_2 \\ v_{\tilde{S}} & s_2 \le e \le s_3 \\ \frac{e - s_3 + v_{\tilde{S}}(s_3 - e)}{s_4 - s_3} & s_3 \le e \le s_4 \\ 1 & \text{otherwise} \end{cases}$$

Definition 2.3[17]Let $\tilde{S} = \left\{\left\langle s_1, s_2, s_3, s_4\right\rangle : w_{\tilde{S}}, u_{\tilde{S}}, v_{\tilde{S}}\right\}$ be a SVTrNN, then the score function $S(\tilde{S}^N)$ is defined as

$$S(\tilde{S}) = \frac{1}{12}(s_1 + s_2 + s_3 + s_4)(2 + w_{\tilde{S}} - u_{\tilde{S}} - v_{\tilde{S}})$$

METHODOLOGY

Mathematical Model for Neutrosophic Fuzzy Fractional Lateral Transshipment Problem (NFFLTrP)

NFFLTrPis formulated to optimize the transportation of variable quantities of a single homogeneous product from sources to depots, allow lateral shipments among depots, and finally distribute to destinations, with the objective of minimizing the total fractional transshipment cost. Consider a fractional lateral transshipment problem with I

sources, K destinations and J depots. Let $\tilde{c}_{ij}^N, \tilde{e}_{ij}^N$ denote the transportation cost and income for unit product from plant to depot (stage 1), $\tilde{s}_{hj}^N, \tilde{h}_{hj}^N$ denotethe transportation cost and income for unit product from depot to depot (stage 2). $\tilde{d}_{jk}^N, \tilde{f}_{jk}^N$ denote the transportation cost and income for unit product from depot to customer (stage 3).Also, $\tilde{a}_i^N, \tilde{b}_j^N$ are the supply and demand in source and destination. $\tilde{g}_j^N$ be denoted as a capacity at depot. The total amount of product flowing into each depot must match the total amount flowing out. Products can be shipped from any plant to any depot and from any depot to any customer, but shipments cannot occur directly between other location pairs. x_{ij}, w_{hj}, y_{jk} are defined as the amount products transport from i^{th} source to j^{th} depot, j^{th} depot to h^{th} depot and h^{th} depot to k^{th} destination point. The mathematical model for neutrosophic fuzzy fractional lateral transshipment problem is defined as follows:

$$\min Z(x,y) = \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\tilde{c}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J}\tilde{s}_{hj}^N w_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K}\tilde{d}_{jk}^N y_{jk}}{\sum_{i=1}^{I}\sum_{j=1}^{J}\tilde{e}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J}\tilde{h}_{hj}^N w_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K}\tilde{s}_{jk}^N y_{jk}} \quad (1)$$

Subject to (2)

$$\sum_{j=1}^{J} x_{ij} \le \tilde{a}_i^N, i \in \{1,2,3,...,I\} \quad (3)$$

$$\sum_{j=1}^{J} y_{jk} \le \tilde{b}_k^N, k \in \{1,2,3,...,K\} \quad (4)$$

$$\sum_{i=1}^{I} x_{ij} + \sum_{h=1}^{J} w_{hj} \le \tilde{g}_j^N, j \in \{1,2,3,...,J\} \quad (5)$$

$$\sum_{i=1}^{I} x_{ij} + \sum_{h=1}^{J} w_{hj} = \sum_{h=1}^{J} w_{jh} + \sum_{k=1}^{I} y_{jk}, j \in \{1,2,3,...,J\} \quad (6)$$

$$x_{ij}, w_{hj}, y_{jk} \ge 0, i \in \{1,2,3,...,I\}, h \in \{1,2,3,...,J\}, j \in \{1,2,3,...,J\}, k \in \{1,2,3,...,K\} \quad (7)$$

In the objective function (1), the numerator and denominator represent the transportation cost, income from the plant to the depot, between depots, and from the depot to the customer. Equations (2) and (3) denote the supply and demand constraints, respectively. Equation (5) ensures the capacity restriction at each depot. Constraint (6) represents the flow conservation at each depot. Finally, equation (7) describes the non-negativity constraints.

Deterministic model for neutrosophic fuzzy fractional lateral transshipment problem

The mathematical formulation of the fractional lateral transshipment problem under a neutrosophic fuzzy environment is highly complex and difficult to solve directly. To address this, a ranking method is used to convert the proposed fuzzy model into a deterministic one. which is described as follows.

$$\min Z(x,w,y) = \frac{\sum_{i=1}^{I}\sum_{j=1}^{J}\tilde{c}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J}\tilde{s}_{hj}^N w_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K}\tilde{d}_{jk}^N y_{jk}}{\sum_{i=1}^{I}\sum_{j=1}^{J}\tilde{e}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J}\tilde{h}_{hj}^N w_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K}\tilde{s}_{jk}^N y_{jk}} \quad (8)$$

Subject to (9)

$$\sum_{j=1}^{J} x_{ij} \le \Re(\tilde{a}_i^N), i \in \{1,2,3,...,I\} \quad (10)$$

$$\sum_{j=1}^{J} y_{jk} \le \Re(\tilde{b}_k^N), k \in \{1,2,3,...,K\} \quad (11)$$

$$\sum_{i=1}^{I} x_{ij} + \sum_{h=1}^{J} w_{hj} \le \Re(\tilde{g}_j^N), j \in \{1,2,3,...,J\} \quad (12)$$

$$\sum_{i=1}^{I} x_{ij} + \sum_{h=1}^{J} w_{hj} = \sum_{h=1}^{J} w_{jh} + \sum_{k=1}^{I} y_{jk}, j \in \{1,2,3,...,J\} \quad (13)$$

$$x_{ij}, w_{hj}, y_{jk} \ge 0, i \in \{1,2,3,...,I\}, h \in \{1,2,3,...,J\}, j \in \{1,2,3,...,J\}, k \in \{1,2,3,...,K\} \quad (14)$$

SOLUTION PROCEDURE

In this section, Charnes Cooper variable transformation method has been discussed to solve the deterministic model for finding the optimal compromise solution using Lingo Software as follows

Charnes-Cooper variable transformation method

The proposed deterministic model involves the fractional objectives, the Charnes-Cooper transformation [19]is applied to convert into an equivalent linear programming model. inequality

$\sum_{i=1}^{I}\sum_{j=1}^{J} \tilde{e}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J} \tilde{h}_{hj}^N w_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K} \tilde{s}_{jk}^N y_{jk} \ge 0$ is true for every x_{ij}, w_{hj}, y_{jk}.

Assume that $s = \dfrac{1}{\left(\sum_{i=1}^{I}\sum_{j=1}^{J} \tilde{e}_{ij}^N x_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J} \tilde{h}_{hj}^N x_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K} \tilde{s}_{jk}^N x_{jk}\right)}$, $u_{ij} = sx_{ij} \ge 0$, $z_{hj} = sw_{hj} \ge 0$, $v_{jk} = sy_{jk} \ge 0$ which are used to convert the deterministic model into the following equivalent linear model.

$$\min Z(u,z,v) = \sum_{i=1}^{I}\sum_{j=1}^{J} \Re(\tilde{c}_{ij}^N) u_{ij} + \sum_{h=1}^{J}\sum_{j=1}^{J} \Re(\tilde{s}_{hj}^N) z_{hj} + \sum_{j=1}^{J}\sum_{k=1}^{K} \Re(\tilde{d}_{jk}^N) v_{jk} \quad (15)$$

Subject to (16)

$$\sum_{j=1}^{J} u_{ij} \le \Re(\tilde{a}_i^N) s, i \in \{1,2,3,...,I\} \quad (17)$$

$$\sum_{j=1}^{J} v_{jk} \le \Re(\tilde{b}_k^N) s, k \in \{1,2,3,...,K\} \quad (18)$$

$$\sum_{i=1}^{I} u_{ij} + \sum_{h=1}^{J} z_{hj} \le \Re(\tilde{g}_j^N) s, j \in \{1,2,3,...,J\} \quad (19)$$

$$\sum_{i=1}^{I} u_{ij} + \sum_{h=1}^{J} z_{hj} = \sum_{h=1}^{J} z_{jh} + \sum_{k=1}^{I} v_{jk}, j \in \{1,2,3,...,J\} \quad (20)$$

$$u_{ij}, z_{hj}, v_{jk} \ge 0, s > 0, i \in \{1,2,3,...,I\}, h \in \{1,2,3,...,J\}, j \in \{1,2,3,...,J\}, k \in \{1,2,3,...,K\} \quad (21)$$

RESULT AND DISCUSSION

Numerical Example

Consider an automobile manufacturing company produces automobiles at two production sites (P_1, P_2), transports them to three retail stores (R_1, R_2, R_3), and subsequently distributes them to six customers $(C_1, C_2, C_3, C_4, C_5, C_6)$ [9]. To enhance supply chain flexibility, responsiveness, lateral transshipment allowed between the retail stores. The company wants to maximize the profit by minimizing the shipping cost through the strategic use of lateral transshipments. Due to market unpredictability, transportation cost, income, supply, and demand are represented

using trapezoidal neutrosophic fuzzy numbers. Then neutrosophic fuzzy parameters are converted into crisp values using ranking function which is defined in definition 2.3. defuzzied neutrosophic transportation cost income, supply, and demand are presented in table [1,8].

Transportation cost from plant to depot (Stage 1)(Table 1)
Income from plant to depot (Stage 1) (Table 2).
Transportation cost from depot to depot (Stage 2) Table 3.
Income from depot to depot (Stage 2) Table 4.
Transportation cost from depot to customer (Stage 3) Table 5.
Income from depot to customer (Stage 3) Table 6.
Supply and Demand Table 7.
Capacity of Depot Table 8.

By using method prescribed in 4.1, the obtained optimum solutions of deterministic fractional lateral transshipment problem using Lingo Global Solver (20.0) are presented in [Table 9.]. Consequently, the comparison between fractional lateral transshipment problem and fractional direct shipment problem is detailed in [Table 10].

CONCLUSION

The supply chain management plays a significant role in the modern world by managing the complete flow of goods and services from raw materials to the final consumer. Investigating lateral fractional transshipment has become a crucial aspect of strengthening logistics operations, aiming to balance inventory, minimize stockouts, and enhance customer service. To address uncertainties in real-world scenarios, the proposed model is formulated within a neutrosophic fuzzy environment. It becomes the neutrosophic fuzzy fractional lateral transshipment problem, which introduces additional challenges due to fuzziness and the non-linearity of the fractional objective function, making it difficult to solve exactly. All parameters are converted into crisp using ranking function. After that, the deterministic model solved using Charner Cooper transformation method. The case study has been discussed for both fractional lateral/direct transshipment problem. The obtained solutions for fractional lateral transshipment show reduction in the objective function than the ordinary fractional transshipment problem. For future research, the proposed problem can be extended to solve multi objective fractional transshipment problem.

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Table 1: Transportation Cost From Plant to Depot (Stage 1)

	R_1	R_2	R_3
P_1	$\{(275,300,375,400) : 0.6,0.4,0.2\}$	$\{(165,220,280,340) : 0.7,0.5,0.2\}$	$\{(340,385,440,485) : 0.5,0.3,0.2\}$
P_2	$\{(325,360,410,450) : 0.6,0.4,0.2\}$	$\{(265,295,335,380) : 0.8,0.5,0.3\}$	$\{(246,280,326,360) : 0.7,0.5,0.2\}$

Table 2: Income From Plant to Depot (Stage 1)

z	R_1	R_2	R_3
P_1	$\{(635,735,830,935) : 0.6,0.4,0.2\}$	$\{(640,735,835,940) : 0.5,0.3,0.2\}$	$\{(550,650,700,800) : 0.5,0.3,0.2\}$
P_2	$\{(300,600,900,1200) : 0.7,0.4,0.3\}$	$\{(300,600,900,1200) : 0.5,0.3,0.2\}$	$\{(450,560,670,750) : 0.7,0.4,0.3\}$

Table 3: Transportation Cost From Depot To Depot (Stage 2)

	R_1	R_2	R_3
R_1	0	$\{(92,131,170,210) : 0.4,0.2,0.2\}$	$\{(100,200,400,500) : 0.7,0.4,0.3\}$
R_2	$\{(92,131,170,210) : 0.4,0.2,0.2\}$	0	$\{(150,200,250,300) : 0.5,0.3,0.2\}$

R_3	$\{(100,200,400,500):0.6,0.4,0.2\}$	$\{(150,200,250,300):0.5,0.3,0.2\}$	0

Table 4: Income From Depot to Depot (Stage 2)

	R_1	R_2	R_3
R_1	0	$\{(305,605,905,1200):0.6,0.4,0.2\}$	$\{(660,745,845,960):0.5,0.3,0.2\}$
R_2	$\{(620,725,835,940):0.5,0.3,0.2\}$	0	$\{(620,728,836,940):0.5,0.3,0.2\}$
R_3	$\{(660,745,845,960):0.5,0.3,0.2\}$	$\{(620,725,835,940):0.5,0.3,0.2\}$	0

Table 5: Transportation Cost From Depot to Customer (Stage 3)

	C_1	C_2	C_3
R_1	$\{(335,385,440,475):0.6,0.4,0.2\}$	$\{(330,380,440,470):0.5,0.3,0.2\}$	$\{(315,365,425,470):0.6,0.4,0.2\}$
R_2	$\{(310,360,420,470):0.7,0.4,0.3\}$	$\{(310,355,415,465):0.5,0.3,0.2\}$	$\{(310,360,420,470):0.7,0.4,0.3\}$
R_3	$\{(325,375,440,465):0.5,0.3,0.2\}$	$\{(330,380,440,470):0.7,0.4,0.3\}$	$\{(335,385,440,475):0.6,0.4,0.2\}$
	C_4	C_5	C_6
R_1	$\{(330,380,440,470):0.5,0.3,0.2\}$	$\{(330,380,440,470):0.5,0.3,0.2\}$	$\{(335,385,440,475):0.6,0.4,0.2\}$
R_2	$\{(315,365,425,470):0.7,0.4,0.3\}$	$\{(340,390,445,478):0.8,0.5,0.3\}$	$\{(320,370,430,470):0.5,0.3,0.2\}$
R_3	$\{(320,370,430,470):0.5,0.3,0.2\}$	$\{(330,380,440,470):0.5,0.3,0.2\}$	$\{(335,385,440,475):0.6,0.4,0.2\}$

Table 6: Income From Depot to Customer (Stage 3)

	C_1	C_2	C_3
R_1	$\{(635,735,830,935):0.4,0.3,0.1\}$	$\{(640,735,835,940):0.5,0.3,0.2\}$	$\{(660,745,845,960):0.6,0.4,0.2\}$
R_2	$\{(635,735,830,935):0.4,0.3,0.1\}$	$\{(635,735,830,935):0.4,0.3,0.1\}$	$\{(305,605,905,1200):0.6,0.4,0.2\}$
R_3	$\{(635,735,830,935):0.4,0.3,0.1\}$	$\{(630,730,830,930):0.5,0.4,0.1\}$	$\{(625,725,830,925):0.6,0.4,0.2\}$

	C_4	C_5	C_6
R_1	$\{(615,715,820,925):0.7,0.5,0.2\}$	$\{(660,755,855,970):0.5,0.3,0.2\}$	$\{(625,725,830,930):0.5,0.3,0.2\}$
R_2	$\{(645,745,830,945):0.5,0.3,0.2\}$	$\{(625,725,830,930):0.5,0.3,0.2\}$	$\{(625,725,830,930):0.5,0.3,0.2\}$
R_3	$\{(665,750,850,960):0.7,0.3,0.4\}$	$\{(640,735,835,940):0.6,0.4,0.2\}$	$\{(635,735,830,935):0.6,0.4,0.2\}$

Table 7: Supply and Demand

$\tilde{a}_1^N=\{(950,1200,1400,1625):0.5,0.3,0.2\}$	$\tilde{a}_2^N=\{(1300,1500,1800,2150):0.6,0.4,0.2\}$
$\tilde{b}_1^N=\{(150,235,300,350):0.7,0.4,0.3\}$	$\tilde{b}_2^N=\{(350,500,600,700):0.5,0.3,0.2\}$
$\tilde{b}_3^N=\{(175,250,330,400):0.7,0.5,0.2\}$	$\tilde{b}_4^N=\{(175,250,350,470):0.6,0.4,0.2\}$
$\tilde{b}_5^N=\{(275,300,375,400):0.8,0.5,0.3\}$	$\tilde{b}_6^N=\{(490,600,710,750):0.5,0.3,0.2\}$

Table 8: Capacity of Depot

$\tilde{g}_1^N=\{(710,910,1050,1350):0.5,0.3,0.2\}$	$\tilde{g}_2^N=\{(710,910,1050,1350):0.5,0.3,0.2\}$	$\tilde{g}_3^N=\{(710,910,1050,1350):0.5,0.3,0.2\}$

Table 9: Fractional Lateral Transhipment Problem

Model	Optimum Solution
Neutrosophic fractional lateral transshipment Problem	$x_{11}=670, x_{12}=191, x_{22}=285.1, x_{23}=670, w_{12}=193.5, y_{12}=59.5,$ $y_{13}=192.52, y_{15}=224.98, y_{34}=290, Z(x,w,y)=0.467$

Table 10: Comparison Between Fractional Lateral Transshipment Problem and Fractional Direct Shipment Problem

Model	Optimum Solution
Neutrosophic fractional lateral transshipment Problem	$x_{11}=231.5, x_{12}=631.01, x_{22}=39.5, x_{23}=670.5, w_{21}=438.9,$ $y_{12}=118.5, y_{13}=192.48, y_{15}=225, y_{31}=172, y_{34}=207.5, y_{36}=290.5$ $Z(x,w,y)=0.4395$
Neutrosophic fractional direct transshipment Problem	$x_{12}=862.5, x_{22}=709.99, y_{21}=172.5, y_{22}=350, y_{23}=192.5,$ $y_{24}=207.5, y_{25}=225, y_{26}=425\ Z(x,w,y)=0.8649$

REVIEW ARTICLE

Emerging Role of Artificial Intelligence in Forensic Odontology - A Review

Shubhangi Mani[1], Sweta Prakash Shele[2]*, Deepak Singh[3] and Manas Bajpai[4]

[1]Professor, Department of Orthodontics and Dentofacial Orthopaedics, Rural Dental College and Hospital, Pravara Institute of Medical Sciences, (Deemed to be University), Loni, Maharashtra, India.
[2]Senior Lecturer, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Rural Dental College and Hospital, Pravara Institute of Medical Sciences, (Deemed to be University), Loni, Maharashtra, India.
[3]Senior Lecturer, Department of Oral and Maxillofacial Surgery, Rural Dental College and Hospital, Pravara Institute of Medical Sciences, (Deemed to be University), Loni, Maharashtra, India.
[4]Professor and Head of Department, Department of Oral and Maxillofacial Pathology and Oral Microbiology, Rural Dental College and Hospital, Pravara Institute of Medical Sciences, (Deemed to be University), Loni, Maharashtra, India.

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***Address for Correspondence**
Sweta Prakash Shele
Senior Lecturer,
Department of Oral and Maxillofacial Pathology and Oral Microbiology,
Rural Dental College and Hospital,
Pravara Institute of Medical Sciences,
(Deemed to be University),
Loni, Maharashtra, India.
E.Mail: swetadentist810@gmail.com

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ABSTRACT

Forensic odontology, the application of dental science to legal investigations, relies heavily on the comparison of dental records and remains recovered from crime scenes, mass disasters, and identification efforts. Traditionally, this process is time-consuming, subjective, and requires specialized expertise. Artificial intelligence (AI), especially machine learning, offers a promising avenue to enhance the accuracy, speed, and efficiency of various aspects of forensic odontology. This paper explores the current and potential roles of AI in this field, discussing its advantages, limitations, and ethical considerations.

Keywords: Artificial intelligence, Forensic odontology, Human Identification, Forensic Sciences

INTRODUCTION

Forensic odontology plays a critical role in identifying unknown individuals, particularly in situations where other methods like fingerprint analysis or DNA profiling are compromised[1]. The unique characteristics of dentition, including tooth morphology, restorations, and anomalies, serve as a valuable identifier[2]. Experienced forensic odontologists meticulously compare pre-mortem dental records with post-mortem findings to establish a match[2,3]. However, the inherent subjectivity in visual comparison, the scarcity of expert odontologists, and the need for rapid identification in mass disaster scenarios underscore the need for innovative approaches. Artificial intelligence (AI), with its ability to automate complex tasks, analyze large datasets, and identify patterns, is poised to revolutionize forensic odontology[4,5,6].

Current and Potential Applications of AI in Forensic Odontology

AI's potential in forensic odontology spans several key areas

Dental Image Analysis and Automation

Radiograph analysis: AI algorithms can be trained to automatically detect and measure anatomical landmarks on dental radiographs (e.g., orthopantomograms, periapical radiographs) [4]. This minimizes inter-observer variability and facilitates the accurate comparison of pre- and post-mortem radiographs[].7 Machine learning models can be used to identify specific dental features, such as root canal fillings, crowns, or other restorations, significantly reducing the time required for manual examination[5,7].

3D reconstruction: AI can assist in creating three-dimensional models of skulls and dentition from CT scans, enabling more accurate and detailed comparisons. 8 These models can be rotated and manipulated, offering a more comprehensive view than traditional two-dimensional radiographs[5,6,7,8].

Image enhancement: AI can be used to enhance poor-quality images, improving visibility and facilitating analysis of subtle features. This is particularly useful in cases where post-mortem changes or trauma have compromised the integrity of the dental remains[8].

Automated Dental Charting and Record Integration

Automated chart generation: AI can analyze dental radiographs and clinical photographs to automatically generate dental charts, which document the presence or absence of teeth, restorations, and other anatomical features. This streamlines the process of creating a comprehensive dental profile[9].

Information retrieval: AI-powered systems can efficiently search and retrieve relevant dental records from large databases, accelerating the identification process. Natural Language Processing (NLP) can be used to extract key information from unstructured text within dental records[7,9,10].

Data integration: AI can integrate data from various sources, including dental records, photographs, CT scans, and DNA profiles, providing a holistic view of the individual[10].

Bite Mark Analysis

Bite mark feature extraction: AI can be trained to automatically extract relevant features from bite mark images, such as the shape, size, and arrangement of teeth. This reduces subjectivity in bite mark analysis and facilitates objective comparison with suspect dentition[10,11].

Bite mark matching: AI algorithms can compare bite mark patterns with dental casts or impressions to assess the probability of a match. This can assist in narrowing down potential suspects and providing valuable evidence in criminal investigations[11].

Distortion correction: AI can correct for distortions in bite mark images caused by skin elasticity and other factors, improving the accuracy of comparisons[11].
A schematic picture shows the algorithm of AI to analyse bite marks (Figure 1).

Age Estimation
Dental age estimation: AI can analyze dental development patterns on radiographs to estimate the age of an individual, particularly useful in cases involving unidentified children or adolescents. Machine learning models can be trained on large datasets of dental radiographs with known ages to predict age with greater accuracy[12].

Mass Disaster Management
Rapid identification: In mass disaster scenarios, AI can accelerate the process of identifying victims by automatically comparing dental records with post-mortem findings[12,13,14]. AI-powered systems can prioritize cases based on the likelihood of a match, enabling authorities to quickly identify victims and notify their families[13].

Data management: AI can streamline the management of large volumes of data generated during mass disaster events, including dental records, photographs, and DNA profiles[13,15].

Advantages of AI in Forensic Odontology
AI may cause reduction of subjective bias and human error through automated analysis. AI enables faster processing of dental records and images, leading to quicker identification. Standardized analysis protocols ensure consistent results across different examiners. Automation can reduce the need for highly specialized personnel and expensive equipment[9,11,15,16,17].AI-driven insights can provide objective support for expert opinions[16]. Advantages of AI over conventional methods in Forensic identification (Figure 2)

Limitations and Challenges
Data Availability and Quality: AI models require large, high-quality datasets for training. The availability of standardized dental records and radiographs can be a limiting factor.
Algorithm Bias: AI models can be biased if trained on skewed or unrepresentative data. This can lead to inaccurate or discriminatory results.
Explainability and Transparency: Black-box AI models can be difficult to interpret, raising concerns about transparency and accountability.
Ethical Considerations: The use of AI in forensic odontology raises ethical questions regarding privacy, data security, and the potential for misuse.
Regulatory Framework: Clear regulatory frameworks are needed to ensure the responsible and ethical use of AI in forensic odontology.
Acceptance by the Legal System: The admissibility of AI-generated evidence in court requires careful consideration and validation[10,18].

Ethical Considerations
The implementation of AI in forensic odontology must be guided by ethical principles to ensure fairness, transparency, and accountability. Key ethical considerations include
Data Privacy: Protecting the privacy of dental records and other sensitive information.
Algorithmic Bias: Mitigating the risk of bias in AI algorithms.
Transparency and Explainability: Ensuring that AI-driven decisions are transparent and explainable.
Human Oversight: Maintaining human oversight and judgment in the interpretation of AI results.
Accountability: Establishing clear lines of accountability for the use of AI in forensic odontology[19].

CONCLUSION

Artificial intelligence holds immense potential to transform forensic odontology, enhancing the accuracy, speed, and efficiency of identification processes. While several limitations and challenges remain, ongoing research and development are addressing these issues. As AI technology continues to evolve, forensic odontologists will need to adapt and integrate these tools into their practice. Ultimately, the successful implementation of AI in forensic odontology will depend on careful consideration of ethical implications, rigorous validation of AI algorithms, and close collaboration between AI researchers and forensic experts. By embracing AI responsibly, forensic odontology can further enhance its role in identifying individuals, solving crimes, and providing closure to grieving families. Future research should focus on developing robust, explainable, and unbiased AI models that can assist forensic odontologists in making accurate and reliable identifications.

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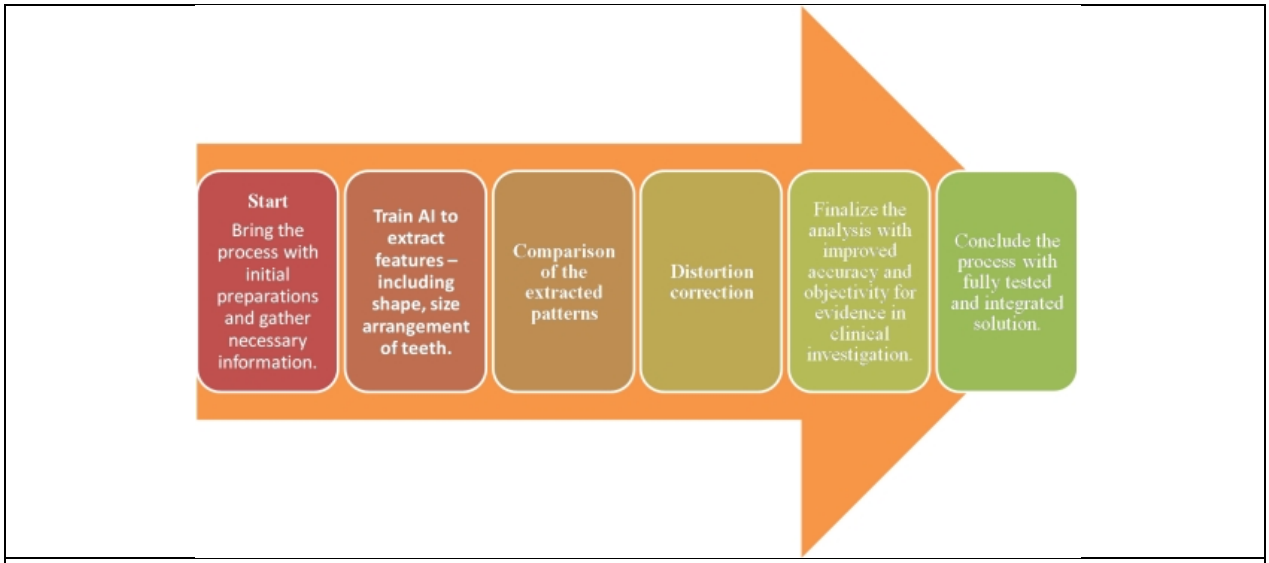


Figure 1: A schematic picture shows the algorithm of AI to analyse bite marks.

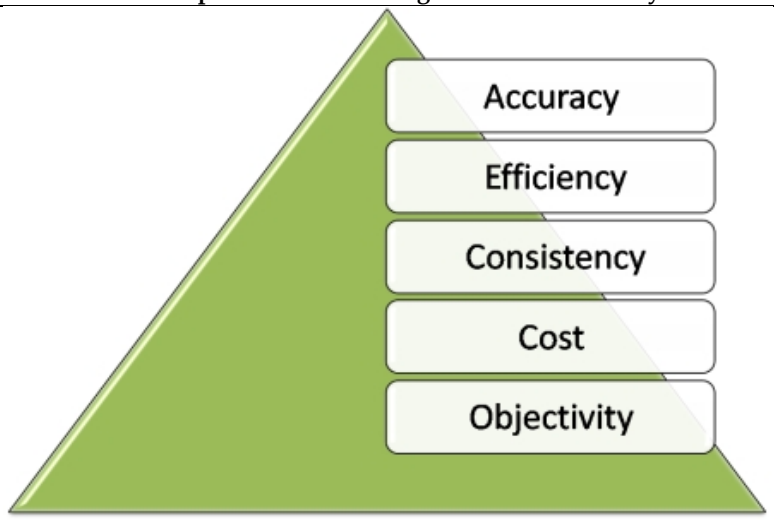


Figure 2: Advantages of AI over conventional methods in Forensic identification

RESEARCH ARTICLE

A Study to Compare the Effect of Breath Stacking Technique versus Diaphragmatic Breathing on Pulmonary Functions among Obese Individuals – An Interventional Study

Haseena Makrani[1]*, Pragna Gondaliya[2], Zishan Khan Belim[3] and Apeksha Barevadiya[4]

[1]Assistant Professor, Shama Institute of Medical Sciences, Danilimda, (Affiliated to Gujarat University), Ahmedabad, Gujarat, India.
[2]Associate Professor, Shree K.K.Sheth Physiotherapy College, (Affiliated to Saurashtra University), Rajkot, Gujarat, India.
[3]Assistant Professor, SKUM College of Physiotherapy, (Affiliated to Gujarat University), Ahmedabad, Gujarat, India.

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***Address for Correspondence**
Haseena Makrani
Assistant Professor,
Shama Institute of Medical Sciences, Danilimda,
(Affiliated to Gujarat University),
Ahmedabad, Gujarat, India.
E.Mail: haseenamakrani8697@gmail.com

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ABSTRACT

In many places of the world, obesity becomes a significant public health concern. An obese person's respiratory mechanics are altered, their lung and chest wall compliance are diminished, their respiratory muscle strength and endurance are decreased, their pulmonary gas exchange is reduced, and their ability to exercise is limited. One breath is stacked on top of another in the breath stacking technique, which is then followed by a brief hold and expiration. Breathing that is slow and rhythmic is known as diaphragmatic breathing. This study sought to examine the effects of diaphragmatic breathing and the breath stacking technique on FVC, FEV1, and FEV1/FVC in obese persons.

Keywords: Obesity, Breath stacking technique, WHR, FVC, FEV1.

INTRODUCTION

In India, obesity has emerged as a serious health concern. Obesity is described by WHO as "a disorder with excessive fat." buildup in the body to the point where it negatively impacts health and wellness[1]. Waist-hip ratio (WHR) and waist circumference (WC) are employed as indicators of abdominal obesity, whereas weight and body mass index (BMI) is utilized to gauge overall adiposity. The waist-to-hip ratio, which measures how much body fat is distributed

from the abdomen to the lower body, is calculated by dividing the waist circumference by the hip circumference at its widest point[2]. The WHO defines central obesity as having a waist-to-hip ratio (WHR) of≥0.90for males and ≥0.85 for women[3]. It is thought that abdominal obesity affects the mechanical properties of pulmonary function by altering lung compliance, a labour of breathing, and elastic recoil. The relationship between the diaphragm, chest wall, and lungs is changed as fat builds up. As a result, acquiring too much weight may have an impact on one's ability to exercise as well as their pulmonary function, small airway function, expiratory flow limitation, changes to their breathing patterns, reduced respiratory muscle strength and endurance, and pulmonary gas exchange[4]. A screening procedure for overall respiratory health is spirometry. The forced vital capacity (FVC) and forced expiratory volume are the primary outcomes of spirometry (FEV). The forced expiratory volume (FEV) is the forced volume of inspiration forced to expire in t seconds. To categorize the severity of obstructive lung disorders, forced expiratory volume in the first second (FEV_1) is used[5]. The amount of gas expelled in the residual volume at maximal inspiration is known as the vital capacity (VC). The FVC is similar, but the patient is exhaling as quickly and forcefully as possible. An FEV_1/FVC ratio of more than 0.70 with both FEV_1 and FVC above 80% of the expected value is considered normal spirometry data. Spirometric abnormalities in patients with mild to moderate obesity represent systemic restraint defects, whereas severe & morbid obesity represents true airflow obstruction. The mechanism may be related to small airway disintegration brought on by decreasing lung volumes with increasing weight problems, or it may be unrelated[6]. A quick approach that addresses both trapped secretions and oxygen intake is breath stacking. Marini and colleagues first described it in 1986, and it involves encouraging someone to take deep, spaced-out breaths while stacking each one on top of the previous one. Encouraging the patient to breathe in slowly and stacking breaths as tolerated, this approach enables the lungs to take in more air than usual inspiration. It is then followed by a brief hold before gradual expiration.[7] Yet, no research has been identified that compares diaphragmatic breathing and the breath stacking technique on pulmonary functions in obese people.So, the goal of this study is to ascertain the impact of the breath stacking technique and diaphragmatic breathing in obese adults and then to compare the impact of each.

OBJECTIVES OF THE STUDY

- To find out the effect of the Breath stacking technique on FVC, FEV_1, and FEV_1/FVC ratio in obese individuals.
- To find out the effect of Diaphragmatic breathing on FVC, FEV_1, and FEV_1/FVC ratio in obese individuals.
- To compare the effect of Breath stacking and Diaphragmatic breathing on FVC, FEV_1, and FEV_1/FVC ratio in obese individuals.

Hypothesis

Experimental hypothesis

There is a significant difference between the effect of the Breath stacking technique and Diaphragmatic breathing on pulmonary functions in obese individuals.

Null hypothesis

There is no significant difference between the effect of the Breath stacking technique and diaphragmatic breathing on pulmonary functions in obese individuals.

MATERIALS AND METHODS

Study design: An interventional study.
Study setting: Shri K. K. Sheth Physiotherapy College, Rajkot
Source of data: In and around Rajkot city
Study population: Obese individuals.
Sampling technique: Purposive sampling for selection and group allotment of a subject by simple random sampling.
Study duration: 6 days /week for 2 weeks.
Sample Size: 46 obese individuals were selected for the study.

$n = 2\frac{S^2(Z1+Z2)^2}{(M1-M2)^2}$

Where, M1= Standard deviation of Intervention1, M2= Standard deviation of intervention 2
Z1= Z value associated with alpha, Z2 = Z value associated with beta
46 obese people between the ages of 35 and 55 participated in the study after the ethical committee gave its clearance. Those who were willing to participate in the study were asked to sign a written consent form after being fully informed of its goals and methods. (ANNEXURE 10.1). Purposive sampling was used to pick the subjects. The subjects were divided into groups using just random sampling. Name, Age, Sex, Height, Weight, BMI, WHR, Personal History, History of Respiratory Illness, and PFT measurements were all noted for each individual. (ANNEXURE-10.2).

Inclusion Criteria
- Age group: 35-55 years
- Gender: both male and female
- Subjects with BMI: ≥ 25kg/m²
- Subjects with waist-hip ratio ≥ 0.80 women, men ≥ 0.95.

METHODOLOGY

46 obese individuals were selected for the study fulfilling the inclusion and exclusion criteria. Subjects were assigned into two groups by simple random sampling.

Group A: 23 subjects had received the breath stacking technique.
- Sit comfortably and loosen restricting clothing or belts.
- Take in a full breath through the nose, allowing the stomach and lower ribcage to expand outward.
- Trap this breath by closing the vocal cords.
- Take another short breath and trap it.
- Take a third breath until your lungs are full.
- Hold the breath for 2 to 3 seconds.
- Let all the air out of the lungs through the mouth making a shushing sound.
- Take rest for a minute before repeating.
- Repeat for 3-5 cycles of BS. (For 10-15 minutes 6 days/week for 2 week.

Group B: 23 subjects had received diaphragmatic breathing.
- Subjects were asked to sit in a semi-fowler position.
- They were told to place their hand below the anterior costal margin and feel the movement occurring.
- Then the subjects were told to breathe in slowly and deeply through the nose allowing the abdomen to rise slightly and then relax and exhale slowly through the mouth.
- This breathing technique was performed for 10-15 minutes such that the patient takes 6 to 8 deep breaths per minute.
- This breathing technique was given for5 days/week for 2 weeks.
- Pre and post-pulmonary function test was taken as per the standard outlined by the American Thoracic Society.
- For measurement of FEV1, FVC, and FEV1/FVC ratio, patients were asked to take the deepest breath as much as possible then place the mouthpiece in their mouth with lips sealing it, and immediately exhale hard and fast for as long as possible, preferably at least 6 seconds followed by a rapid inspiration from the mouthpiece.
- Three trials were given for each procedure and the best trial was selected. The trial was considered “unacceptable” If it showed evidence of cough, early termination of expiration, or inconsistent effort.
- After the collection of data, pre, and post-values for FEV1, FVC, and FEV1/FVC were analyzed by statistical software and results were found.

RESULTSAND DISCUSSION

- All statistical analysis was done using SPSS 26.0 software. Before applying the statistical test, data were screened for normal distribution using the Shapiro-Wilk test.
- All the outcome measure was analyzed at baseline and 2 weeks of intervention using an appropriate statistical test. The level of significance was kept at 95% changes in the outcome measure were analyzed within-group as well as between-group.

Interpretation

- In group-A, a significant difference was found in pre & post-FVC (l) ($Z = 4.198$, $P < 0.05$), FEV_1 (l/s), ($Z = 4.198$, $P < 0.05$), and FEV_1/FVC ($Z = 2.805$, $P < 0.05$).Which is shown in Graph 1 and table 1.
- In group B significant difference was found in pre & post-FVC (l) ($Z = 4.199$, $P < 0.05$), FEV_1 (l/s), ($Z = 4.198$, $P < 0.05$), and FEV_1/FVC ($Z = 3.182$, $P < 0.05$).Which is shown in Graph 2 and table 2.

Inter Group comparison of Group A and Group B suggested that there was a significant difference for FVC (l) ($U = -3.374$, $P < 0.05$) and FEV_1(l/s), ($U = -3.462$, $P < 0.05$) and not a significant difference for FEV_1/FVC ($U = 0.438$, $P > 0.05$) between the two Groups. Which is shown in Graph 3 and table 3.
Hence, the result of this study supports the experimental hypothesis.

DISCUSSION

The study intended to find out and compare the effect of the Breath stacking technique and diaphragmatic breathing on pulmonary function tests in obese individuals. The result of this study supports the experimental hypothesis. Thereasonforthereductioninpulmonaryfunctioninobeseindividualsisthetrunkfat,whichmaycompressthethoraciccavity andrestrictthediaphragmaticmovement,resultinginreducedverticaldiameterofthethoraciccavity.Itreducesthecomplian ceofthelungsand the thoracic cavity resulting in increased load on the respiratory muscles. This may end up with are duction in lung volumes and flowrates. [8]. During normal breathing after a particular degree of stretching, pulmonary stretch receptors in alveoli are stimulated and send an impulse to respiratory centers for inhibition of inhalation and to begin with exhalation. Yet, when breathing diaphragmatically, the lung expands significantly and the alveolar walls are stretched to their greatest degree. The stretch receptors are thus trained to with stand more and more stretching, so that the duration at which inhibitory neural impulse generated by stimulation of such pulmonary stretch receptors increases, thus it improves ventilation.[9]. Breath stacking allows larger mobilization of inspired air. The completion of slow, deep inspirations, followed by a post-inspiratory pause, with a pause of at least five seconds, allows the air to disperse itself uniformly. The breath-stacking increases the amplitude and duration of thoracic expansion. During airway occlusion, the central drive increases gradually and Maximum inspirations cause increases of the trans-pulmonary pressure and the post-inspiratory pause, with the maintenance of this raised pressure, contributes to the increases of the PaO2, presumably through the recruitment of the collapsed alveoli.[10]

CONCLUSION

Breath stacking technique and diaphragmatic breathing both were effective in improving pulmonary functions in obese individuals after performing breathing techniques for 6 days/week for 2 weeks and there was a significant difference between both groups and breath stacking technique was more effective than diaphragmatic breathing in improving pulmonary function in obese individuals.

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Table 1:Intergroupcomparisonoffvcin Group A & B

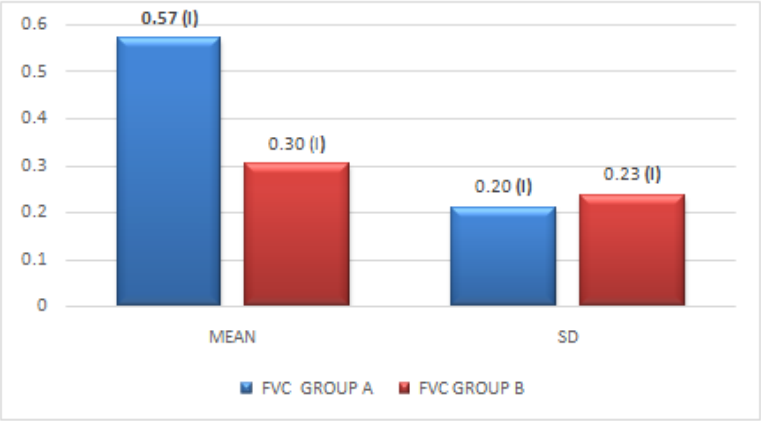
PARAMETERS	MEAN ± SD		U	P	RESULT
FVC GROUP A	0.57	0.20	-3.374	< 0.05	SIGNIFICANT
FVC GROUP B	0.30	0.23			

Table 2: Intergroup Comparison of FEV_1 IN Group A & B.

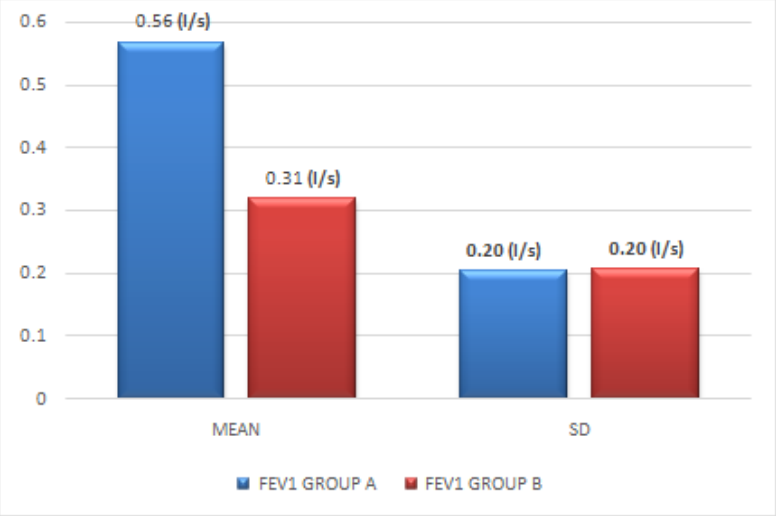
PARAMETERS	MEAN ± SD		U	P	RESULT
FEV_1IN GROUP A	0.56	0.20	-3.462	<0.05	SIGNIFICANT
FEV_1INGROUP B	0.31	0.20			

Table 3: Intergroup Comparison of Fev_1/Fvc Group A & B.

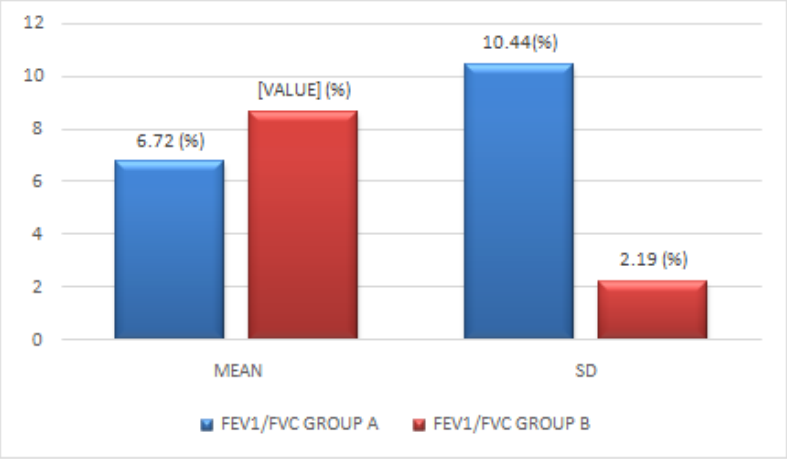
PARAMETERS	MEAN +- SD		T	P	RESULT
FEV_1/FVC GROUP A	6.72	10.44	0.438	>0.05	NOT SIGNIFICANT
FEV_1/FVC GROUP B	8.65	2.19			



Graph. 1: Intergroup Comparison of FVC in Group A & B



Graph. 2: Intergroup Comparison of FVC in Group A & B



Graph. 3: Intergroup Comparison of FEV_1/ FVC Group A & B.

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RESEARCH ARTICLE

Green Chemistry based Colorimetric Methods and UV Spectrophotometric Method for the Assessment Firocoxib in Formulation

K.Swaroopa Rani[1]*, T. Sankruthya[2] and M. Thanmai[2]

[1]Teaching Faculty, Department of Pharmacy, SKU College of Pharmaceutical Sciences (Affiliated to Sri Krishnadevaraya University), Ananthapuramu, Andhra Pradesh, India.
[2]Student, Department of Pharmacy, SKU College of Pharmaceutical Sciences (Affiliated to Sri Krishnadevaraya University), Ananthapuramu, Andhra Pradesh, India.

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***Address for Correspondence**
K.Swaroopa Rani
Teaching Faculty,
Department of Pharmacy,
SKU College of Pharmaceutical Sciences
(Affiliated to Sri Krishnadevaraya University),
Ananthapuramu, Andhra Pradesh, India.
E.Mail: swarooppariper@gmail.com

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ABSTRACT

This design includes effective and accessible UV Spectrophotometric and colorimetric ways for the Estimation of Firocoxib in bulk and retailed expression, which is simple, precise, accurate and provident. originally, standard and sample stock results were prepared by using DMF. The absorbance of firocoxib medicine result is measured at 410 nm, 415 nm in system A and system B independently, in UV range absorbance maxes is measured at 290nm. The working standard results were prepared with reagents Fecl3 and k2Cr2O7. This system validated for Linearity, Accuracy, Precision, Ruggedness, Robustness, LOD, LOQ and Assay as per ICH guidelines.

Keywords: Firocoxib, UV Spectroscopy[4], Colorimetry, Method development[5], System confirmation[6,7], Ferric chloride and Potassium dichromate.

INTRODUCTION

The medicine Firocoxib is a nonsteroidal anti-inflammatory medicine (NSAIDS). It's used to treat pain and inflammation in Dogs and nags. Acts by inhibiting cyclo oxygenase-2. The UV Spectrophotometric and Colorimetric styles for estimation of Firocoxib weren't developed ahead and this study develop easy and provident styles.

Structure(Fig.10)

MATERIALS AND METHODS

Materials
Chemicals (Table: 28)

Instruments
- Weighing balance
- UV Spectrophotometer
- Colorimeter
- Micropipette
- Volumetric flask
- Measuring cylinder
- Beaker

Method development
Colorimetry
Selection of solvent
Solubility studies were conducted to the firocoxib with many solvents. It was set up that firocoxib was freely answerable in Dimethyl Formamide.

Selection of reagent
Reagent was named grounded on the chemical structure of Firocoxib. Linearity of medicine was set up with ferric chloride, Potassium permanganate in system A and system B independently.

Preparation of standard stock solution
Standard firocoxib of 10 mg was counted and transfer into a 10 ml volumetric beaker and dissolved in DMF. The beaker was shaken and volume was made up to the mark with DMF to give a result 1000µg/ml (stock result).

Method A
Determination of maximum absorbance wavelength of 10µg/ml solution
Stock result of firocoxib was further mixed with DMF to get attention of 10µg/ml and 1ml of reagent was added. Absorbance was checked at colourful wavelengths and it was set up that 410nm is the maximum absorbance wavelength[10].

Selection of attention of reagent
Colourful trails were made with 1,2, 3,4, 5, 6, and 7(mg/ ml) reagent results. More linearity was attained with 3(mg/ ml) reagent result in system A.

Effect of volume of reagent on the linearity of coloured complex
Colourful working standard results are prepared using different volumes of 3(mg/ml) reagent and absorbance is measured. Finest result was attained with volume 1 ml reagent result [11,12].

Effect of time on the linearity of the colour complex
Absorbance of the coloured complex result is observed in a series with time intervals of 15 minutes and the linearity is observed from starting up to 90 minutes.

Selection of logical concentration range

From standard stock result of Firocoxib medicine, applicable aliquots 10 to 130µg/ml independently were pipetted out into a 10 ml volumetric beaker. To each sample add 1 ml of 3(mg/ml) reagent result and made up to the mark with DMF to attained working standard results of attention from 20- 130µg/ ml. Absorbance of these samples were measured at 410nm.

Construction of calibration curve

From the attained absorbance values estimation wind was constructed. Regression equation and correlation measure (R^2) are determined.

METHOD-B

Determination of maximum absorbance wavelength of 10mg/ml result

Stock result of Firocoxib was further adulterated with DMF to get attention of 10µg/ml and 2 ml of reagent was added. Absorbance was checked out at wavelength of 415nm is the maximum absorbance wavelength in system B (Potassium dichromate).

Selection of concentration of reagent

Colourful trails were made with 1, 2, 3,4(mg/ml) reagent. More linearity was attained with 2(mg/ ml) reagent result in this system.

Effect of volume of reagent on linearity of colour complex

Linearity of coloured complex was measured with different volumes of reagent. It was set up that 2 ml of reagent gives linearity.

Effect of time on the linearity of colour complex

The colour of medicine result with reagent replied after 10 min stability of complex was set up to 125 min.

Selection of logical concentration range

From standard stock result of firocoxib medicine, applicable aliquots µg/ml independently were pipetted out into a 10 ml volumetric beaker and 1 ml of reagent was added and made up to the mark with DMF to attained working standard results of attention from 10- 100µg/ ml. Absorbance for these results were measured at 415nm.

Construction of calibration curve

From standard firocoxib stock result attention of 10- 100µg/ml in system B were prepared. Absorbance value of a result in system B were measured at 415nm. From the absorbance value, estimation wind was constructed. Retrogression equation and correlation measure(R^2) are determined[13].

Method development of UV spectrophotometry

Method development involves the following types

Determination of maximum absorbance wavelength of 10µg/ml result:

Stock result of firocoxib was further adulterated with DMF to get attention of 10- 50µg/ ml. Absorbance was checked out at colourful wavelengths and it was set up that 291nm is the maximum absorbance wavelength[14].

Selection of concentration range

From standard stock result of firocoxib, applicable aliquots, 100µg/ml independently were pipetted out in a 10 ml volumetric beaker and was made up to with DMF attained working standard results of attention from 10- 100µg/ ml. Absorbance for these results were measured at 291nm.10- 50µg/ ml attention were showing direct values.

Construction of calibration curve

From standard firocoxib stock result attention of 10-50µg/ ml. Absorbance value of each result against different attention and DMF as a blank were measured at 291nm. From the absorbance value estimation wind was constructed. Regression equation and correlation measure(R^2) were determined.

METHOD VALIDATION

Linearity

Method A

From standard-issue stock result of Firocoxib applicable aliquots of µg/ml independently where pipetted out in 10 ml volumetric steins and append 1 ml of 3(mg/ml) $FeCl_3$ reagent and were made up with DMF to gain working standard-issue results of attention from 10- 100µg/ ml. Absorbance for these results was measured at 420nm. This attention was showing off direct valuations. From that absorbance value, the regression equation and correlation measure(R^2) are determined[15].

Method B

From standard-issue stock result of firocoxib, applicable aliquots of 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120µg/ml independently where pipette out in 10 ml volumetric steins and append 2 ml of 2% w/v were made up with DMF to gain working standard-issue results of attention from 10- 120µg/ ml. Absorbance for these results was measured at 415nm. This attention was showing off direct valuations. From that absorbance value, the regression equation and correlation measure(R^2) are determined.

Precision

Precision of styles was studied as intraday and inter day was performed by analyzing the three nonidentical attention of medicine for three moments in the same day. Inter-day perfection was performed by analyzing three nonidentical attentions of medicine for three days in a week. Three nonidentical attention were LQC, MQC, and HQC[16].

Accuracy

The accuracy of proffered methods was assessed by reclamation inquiries at three nonidentical situations i.e. 80%, 100%, and 120%. The reclamation inquiries were carried out by adding known quantum of standard-issue result of the medicine to the pre analyzed phrasings, results were also analyzed by professional styles [17,18].

Ruggedness

Absorbance valuations were taken by two judges with the same instrument and with the two instruments by the same judges[19,20].

Robustness

The robustness is a parameter that has been estimated in confirmation inquiries of logical styles, which has been outlined as the capacity of procedure to produce unprejudiced effects in the presence of fragile changes in the experimental conditions[21,22]. Robustness of the eluded procedure was assessed by assessing the influence of fragile variation in experimental variables (measure of reagent, temperature, and heating time). The effects offered. The robustness of the proposed system was determined by(a) changing the pH of the media by ± 0.1 unit and(b) changing the attention of the reagent by ± 0.1 unit.

Limit of Detection and Limit of Quantification

LOD is the smallest attention of analyte in a sample that can be detected but not inescapably quantified. LOW is the smallest attention of analyte in the sample that can be quantitatively determined with perfection and delicacy [23,24].
LOD and LOQ was calculated from direct wind utilizing following formulas
LOD = 3.3 σ/ slope,
LOQ= 10 σ/ pitch,

Where σ = the standard-issue divination of the reaction and S = slope of calibration curve.

RESULTS

Method -A

Table 1: Sequel of attention of reagent($FeCl_3$)

S.NO	CONCENTRATION %(mg/ml)	ABSORBANCE
1	1	0.039
2	2	0.048
3	3	0.384
4	4	0.041
5	5	0.052
6	6	0.107
7	7	0.089
8	8	0.143
9	9	0.161
10	10	0.179

Table 2: Effect of volume of Ferric chloride

S.no	Volume (ml)	Absorbance
1	0.5	0.101
2	0.6	0.152
3	0.7	0.267
4	0.8	0.387
5	0.9	0.668
6	1	0.859
7	2	0.786
8	3	0.785
9	4	0.584
10	5	0.524

Table 3: Effect of time on colored complex

S.NO	Time(min)	Absorbance
1	0	0.048
2	15	0.052
3	30	0.625
4	45	0.602
5	60	0.592
6	75	0.586
7	90	0.515
8	105	0.425
9	120	0.38
10	135	0.36

Linearity

Table 4: Linearity of Firocoxib with Ferric chloride reagent in colorimetry[25]

S.no	Concentration(µg/ml)	Absorbance±S. D	%RSD
1	10	0.066 ± 0.0009	1.36
2	20	0.088 ±0.00075	0.8
3	30	0.09 ±0.0015	1.66
4	40	0.105 ±0.0012	1.14
5	50	0.116 ±0.0021	1.72
6	60	0.119 ±0.0022	1.84
7	70	0.13 ±0.0022	1.69
8	80	0.151±0.0018	1.19
9	90	0.169 ±0.0023	1.36
10	100	0.172 ±0.0015	1.87
11	110	0.202±0.0015	0.74
12	120	0.214±0.00218	1.01
13	130	0.225±0.0034	1.51

Table 5: Recovery studies of Firocoxib with Ferric chloride reagent[26]

Sl.no	Name of the drug	Amount of sample(µg/ml)	Recovery level	Amount of drug added (µg/ml)	Total amount found	%Recovery	%RSD
1	Firocoxib	30	50	15	13.021±0.256	81.33	1.966
			100	30	30.42 ±0.421	101.4	1.383
			150	45	42.865±0.583	95.25	1.360

Table 6: Precision of Firocoxib with Ferric chloride reagent

Sl.no	Sample	Intra day	Inter day
1	LQC (lower quality control)	0.319± 0.008	0.42125±0.00685
2	MQC (middle quality control)	0.381±0.01	0.379±0.0051
3	HQC (high quality control)	0.441±0.010	0.46395±0.0074

Table 7: Ruggedness of Firocoxib with Ferric chloride reagent

S.no	Sample	Same instrument different analyst
1	LQC	0.291±0.0016
2	MQC	0.3147±0.0028
3	HQC	0.4033±0.0071

Table 8: Assay of Firocoxib with Ferric chloride reagent[27]

Drug	Label claim	Amount found	% Recovery	%RSD
Firocoxib	57mg	57.2	102.04	0.28

Table 9: Robustness of Firocoxib with Ferric chloride reagent

Parameters	Sample	Absorbance ± SD	% RSD
Wave length (405nm)	LQC	0.291±0.0016	0.5
	MQC	0.3147±0.0028	0.8
	HQC	0.4033±0.0071	1.76
Wave length (410nm)	LQC	0.088±0.00075	0.8
	MQC	0.116±0.0021	1.7

	HQC	0.151±0.0018	1.19
Wave length (415nm)	LQC	0.2142±0.0032	1.49
	MQC	0.258±0.0012	0.46
	HQC	0.368±0.0045	1.22

Table 10: LOD and LOQ of Firocoxib with Ferric chloride reagent[28]

LOD	2.28**(µg/ml)**
LOQ	6.92**(µg/ml)**

Method -B

Table 11: Sequel of Concentration of reagent

S.no	Concentration of reagent(µg/ml)	Absorbance
1	0.1	0.005
2	O.2	0.052
3	0.3	0.034
4	0.4	0.028
5	0.5	0.022
6	0.6	0.016
7	0.7	0.015
8	0.8	0.02
9	0.9	0.012
10	1	0.01

Table 12: Sequel of measure of Potassium dichromate

S.no	Volume of reagent (ml)	Absorbance
1	0.5	0.001
2	0.6	0.002
3	0.7	0.0025
4	0.8	0.018
5	0.9	0.021
6	1	0.024
7	2	0.064
8	3	0.058
9	4	0.056
10	5	0.042

Table 13: Sequel of time on colored complex

S.No	Effect of Time (min)	Absorbance
1	15	0.012
2	30	0.032
3	45	0.142
4	60	0.258
5	75	0.268
6	90	0.283
7	105	0.312
8	120	0,318
9	135	0.328
10	150	0.264

Table 14: Linearity of Firocoxib with Potassium dichromate reagent

S.no	Concentration(µg/ml)	Absorbance ± Std dev	%RSD
1	10	0.169 ± 0.001	0.5
2	20	0.122 ±0.0015	1.22
3	30	0.169± 0.001	0.5
4	40	0.196 ±0.0018	0.9
5	50	0.223 ±0.0015	0.6
6	60	0.24 ±0.0008	0.3
7	70	0.253±0.0008	0.3
8	80	0.296 ±0.004	1.3
9	90	0.302 ±0.006	1.6
10	100	0.36 ±0.005	1.3

Table 15: Accuracy studies of Firocoxib with Potassium dichromate reagent

Sl.no	Name of the drug	Amount of sample	Recovery level	Amount of drug added	Total amount found	%Recovery	%RSD
1	Firocoxib	60	50	30	26.23±0.083	87.43	0.31
			100	60	52.86±0.12	88.1	0.22
			150	90	88.685±0.31	98.53	0.34

Table 16: Precision of Firocoxib with Potassium dichromate reagent

Sl.no	Sample	Intra day	Inter day
1	LQC	0.201±0.0021	0.128±0.0012
2	MQC	0.247±0.0018	0.200±0.0038
3	HQC	0.4582±0.0036	0.202±0.00466

Table 17: Ruggedness of Firocoxib with Potassium dichromate reagent

S.no	Sample	Same instrument different analyst
1	LQC	0.2918±0.016
2	MQC	0.2855±0.004
3	HQC	0.356±0.0025

Table 18: Assay of Firocoxib with Potassium dichromate reagent

Drug	Label claim	Amount found	% Recovery	%RSD
Firocoxib	57mg	56.9 mg	99.83	0.22

Table 19: Robustness of Firocoxib with Potassium dichromate reagent

Parameters	Sample	Absorbance ± SD	% RSD
Wave length (410nm)	LQC	0.1332±0.0022	1.65
	MQC	0.206±0.0031	1.50
	HQC	0.159±0.0028	1.76
Wave length (415nm)	LQC	0.31 ±0.0037	1.19
	MQC	0.35±0.0058	1.65
	HQC	0.161±0.0017	1.05
Wave length (420nm)	LQC	0.253±0.0035	1.38
	MQC	0.165±0.0020	1.21
	HQC	0.103±0.0020	1.94

Table 20: LOD and LOQ

LOD	0.88
LOQ	2.6

UV Spectrophotometric method

Table 21: Linearity of Firocoxib

S.no	Concentrating (µg/ml)	Absorbance ± Std dev	%RSD
1	10	0.412±0.00461	1.11
2	20	0.507±0.0017	0.341
3	30	0.596±0.00293	0.491
4	40	0.701±0.0066	0.944
5	50	0.794±0.00508	0.639

Accuracy

Table 22: Reclamation inquiries of Firocoxib

S.no	Name of the drug	Amount of sample	Recovery level	Amount of drug added	Total amount found	%Recovery	%RSD
1	Firocoxib	30	50	15	13.028±0.156	86.85	1.19
			100	30	29.12±0.21	97.06	0.72
			150	45	45.352±0.52	100.7	1.14

Precision

Table 23: Precision of Firocoxib in UV Spectrophotometry

S.no	sample	Intra day	Inter day
1	LQC	0.2472±0.00675	0.3908±0.0046
2	MQC	0.81505±0.0029	0.06853±0.00435
3	HQC	0.824±0.0086	0.7516±0.0058

Ruggedness

Table 24: Ruggedness of Firocoxib in UV Spectroscopy[29]

S.no	Sample	Same instrument different analyst
1	LQC	0.2526±0.0034
2	MQC	0.48385±0.0051
3	HQC	0.77835±0.0102

Assay

Table 25: Assay of Firocoxib in UV spectroscopy

Drug	Label claim	Amount found	% Recovery	%RSD
Firocoxib	57mg	57.90	99.28	1.24

Robustness

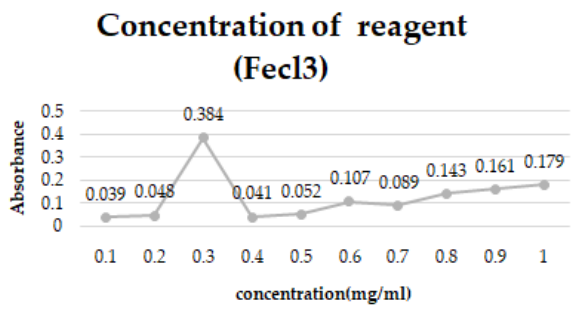
Table 26: Robustness of Firocoxib in UV spectroscopy[30]

Parameters	Sample	Absorbance ± SD	% RSD
Wave length (293nm)	LQC	0.933±0.0051	0.546
	MQC	0.758±0.0054	0.712
	HQC	0.768±0.0062	0.807

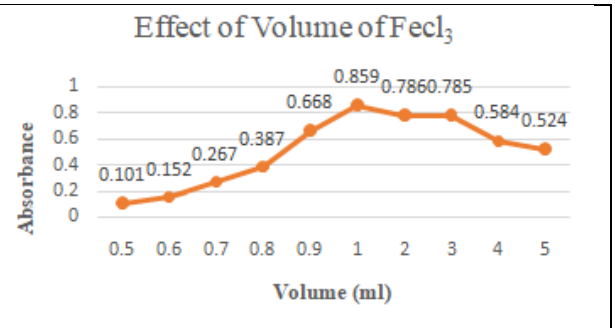
Wave length (291nm)	LQC	0.458±0.0067	1.46
	MQC	0.815±0.0091	1.12
	HQC	0.824±0.0086	1.04
Wave length (287nm)	LQC	0.9342±0.0027	0.289
	MQC	0.7414±0.0042	0.566
	HQC	0.887±0.011	1.24

Table 27: LOD and LOQ of Firocoxib

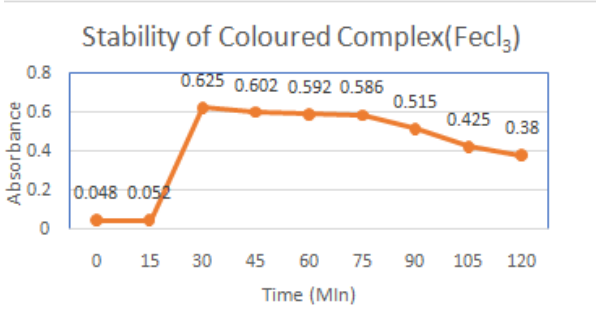
LOD	0.584
LOQ	1.77



Graph 1: Effect of concentration of $Fecl_3$



Graph2: Effect of volume of Ferric chloride reagent



Graph 3: Stability of colored complex with Ferric chloride

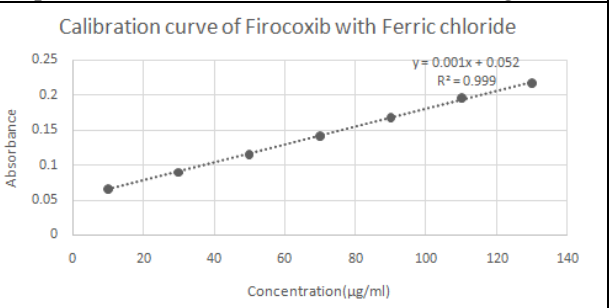


Figure 4: Calibration curve of Firocoxib with Ferric chloride reagent

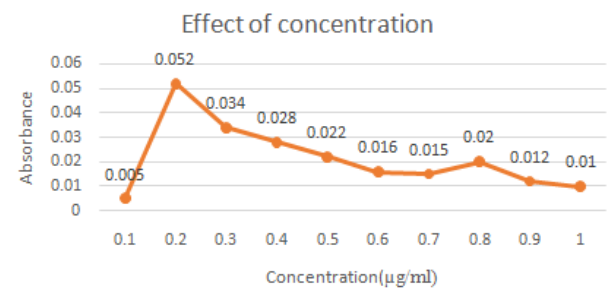


Figure 5: Sequel of concentration of Potassium dichromate

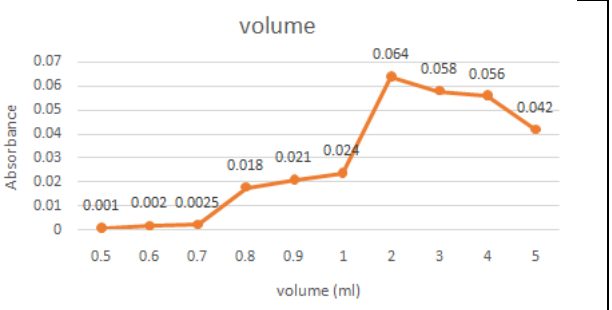


Figure 6: Effect of volume of reagent Potassium dichromate

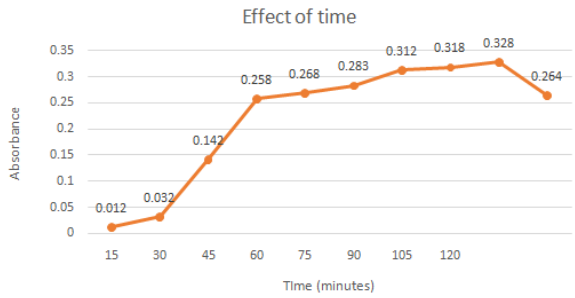


Figure 7: Sequel of time on colored deal

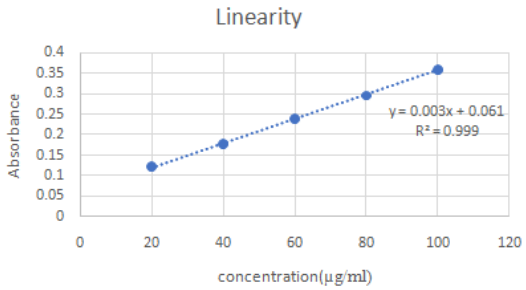


Figure 8: Calibration curve of Firocoxib with Potassium dichromate reagent

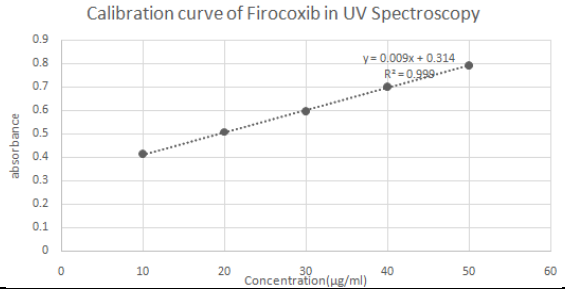


Figure 9: Calibration curve of Firocoxib by UV Spectrophotometry

DISCUSSION

All the styles were validated and developed as per ICH companion lines. The styles for the estimation of firocoxib medicine in brunt and retailed lozenge shape were set up to be accurate, simple, precise, robust, sensitive and reproducible. These styles were validated in tours of linearity, perfection, delicacy, robustness, ruggedness, LOD, LOQ and particularity. The medicine is followed the Beer's lambert's law over the attention range from 20-130(µg/ml)10-
100(µg/ml),50(µg/ml)in A,B independently andUVSpectroscopy utilizing retrogression dissection the direct equation Y = 0.0013 x 0.0523, 0.0013 x 0.0523, 0.0096x 0.3146 with correlation measure of r2 = 0.9995 in all styles independently 23,24. The perfection effects show off RSD lower than 2 at each position in all styles, which easily shows that the system is precise enough for the dissection of Firocoxib. The delicacy of the styles was chorded by reclamation inquiries. The robustness and ruggedness inquiries make known that the styles is able robust and inured. The LOD and LOQ valuations demonstrate that perceptivity of the styles. Determination of Firocoxib medicine in brunt and retailed expression of two brands showed off, content of Firocoxib were veritably close to the labelled quantum. The RSD valuations in all the parameters were within the respectable limitations. The UV Spectrophotometric system is simple, sensitive and accurate.

ACKNOWLEDGEMENT

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CONCLUSION

The above developed methods are simple, accurate and ecofriendly and can be used routinely in quality control labs.

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Table 28: Chemicals and Manufactures

S. No	Chemicals	Manufacturers
1	Firocoxib	Sequent Scientific Ltd, Mangalore.
2	Ferric chloride reagent	MOLY CHEM
3	Potassium dichromate	SDFCL
4	Dimethyl Formamide	SDFCL

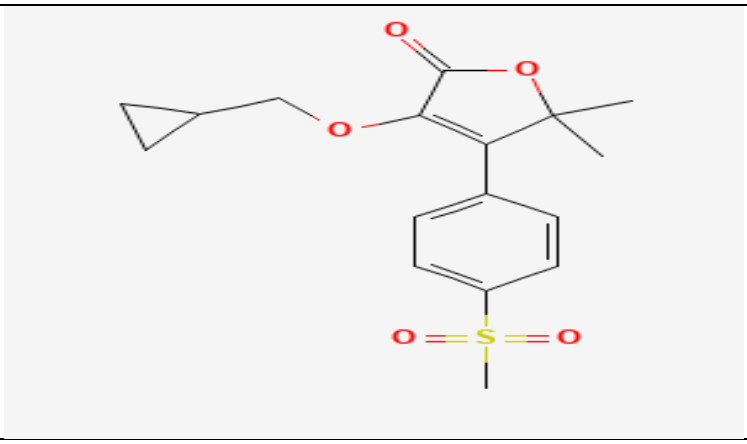


Figure 10 : Chemical structure of Firocoxib

RESEARCH ARTICLE

Identification of Physical Fitness in Postmenopausal Women

Zarana Khamar[1]*, Arvind Kumar[2] and Tvisha K. Patel[3]

[1]Ph.D Scholar (Enrollment No.: 2111705006), Venus Institute of Physiotherapy, (Affiliated to Swarrnim Startup and Innovation University), Gandhinagar, Gujarat, India.
[2]Dean, Faculty of Allied and Health Care, Gokul Global University, Patan, Siddhpur, Gujarat, India.
[3]Ph.D Scholar (Enrollment No.: 2111705005), Venus Institute of Physiotherapy, (Affiliated to Swarrnim Startup and Innovation University) Gandhinagar, Gujarat, India.

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***Address for Correspondence**
Zarana Khamar
Ph.D Scholar (Enrollment No.: 2111705006),
Venus Institute of Physiotherapy,
(Affiliated to Swarrnim Startup and Innovation University),
Gandhinagar, Gujarat, India.
E.Mail: zarana20057@gmail.com

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ABSTRACT

The term menopause means 12 months of amenorrhea from the final menstrual date. Physical fitness defined as series of elements associated with health and its performance of activity of daily living which represents one's ability to execute operational capability to perform physical activity. In postmenopausal women hormone reduction associated with reduce muscle strength, mass, agility, flexibility which affects activities of daily living. Thus, the purpose of the study was to find out the physical fitness in postmenopausal women. It was an observational study for which 125 postmenopausal women were taken. Inclusion Criteria were postmenopausal women, aged 45-55 years, willing to participate for the study, who do not exercise regularly, able to understand English language. Exclusion Criteria were OA of any joint, women with hysrectomy, women on hormone replacement therapy, unable to understand English language. For evaluation of the physical fitness the Senior fitness test was used. The data was analyzed using SPSS 20 version. The mean age of the postmenopausal women was 50±3 years. The mean score of the chair stand test was 15.26, the biceps curl test 13.17, 6-minute walk test 500.20, the chair sit and reach test 25.00, the back scratch test 15.56, the 2.45M up and go test 8.99.There was reduce physical fitness in terms of reduce flexibility, upper limb and lower limb strength, agility, aerobic endurance.

Keywords: Menopause, Physical Fitness, Senior Fitness Test, Postmenopausal Women

INTRODUCTION

The term menopause means 12 months of amenorrhea or no menses from the final menstrual date. It is the final phase of women's reproductive life which is part of normal aging process. Its further divides into three phases: perimenopause, premenopause and postmenopause. Each phase has different characteristics and effect on women's wellbeing. The changes occur at the time of menopause is due to decline hormone level in blood mainly estrogen. During the menopause various impleasure symptoms experienced by the women such as vasomotor, hot flashes, fatigue, joint problem, psychiatric, sleep disturbances, urogenital atrophy which is also a part of normal aging process [1]. The term menopause is known as the last menstruation, generally lasts between 46 and 52 years, and marks as aging of a woman's reproductive system [1]. Hormonal changes associated with menopause starts many years prior to menopause and linked with gradual increase in follicle-stimulating hormone (FSH) and sudden decrease in systemic female sex steroids (estradiol and estrone) 6 months around the menopause. At the time of menopause different symptoms affect the quality-of-life women along with these symptoms leads to decrease in physical fitness which is also associated with increase in age. Physical fitness defined as series of elements associated with health and its performance of activity of daily living which represents one's ability to execute operational capability to perform physical activity [2]. There is gender related decrease in physical activity performance, which is markley seen in females than in males at their middle age which is mainly because of females have rapid change in hormone level in menopausal life[3]. Menopause-induced changes in sex hormones like estrogen can lead to a decline in physical activity, and this, in turn, may impact skeletal muscle function and overall performance. The decline in spontaneous physical activity (PA) that occurs with menopause could be partly due to the loss of estrogen's neuroprotective effects, which might make women feel less motivated or experience increased fatigue. The estradiol replacement can reverse this decline are quite insightful. In rodents, estradiol replacement has been shown to improve motor function and potentially increase PA, suggesting that hormone therapy could be beneficial in mitigating some of the physical activity-related changes seen during menopause. The decline in physical performance during menopause may partly be a result of reduced physical activity (PA) and hormonal changes. That makes sense—when estrogen levels drop during menopause, it can affect muscle strength, endurance, and overall energy levels. The reduced spontaneous PA, along with the loss of estrogen's positive effects on muscle mass and metabolism, could contribute to the observed decline in physical performance.

This could be a multifactorial issue, where hormonal changes not only affect muscle tissue directly but also influence factors like mood, sleep, and joint health, which in turn impact physical activity levels[3]. The symptoms which commonly seen during process of aging were reduction in muscle strength, decline in cardiovascular and logical capacity with reduce joint mobility. These symptoms were more evident in postmenopausal women because of osteoporosis and sarcopenia. The physical fitness associated with age can be evaluated by various fitness tests. The physical fitness consists of various parameters such as cardiorespiratory endurance, muscular strength, flexibility, balance, agility and body composition. These parameters can be evaluated and improved by well-structured exercise program[4]. To perform activities of daily activity the physical performance is necessary. Reduce walking speed and mobility restrictions are due to reduction in muscular strength and power associated with increase age. This can be mainly seen in later age of life but in women it can begin in her midlife because of change in hormonal level during menopausal life[5]. Hormone mainly estrogen majorly takes part in keeping skeletal muscle homeostasis and its action in postmenopausal women have reduced muscle mass, muscle power and muscle strength compare to premenopausal women[5]. In postmenopausal women reduction in physical activity mainly due to sedentary lifestyle which can be associate with increase in age also. Alternatively, further longevity, low morbidity and mortality can be visualized in physically active individuals. Postmenopausal women with inactive lifestyle have reduce resistance, strength and lower limb muscle velocity. Along with this, reduction in walking speed is due to muscular weakness and reduce cardiorespiratory capacity which results in less safety. It also causes reduce walking frequency in daily routine, and results in more time of incactivity [6]. The physical fitness known as the functional capacity to carry out day to day activities by their own in the absence of exhaustion. Increase age associated with decline in physical fitness which includes strength, agility, endurance and flexibility results in problems in routine

activities and normal activity performance [7]. There are various validated tools used to assess the physical fitness from them we used the senior fitness test developed by Rikli and Jones. The Senior Fitness Test is a functional test of muscular strength, endurance, balance and flexibility. The senior fitness test includes the chair stand test, the biceps curl test, the 6 – minute walk test, the chair seat and reach test, the back scratch test, the 2.45-m Up-and-Go test to asses various physical fitness measures in the postmenopausal women [8]. Menopause itself is period of 4-6 years in women life depending upon individual. So, most of the time of women spend in her postmenopausal phase. To live functionally independent in later years of life is most important for that one has to be physically fit. Reduction in the fitness results in institutilization, long term illness and mortality. Estrogen deficit in postmenopausal phase results in increase fat mass, reduction in bone mineral density and lean body mass [9]. This leads to reduction in motor skills, flexibility, speed, muscle strength which interferes with activities of daily living. Thus, the aim of the study was to find out the physical fitness in postmenopausal women.

MATERIALS AND METHODS

After getting the ethical clearance from the institutional ethical committee the study was conducted. It was an observational study done between 1 February 2024 to 1 April 2024 in Ahmedabad, Gujarat. CTRI registration was obtained and number of CTRI registration is CTRI/2024/01/062074.For this observational study 125 postmenopausal women between 45-55 years were taken. The physical fitness of postmenopausal women was evaluated using the senior fitness test. For the enrollment in the study, inclusion Criteria were postmenopausal women, women aged 45-55 years, willing to participate for the study, who do not exercise regularly, able to understand English language. The postmenopausal who do not meet Exclusion Criteria, OA of any joint, women with hysrectomy, women on hormone replacement therapy, unable to understand English language were excluded from the observational study. Written and informed consent obtained from each participant and their privacy about enrollment in the study was maintained. Two trained physiotherapists were always there for accuracy of the procedure. After explaining and demonstrating the test to the participant the recording of the result was done. The test was carried out in the physiotherapy department with instruments required for it. Rikli and Jones developed the Senior Fitness Test for adults aged over 60 years. It is use to evaluate physical fitness in healthy individuals. It does not require any expensive equipment to measure the physical fitness [8].

It includes total 6 functional test to evaluate physical fitness in postmenopausal women.

1. The Chair Stand Test. This requires people to repeatedly stand up from and sit down on a chair for 30 seconds. The number of stands is recorded. This reflects lower body strength.
2. The Biceps Curl Test. This requires people to repeatedly lift a 5 lb (2.27 kg) weight (for women) for 30 seconds. The number of lifts is recorded. This reflects upper body strength.
3. The 6-minute Walk Test. This is measured in distance (m) and reflects aerobic endurance.
4. The Chair Sit and Reach Test. This is measured in distance (cm) and reflects lower body flexibility.
5. The Back Scratch Test. This is measured in distance (cm) and reflects upper body flexibility.
6. The 2.45-m Up-and-Go test. This is measured in time (seconds) and reflects agility and dynamic balance.

Thus, by the above different test the physical fitness of postmenopausal women was taken. The test was carried out in the physiotherapy department of the college under the supervision of the expert. For the statistical analysis statistical package for the social sciences (SPSS) 20 version was used. The mean age of postmenopausal women was calculated. The mean value of each fitness test was calculated. From the results obtaining from above test the physical fitness of postmenopausal women was calculated.

RESULT

As per the findings of the observational study, the mean age of the postmenopausal women was 50±3. The physical fitness of postmenopausal women was calculated from obtained results of test analysis. The mean score of the chair stand test was 15.26, the biceps curl test 13.17, 6-minute walk test 500.20, the chair sit and reach test 25.00, the back scratch test 15.56, the 2.45M up and go test 8.99.(Table 1)

DISCUSSION

The physical fitness being one of the important aspects for evaluation as age advances here in our study the main reason for its evaluation is the effect of the hormones during the menopausal age. The result of the study shows the mean age of the postmenopausal women included for the observational study was 50±3. This age group is the most prevalent for the menopausal symptoms to begin. The results obtained from our study, indicates overall decline in score of all test measures which shows reduction in physical fitness in postmenopausal women. As per the study done by researcher in it shows the reduction of physical fitness in women than men. This was due to gender prevalent factors which are in inverse relation with that of age. Here the fitness in terms of physique is most commonly affected in the later phase [11]. The lower body strength was evaluated by the chair stand test in this observational study. The result of which indicates reduce lower limb strength in postmenopausal women. Lower extremity strength shows the routine physical activity level due to repeated involvement of limb muscles used in daily routine. A multifactorial affection is involved in the reduction of this strength as a female even under goes the process of parity and ligamentous laxity are most common in that phase. In post-partum period a female might not have gone through proper strengthening protocol which may lead to reduce the strength of lower body in the later life. Thus, it shows the common effect of physical activity deterioration linked between lower limb strength and menopausal status [3]. The similar results found in the study done by slawinska *et al*. (2000) which suggest 50.6% reduction in lower limb strength than the normal data obtained. The other study also shows similar results suggesting decrease in lower body strength compare to upper body strength in women more than a middle age group[3]. Bondarev *et al*. shows postitive results as we found in this study which says reduction in lower body strength is 29.74% in 50 to 80-87 years age.

Along with this, other studies suggest reduction in strength 1% every year in 45-60 years age which increases 1.5% every year after 60 years[10]. Thus, it makes it evident enough to study this particular age group for their physical fitness of lower body strength where the muscles of the entire leg work in a synchrony so as to gain the outcome. This is important for the purpose of mobility. Paterson *et al*. (2007) says reduce lower limb strength more compare to upper limb strength [1]. This could possibly be due to the postures adapted in daily living. The arm strength in postmenopausal women was tested by the biceps curl test which shows reduction in upper limb strength in our population. slawinska *et al*. (2000) says 21.3% reduction in upper limb strength in comparision with normal data obtained from the study[11]. Findings of the recent study shows there was reduction in the muscle strength which was also supported by the results of the other studies which states that there was reduction in muscle mass by 4% in the postmenopausal women compare to premenopausal women which results in reduction of muscle strength at menopause due to loss of muscle mass [5]. The 6- minute walk test was used to evaluate the aerobic endurance of postmenopausal women. Bondarev D. *et al* studied the reduction in exercise tolerance and aerobic capacity in postmenopausal women compared with premenopausal women which is in favour of our recent findings [5]. As evaluated by many researchers the focus on the reduction in physical fitness could be most commonly due to a decline in the mass of an individual muscle which is termed as sarcopenia, which leads to tightness of muscles, a lower strength as well as the endurance alterations in the mass affected. All the three aspects of the physical fitness are evaluated in the present study by various test.

This accounts to identify the lower fitness related to muscle mass alteration. The recent assessment has seen the same and thus could propose that the fitness is reduced identically due to the alteration in the muscles itself. The joint undergoes changes pertaining to the bones where osteoporosis is majorly seen in this age group and further increases linearly with age. The upper and lower body flexibility was assessed by the back scratch test and the chair sit and reach test repectively, the result of which shows decline in upper limb and lower limb flexibility. Ridhi singhavi *et al*. results indicate there was an average flexibility between 40-55 years of age [9]. To measure the agility in postmenopausal women the 2.4 meter up and go test was used. The test results show decline in agility in study population. Other supporting studies finds the similar declines in the agility which was in favour of present study(10). The research done by Bondarev *et al*. indicates there were 46% reduction in agility and balance which was

observed in the females of the same condition[5]. The falling of the outcomes of most of the parameters of physical fitness found in current study was in favour of the other researchers. As per Espeland *et al*. (2007) decrease lower limb strength, aerobic endurance, agility, dynamic balance and flexibility are associated with advancing age and results decline in mobility [12]. The study by Schultz *et al* (1997) decrease in lower limb strength results in reduce functional activities such as getting up from the chair [13]. This is a matter of concern for the population particularly in that age group. It is important to identify the problems prevalent in this cohort so as to further plan the management stratergies. There were many factors responsible for reduction of physical fitness in postmenopausal women like age, gender, associated co-morbidities, but along with them the decrease in physical fitness in postmenopausal women was mainly due to hormone reduction in the blood during the time of menopause which negatively affects their muscle power, strength, flexibility, endurance and agility.

CONCLUSION

After the result obtained from recent study it concluded that there was decline in the physical fitness in postmenopausal women. For the future recommendations further exercise regime can be developed to increase the physical fitness in postmenopausal women to have better quality of
life.

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Conflict of Interest The authors declare that there is no conflict of interests regarding the study or this article.

Author Contributions Dr.Zarana Khamar Tvisha Patel has designed the study with even preparation of the manuscript. The review of the protocol as well as the analysis of the data and interpretation of result was done by Dr. Arvind Kumar. Dr. Tvisha Patel recruited the participants and collected the data.

Ethics approval The ethical approval was obtained post review by the Institute's ethical committee, Venus Institute of Physiotherapy: VIP/2023/EC/111. The trial is registered in Clinical Trial Registry- India: CTRI NO: CTRI/2024/01/062074

Data availability: The data will be available on request, maintaining confidentiality of the patients.

Abbreviation: OA-osteoarthritis,
SPSS- statistical package for the social sciences,
PA- Physical activity,
FSH -follicle stimulating hormone,
QOL- quality of life,
CTRI – clinical trial registry of india,
kg – kilogram,
mtr -meter,
cm – centimeter,
rep – repetation,

sec – seconds,
min – minute

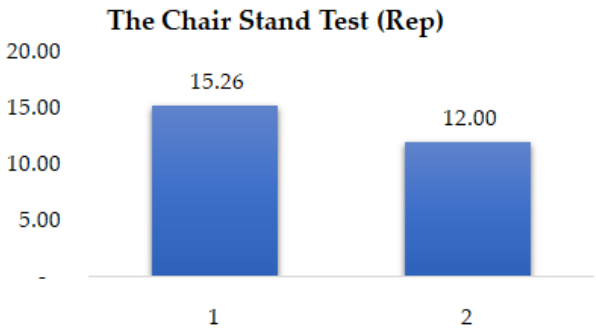
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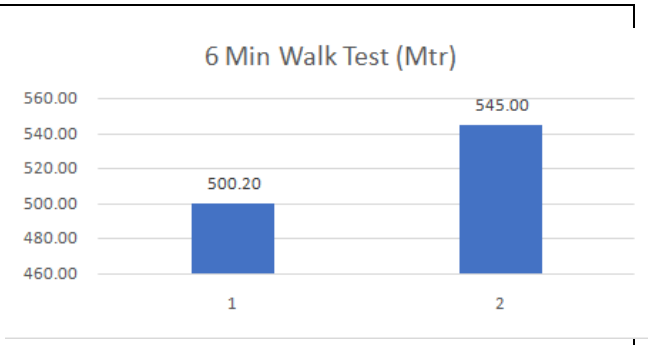
Table 1: Mean test score

TESTS	MEAN
The Chair Stand Test (Rep)	15.26
The Biceps Curl Test (Rep)	13.17
6 Min Walk Test (Mtr)	500.20

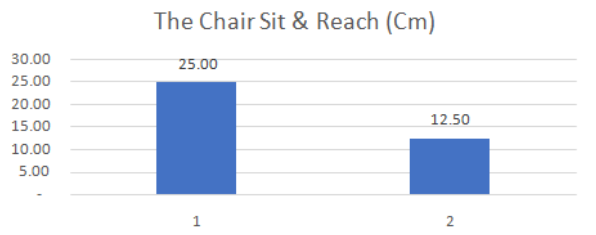
The Chair Sit and Reach (Cm)	25.00
The Back Scratch Test (Cm)	15.56
The 2.45M Up and Go Test (Sec)	8.99



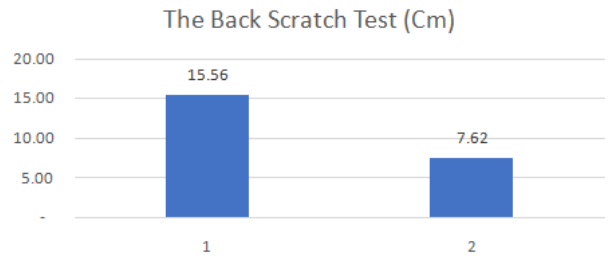
Graph 1: The chair stand test



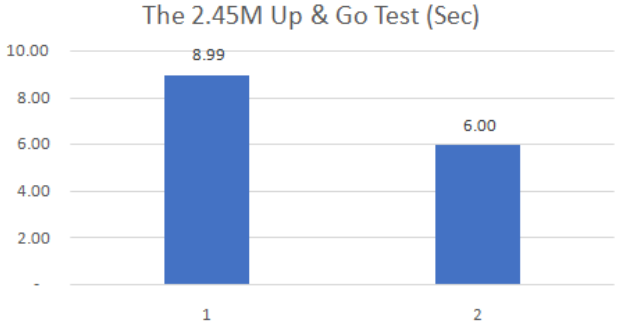
Graph 2: 6-minute walk test



Graph 3: The chair sit and reach test



Graph 4: The back scratch test



Graph 5: The 2.45 M up and go test

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RESEARCH ARTICLE

Characteristics and Synthesis of Amylose Lipid Complex (Resistant Starch Type 5) From Corn (*Zea mays*) Starch

RM. Karthika*

Assistant Professor, Department of Clinical Nutrition and Dietetics, Allied Health Sciences, Dhanalakshmi Srinivasan University, Samayapuram, Tiruchirappalli , Tamil Nadu, India.

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***Address for Correspondence**
RM. Karthika
Assistant Professor,
Department of Clinical Nutrition and Dietetics,
Allied Health Sciences,
Dhanalakshmi Srinivasan University, Samayapuram,
Tiruchirappalli , Tamil Nadu, India.
E.Mail: k123456meenakshi@gmail.com

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ABSTRACT

High-amylose corn starch (RS) is a novel type 5 resistant starch that can be used for the formulation of foods. RS5 was developed by using FFAs to treat high amylose corn starch. It is distinguished by the existence of amylopectin crystallites in RS5 that are long-chain double-helical. The granules of RS5, which are made of a mixture of corn oil and saline, have been subjected to gelatinization and retrogradation on progressive heating/cooling of the aqueous solution. The modified starches are used in both food and non-food industries. The modification of starches is designed to change the gelatinisation characteristics, gelling tendency, and rheological properties of the starches. SEM images revealed the disrupted structure of granulations after processing. Because of its decreased crystallization, the modified starch was shown to be extremely heat stable and to have an anti-staling action in bread and biscuits.

Keywords: Resistant Starch, Carbohydrates, Amylose, Amylopectin, Native (corn) starch

INTRODUCTION

Starch -An Outlook

The primary dietary carbohydrates are starches; sugars make up the remaining types. Potato and cereal products are the examples of plant-based sources of starchy foods.Starch consist of amylase and branched amylopectin molecules in molar ratios of (15% - 25%) and (85% - 75%). The primary components of the starch granule are a combination of two polymers and an essential. Amylase is a linear polymer, and amylopectin is a highly branched polymer (Tester *et al*., 2004).

Amylose

A linear chain of 1α4-linked α-D-glucopyranosyl units makes up amylose. Very few α-D-(1 α 6) branches are present in many amylose molecules; they may appear once every 180–320 units, or 0.3-0.5% of the connections. Amylose molecules typically weigh around 10 g/mol and have a degree of polymerization (DP) of 6000. Both single and double helices can be readily formed by the chains. While the hydrophilic hydroxyl groups are located on the outside of the coil, the majority of the hydrogen atoms are lipophilic and found inside the helix (Whistler and BeMiller, 1997).

Amylopectin

The highly branched polymer amylopectin has a DP of 2 α 10 and an average molecular weight between 10 and 5 α 10g/mol. It is therefore one of nature's biggest polymers. It is composed of thousands of small linear chains of α-(1 α 6)-linked α-D-glucopyranosy l units connected to one another. The branch point linkages constitute 4-5% of the total linkages (An amylopectin molecule consists of main chain, called the C chain, which carries the one reducing end-group and numerous branches, in termed A chains and B chains. Short chains (A chains) of DP 12-16 that can form double helices are arranged in clusters.The clusters comprised 80% to 90% of the chain and are linked by longer chains (B chains) that form the other 10% to 20% of the chains. Most B chains extend into 2 (DP) about 40) or 3 clusters (DP about 70), but some extend into more clusters (DP about110) Thompson, (2000).

Types of resistant starch

Resistant starch is defined as a portion of starch that cannot be digested by amylases in the small intestine and passes to the colon to be fermented by microbiota Resistant starch classification system are based on starch digestive rate. This system divides starches into rapidly digestible starches, slowly digestible starches, and resistant starches based on the results of in vitro digestion as proposed by (Englyst *et al.*,1982). There are currently 5 types of resistant starch as summarized below

Resistant Starch Type 1

According to Hernández, Emaldi, and Tovar (2008), RS1 is the name used to describe RS in which the starch is physically inaccessible to digestion, such as when grains, seeds, or tubers have intact cell walls. Because RS1 is heat stable for the majority of routine cooking processes, it can be used as an ingredient in a large range of traditional dishes (Sajilata, Singhal, and Kulkarni, 2006).

Resistant Starch Type 2

Natural, uncooked starch granules, like raw banana or potato starches, have a low hydrolysis susceptibility due to their crystallinity (Hernández *et al*., 2008). RS2 refers to native starch granules that are shielded from digestion by their shape or conformation. This compact structure explains the refractory nature of RS2, including ungelatinized starch, and restricts the accessibility of digesting enzymes and different amylases. Foods such as bananas include uncooked starch (Sajilata *et al*., 2006). According to Wepner, Berghofer, Miesenberger, and Tiefenbacher (1999), high-amylose maize starch is a special kind of RS2 that maintains its structure and resilience even when several items are processed and prepared.

Resistant Starch Type 3

Materials generated from non-granular starch that are resistant to digestion are referred to as RS3. According to Wepner *et al*. (1999), RS3 forms are typically created when starch granules retrograted According to Hernández *et al*. (2008), retrograted starches, or RS3, can occur in cooked foods that are stored at room temperature or at low temperatures. Accordingly, RS3 is present in most moist-heated foods (Sajilata *et al*., 2006). The thermal stability of RS3 makes it very interesting. This permits its usage as an ingredient in a broad range of traditional dishes and makes it stable in the majority of typical cooking processes (Haralampu, 2000).

Resistant Starch Type 4

RS can be created by chemically altering starch. When starch is modified using octenyl succinic anhydride (OSA), hydrophobic groups are added to the starch molecules, which prevents amylolytic enzymes from attaching to the

starch (Zhang and Hamaker 1998; Han and BeMiller 2007; He *et al*. 2008). Cross-linking of starch is another effective method to prepare RS4 (Woo and Seib 2002; Xie *et al*. 2006; Al-Tamimi *et al*. 2010).

Resistant starch type 5

The single-helical complex formation between amylose and lipids has also been used to develop RS 5 (Gelders *et al*. 2005; Hasjim *et al*. 2010). A RS5 product, containing up to 75% RS (AOAC Method 991.43), has been prepared by complexing partially-debranched high-amylose corn starch with FFAs. In vivo an investigation into human feeding has shown that the RS5 is resistant (Hasjim *et al*. 2010).

The following are the benefits of using amylose-lipid complex as Resistant Starch 5 over other varieties

- Amylose-lipid complexes exhibit greater thermal stability than most native starch granules with B- and C-type polymorphs (RS type 2), such as those found in potatoes and bananas.
- Compared to retrograded amylose (RS type 3) and chemically modified starch (RS type 4), forming amylose-lipid complexes requires less intensive physical or chemical treatment.
- These complexes can spontaneously reassemble during cooling after being heated beyond their dissociation temperature (Biliaderis and Galloway, 2000; Hasjim *et al*., 2010).

Production of resistant granular starch through starch-lipid complex

By promoting the production of amylose-lipid complexes (RS type 5) within starch granules, a novel method has been developed lately to increase the concentration of resistance starch (RS). In order to cleave the branch points in amylopectin, debranching enzymes, such as pullulanase or isoamylase, are applied to inflated starch granules. The long chains that are released from amylopectin then behave similarly to those of amylose, which allows them to efficiently form complexes with fatty acids (FAs). Because of its high amylose content and profusion of long amylopectin branches, high-amylose maize starch VII (HA7) was chosen for this technique (Jane *et al*., 1999; Li *et al*., 2008). An HA7 starch suspension is first heated to a high temperature in order to create the complex, and then it is enzymatically treated with either pullulanase (PUL) or isoamylase (ISO). Enzymatic debranching is followed by the extraction.

Application of RS Type 5

RS is present in up to 75% of the (ISO-treated HA7)-FA and (PUL-treated HA7)-FA complex products following enzyme digestion at 100 C in excess water using the AOAC Method 991.43. According to the findings, RS type 5 in the (ISO-treated HA7)-FA and (PUL-treated HA7)-FA complex products is thermally stable and, as a result, appropriate for heating-intensive food processing. Furthermore, an ISO-treated HA7-PA complex product's heat stability has been evaluated in bread-making. 72% (dry basis, db) of the wheat flour in a white bread recipe was swapped out with ISO-treated HA7)-PA complex product (60%, db) and wheat gluten (12%, db) (Hasjim *et al*., 2010).

The analytical result of the RS content of the bread containing RS type 5, determined using the AOAC Method 991.43, was 34.4% (db), which was similar to the value calculated (33.6%, db) from the RS contents of the wheat flour, (ISO-treated HA7)-PA complex product and wheat gluten used to make the bread. Although bread made with such a high content of RS type 5 was not intended for a desirable texture, the result proves that RS type 5 is more heat stable and its enzyme resistance remains in the bread after baking (Tufvesson *et al*., 2003).

Properties of RS 5

The formation of a single-helical complex between amylose and lipids makes it resistant to enzymatic hydrolysis. Starch's digestibility is further decreased by the amylose-lipid complex (ALC), which also prevents starch granules from expanding. Monoglycerides, phospholipids, long-chain alcohols, and free fatty acids (FFAs) are among the lipids that have been demonstrated to form a helical complex with amylose. According to Jane and Robyt (1984) and Gelders *et al*. (2005), amylose helical complexes with fatty acids and other substances that possess hydrophobic moiety are resistant to hydrolysis by enzymes.

MATERIALS AND METHODS

Selection of sample

Normal yellow colour high amylose corn starch (*Zea mays*) was purchased from the local market in Salem, Tamil Nadu, The starch sample was sealed in polyethylene bags for further analysis and stored at normal room temperature. Pullulanase enzymes from bacillus acidopullulytic enzyme (promozyme 400 L) was purchased from SD-Fine chemical companystearic acid was purchased from sigma chemical company, USA and it was used for analysis.

Preparation of Resistant starch

Type 5 resistant starch was made using a modified version of Hasjim *et al*. (2010)'s methodology. Following a one-hour stirring preheating period at 80°C, a starch suspension (10%, w/w, dry basis, db) was debranched using pullulanase (5 units/g starch, dry starch basis, dsb) at 60°C for twenty-four hours. After adding corn oil and 10% w/w stearic acid to the debranched-starch suspension, the mixture was vigorously stirred and kept at 80 °C for an additional hour. Centrifugation was used to recover the RS5, which was subsequently cleaned with a 50% (v/v) ethanol aqueous solution, dried at 37 °C, and crushed into a fine powder.

Chemical composition Of NCS Treated SACS, COCS starch

Starch yield

Starch yield was determined by the following method. The NCS, SACS and COCS of each starch were weighted and it was prepared by the Hasjim *et al*., (2010) method. The following formulas were followed for the starch yield of each starch respectively.

Chemical analysis

The moisture content of native corn starch (NCS), treated (Corn oil and Stearic acid) was determined by (AOAC, 2005), ash (AOAC, 2004), fat (Soxhlet method: Sadasivam and Manickam, 2005), crude fibre (Acid and Alkali digestion method: Sadasivam and Manickam, 2005), total carbohydrate content can be calculated by determining the difference between 100 and the sum of moisture, ash, crude protein, crude fat and crude fibre (Akpapunam and Sefa-Dedeh, 1995; Onyeike and Oguike, 2003; Ponka *et al*., 2005), total energy by multiplying the total carbohydrate content into the one gram (1 g) of pure energy value of Carbohydrate and protein as 4 Kcal, total fat as 9 Kcal. The detailed procedure of chemical composition of starches given in annexure -I.

Amylose Content of NCS and treated SACS, COCS

Total amylose (T-AM) and free amylose (F-AM) content were determined by iodine binding according to Chrastil (1987). Five milliliters of 85% ethanol were added to a test tube containing twenty milligrams of starch (NCS, SACS, and COCS), which was then kept at 60°C for thirty minutes while being periodically mixed to extract the lipids. The supernatant liquors were disposed of after the samples underwent centrifugation. To solubilize the starch, 4.0 ml of deionized water and 2.0 ml of 1.0 M NaOH solution were added to the lipid-free samples. The test tubes were then heated to 100°C for 30 minutes while being stirred occasionally. In a different test tube, 5 ml of 0.5% trichloroacetic acid was combined with 0.1 ml of this solution. The tubes were vortexed, then 0.05 ml of an iodine solution (1.27 g I2 and 3.0 g KI/l) was added and mixed right away. After 30 minutes at 25°C, the absorbance was measured at 620 nm in comparison to an H2O blank. Total (T-AM) values were derived from iodine binding using hot ethanol with and without lipid samples NCS, SACS, and COCS, respectively.

Functional Properties of NCS Treated SACS, COCS starch

Water Absorption Capacity

The Anderson *et al*. (1969) method was used to test the water absorption capacity of treated and native maize starch samples. In short, 0.5 g of the material was combined with 6 ml of distilled water and centrifuged. To determine the dry solids weight, the sample was centrifuged and then held in a water bath at 30 °C with constant stirring for 30

minutes. The suspension was then put in a petridish and dried at 105 °C for 4 hours, and the wet sediment was weighed. The WAC was determined as

WAC = ((Weight of wet starch − Weight of dry starch) / (Weight of dry starch)) × 100

"The water absorption capacity of native corn starch (NCS) and treated corn starch is crucial in the formulation of baby foods due to its functional role in developing ready-to-eat products."

Oil absorption capacity

Using the methodology outlined by Abbey and Ibeh (1988), the Oil Absorption Capacity (OAC) of treated and native corn kernels was measured.1 g of the starch sample was placed in a centrifuge tube of known weight, and 10 ml of oil was added. After 30 minutes of standing, the mixture was centrifuged (3500 g, 15 min), and the supernatant was disposed of. The weight gain was considered the water/oil absorption capacity after the tube and residue were weighed. A collection of information on the areas of application in food formulations is provided by the oil absorption capacity of NCS and treated corn starch.

AOAC = ((Empty weight of the centrifuge - weight of the after centrifuge) / (Weight of the sample)) × 100

Swelling Power

According to Peroni *et al*. (2006), the swelling power of treated and native maize starch samples was calculated. Glass tubes with coated screw covers were filled with 0.2 g of flour sample (dry starch base) and 18 g of distilled water. The tubes were placed in a shaking water bath at 60, 70, 80 or 90ºC for 30 min. After that, the tubes were taken from the bath, dried and weighed. The weight of the mixture was completed to exactly 20 g. The tubes were closed, inverted for homogenization and centrifuged at 1700 g for 5min. The supernatant was removed carefully and swelling power was determined as sediment weight divided by dry weight of flour (g/g), The swelling power of NCS and treated corn starch provide a data on process condition (such as nature of starch materials).

Structural Properties Of NCS Treated SACS, COCS starch

Scanning Electron Microscope (SEM)

A Scanning Electron Microscope (SEM) (JEOL-Model 6390, Japan) was used to examine the granule morphology of flour, native (corn) starch, and treated (corn) samples.On a double-sided tape that was attached to a SEM stub, the starch granules were scattered. After being covered with gold, the samples were put inside the SEM chamber. A scanning electron microscope device operating at a 15 kv accelerating voltage was used to take photomicrographs. The structural and size differences between NCS and treated corn starch are examined by morphological characteristics.

Light microscopy

The experiment was conducted using a polarization microscope (Axioskop 40 Pol/40 A Pol, ZEISS, Oberkochen, Germany) fitted with a 35 mm SLA camera. Since iodine slips inside the amylose coil and forms a rich blue color inclusion complex, it was utilized to analyze the interior structure of starch granules. Before being inspected under a light microscope, 1 ml of 5% starch suspensions were stained with 0.25 ml of an I2–KI diluted solution (2 mg/ml I2 + 20 mg/ml KI). They were then repeatedly washed with distilled water 20 minutes later and dispersed in a 1:1 (v/v) glycerol/water mixture to reduce evaporation and granule movement in the field. The unstained starch's morphology was examined using both ordinary and polarized light. Chen Pei and colleagues (2010). Information on the granule shape and structure of NCS and treated corn starch is provided by light microscopy details.

Statistical Analysis

All determinant values were the average of triplicate values except instrumentation. LSD (Least Square Difference) as post hoc comparison was done to compare the means after one way analysis of variance (ANOVA). All statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS), Version 17.0.

RESULT

Preparation of RS 5

In order to create RS, amylose and lipids have also formed a single-helical complex (Gelders *et al*., 2005; Hasjim *et al*., 2010). FFAs have been complexed with partially-debranched high-amylose corn starch to create an RS5 product that contains up to 75% RS (AOAC Method 991.43). The procedure of Hasjim *et al*. (2010) was followed in the preparation of the RS 5 of SACS and COCS.

Chemical Composition of Native and Treated Corn Starch

A straightforward and practical method of demonstrating the purity of starch extracts is by their chemical composition, where higher starch and lower levels of other components (such as protein, fat, ash, and fiber) are preferred (Ikegwu *et al*., 2010). Table 1 shows the functional properties of the RS 5 corn (Native Corn Starch (NCS)), Stearic acid treated Corn Starch (SACS), Corn Oil treated Corn Starch (COCS) starches. Moisture, Ash, Crude fat and Crude fiber was evaluated. Functional properties are the characteristics that determine the suitability of the food material for specific purpose (Eke-Ejiofor and Owuno, 2014). The starch yield of the treated COCS and SACS 44.4 and 51.1 (%) respectively. The heat treatment affects the starch yield in SACS treated starch than the COCS starch samples. Fan Zhu and Ya-Jane Wang (2013) reported that the Starch complex yield of common starch treated with α-napthol was found to be 53.2 % which it was comparable to the COCS starch 51.1 (%). The starch complex yield of SACS is 44.4 % this starch was less than the native corn starch 51.1 (%). The moisture content of NCS, COCS and SACS was 8.62, 7.63 and 7.56 (%) respectively. Starches treated with stearic acid and corn oil shows reduced moisture content compared to native corn starch (NCS), the higher moisture level was observed in native corn starch (NCS). The moisture content of NCS, SACS and COCS was comparatively low when compared with the results of Shalimar Maize 8.45 (%) reported by Abida Ali *et al.,* (2016). These lower values of moisture content are advantageous in terms of shelf life. The moisture content of these starches was in the range generally accepted for dry products in order to obtain a desirable shelf life (Abida Ali *et al.,* 2016). The ash content of NCS COCS and SACS was 0.09, 0.16 and 0.11 (%) respectively. Normal high amylose corn starch (NCS) shows reduced ash content compared to Starches treated with stearic acid and corn oil, the higher ash content level was observed in corn oil treated starch and stearic acid treated starch. Native corn starches (NCS) was highly purified in nature than the treated starch samples, the treated starch samples slightly higher than the native starch (NCS) this may be due to the reason of addition of stearic acid and corn oil to the high amylose corn starches. Thus ash content of SACS, COCS which clearly depicts the presence of mineral content than that of native corn starches. The ash content of NCS, SACS and COCS was less comparable to the corn cultivars such as PS-43 (0.20%), shalimar maize (0.38%) reported by Abida Ali *et al.,* (2016). The relatively low values reported for ash, lipid and protein contents indicate the purity of starches and these properties met the worldwide industrial standards (Swinkels, 1985), Thomas *et al.,* 1997). The crude fibre content of NCS, SACS and COCS was 0.73, 0.48 and 0.83 respectively. The crude fibre content of COCS was higher than that of NCS and SACS starches. The addition of corn oil into the high amylose starches slightly increase the crude fibre content. compare with NCS and COCS treated starches the SACS starch less in fibre content.

The crude fat content of NCS, SACS and COCS was 0.26, 0.26 and 0.53 respectively. Ihegwuagu *et al* (2009) who reported that the fat content of Corn starch (1%) which was higher than (NCS), (SACS) and (COCS). Abida Ali (2014) who reported the crude fat content of (P-43 cultivars and Shalimar Maize) cultivars of corn starches shows 0.67 and 0.69 (%) which it was higher than NCS, SACS and COCS corn starches. There was no significant difference was observed in the fat content of NCS and SACS corn starches. Crude fat content was higher in the corn oil treated corn starches. It may be due to addition of corn oil to the corn starch slightly increased the crude fat content than the NCS and SACS corn starches. Thus crude fat content was high only in corn oil treated corn starches. NCS, SACS, and COCS have pH values of 6.19, 6.17, and 6.20, in that order. One significant physicochemical characteristic of starch that affects its uses is pH. The pH values of starch treated with stearic acid and maize oil were comparable to those of native and other modified starches. The amylose content of NCS, SACS and COCS was 33.6, 35.2, and 36.8 respectively. Han *et al*., (2003) who reported the amylose content of high amylose corn starches 35.9, 34.5 and 31.3 for

different autoclaving temperatures. Because there were more long chains in high-amylose corn starch chains, they were more prone to thermal breakdown than normal or waxy corn starch chains. Additionally, Kweon *et al*. (2000) found that long linear branches of anomalous amylopectin (38%) and regular linear amylose (33%) made up high-amylose corn starch (ae 7Wx), which included 70% amylose. According to their findings, amylomaize starch that included 50% amylose contained 30% amylose, 40% amylopectin peculiar to amylomaize, and 30% intermediate material, which did not meet the criteria for either amylose or amylopectin. SACS and COCS starches had complex indices of 66.7 and 83.4, respectively. The process that involves the development of a starch-iodine complex and the measurement of starch that is not complexed with lipids is used to quantify starch-lipid complexes and calculate the complexing index (CI). The amount of starch complexed to the iodine is correlated with the absorbance (Kaur and Singh, 2000). The helical cavity of amylose is home to linear complexing agents, such as fatty acids, monoglycerides, or linear alcohols, which typically induce its structure (Nuessli *et al*. 2003). There was a strong correlation between SACS and COCS's amylose concentration and Complex Index. The chemical composition is simple and convenient way to illustrating the purity of the starch extracts, whereby higher starch and lower contents of other components (fat, ash, fibre) are highly desirable (Ikegwu *et al*., 2010). The result indicate that the ash, fat and fibre content of SACS corn starches was comparatively less than the NCS and COCS starches which it clearly indicates that SACS is highly pure than NCS and COCS corn starches. The result also implied that NCS is less pure than COCS and SACS corn starches.

Functional Properties of Native and Treated Corn Starch

The Table 2 shows the water and oil absorption capacity of the RS 5 samples produced from different treated conditions. Water absorption capacity represents the ability of a substance to associate with water under a limited water condition (Abida Ali *et al*., 2016). The water absorption index (WAI) measures the volume occupied by the starch after swelling in excess water, and indicates the integrity of starch in aqueous dispersion Magali Leonel *et al*., (2009). Water absorption capacity (WAC) of native corn starch and treated corn starch was 1.61, 1.37, and 2.01 (g/g) respectively. The RS 5 content mainly composed of retrograded amylose content which is joined tightly by a hydrogen bond. Due to the tight bond within the insoluble crystalline structure RS 5 is not able to form a hydrogen bond with the water molecules (Sajilata *et al*., 2006). The differences in WAC of starches from different types could probably be attributed to the variation in their granule structure. The engagement of hydroxyl groups to form hydrogen and covalent bonds between starch chains lowers WAC (Hoover and Sosulski, 1986). The loose association of amylose and amylopectin molecules in the native starch granules has been observed to be responsible for high WBC (Soni *et al*., 1987). The (WAC) water absorption capacity of stearic acid treated corn starches significantly decreased levels than the native and corn oil treated starches. Hence, RS 5 of stearic acid treated corn starches in food can exert both nutritional benefit and improve the property of the food. The oil-Absorption capacity (OAC) property (Table 1) of the corn RS5 starch samples produced from different treatments. The OAC was used to measure the ability of starches to hold the oil. The oil absorption capacity observed in RS 5 of native corn starch was significantly ($p<0.05$) higher than corn oil and stearic acid treated starches. OAC is the ability of the dry starch to physically bind fat by capillary attraction and it is of great importance, as fat acts as flavor retainer and also increases the mouth feel of foods (Abida Ali *et al*., 2016). According to Hutton and Campbell (1981), food's capacity to absorb water and oil may improve sensory qualities like mouthfeel and flavor retention. Accordingly, this statement suggests that the OAC of NCS was higher than that of COCS and SACS, while the WAC of RS 5 COCS starches was higher than that of NCS and SACS. It demonstrates that the mouthfeel and flavor retention of these COCS and NCS are higher. The physical trapping of oil and the binding of fat to the apolar chain of proteins are the primary mechanisms responsible for oil absorption (Kinsella, 1979).

Functional Properties of Native and Treated Corn Starch

The results of starch swelling power of NCS, SACS and COCS at different temperatures are presented in Table 3. Swelling power of the starch granules was raised gradually from 40°C to 90°C. At higher temperatures (>70° C), a sudden increase in swelling power was observed in all the starch samples. For NCS and treated starch samples, the swelling profile exhibits a consistent general tendency of increasing with temperature. This is an indication of the granules' capacity to absorb water when heated. According to Charles *et al*. (2007), this could be explained by the

breakdown of starch granules at higher temperatures, which causes all of the amylose to be released from the amylopectin network. All of the starch samples had a strong swelling power at higher temperatures. As the temperature rises, the starch granules' absorption of water causes swelling to worsen (Charles *et al*., 2007).Given that an increase in swelling power indicates that a starch is suitable for use as a disintegrant in the pharmaceutical industry (Chowdary *et al*., 2011), NCS, SACS, and COCS seem to be better options for disintegrants in tablet formulation, particularly since tablets are made and used at temperatures where they have superior swelling properties. The application of high amylose corn starch NCS, SACS, and COCS in other industries is confirmed by the fact that high swelling power also leads to high digestibility and the capacity to use starch in solution, indicating improved dietary properties and the use of starch in a variety of dietary applications (Nuwamanya *et al*., 2010). As the temperature increased to 60°C, the solubility (%) of NCS, SACS, and COCS starch increased steadily. At increasing temperatures, the solubility gradually reduced in all of the starch samples. Solubility increased as the temperature increased because of increase in mobility of the starch granules, which facilitated dispersion of starch molecules in water. The change in functional properties of starch with respect to temperature has been attributed due to amylose and amylopectin content of the starch. The results show that increase of straight chain amylose content increases solubility of NCS, SACS and COCS starches. The solubility and swelling power of high amylose corn starch could be explained by its granule size and amylose content.

Indeed, starch with large granules swells rapidly when heated in water and water molecules are bonded to the free hydroxyl groups of amylose and amylopectin by hydrogen bonds (Singh *et al*., 2003). The swelling power and solubility was comparatively less when compared with the cassava and potato starches works reported by Eke-Ejiofor and Owuno, (2014). According to Hoseney (1987), amylose also functions as a diluent and an inhibitor of swelling, particularly when lipids are present and can form insoluble complexes with some of the amylose during swelling and gelatinization. The crystalline bundles known as micells, which are formed by hydrogen bonding between the starch molecules, have been used to demonstrate the associative binding force within the granules (Leach *et al*., 1959). When an aqueous suspension of starch granules is heated, these structures become hydrated and swelling occurs. According to Schoch and Maywald (1968), starches have been classified as high swelling, moderated swelling, restricted swelling, or highly restricted welling. High swelling starches have swelling power of approximately 30% or higher at 95°C. Their granules swell enormously and the internal bonds become fragile toward shear when the starch is cooked in water. Restricted swelling starches have swelling power in the range of 16-20% at 95°C. The cross linkages in their granules reduce swelling and stabilize them against shearing during cooking in water (Galvez and Resurreccion, 1993). The high amylose corn starch NCS, SACS and COCS starch had swelling power of 2-9% respectively. Therefore, the resulting swelling power indicated that the starch of high amylose corn starch was highly restricted type according to Schoch and Maywald (1968).

Structural analysis of Native and Treated Corn Starch

Scanning Electron Micrograph of Native and Treated Corn Starch

Using scanning electron microscopy, morphological analyses of NCS, SACS, and COCS starch granules (Fig. 1, 3, 4) showed surface alterations and granule breakage in SACS and COCS corn starches compared to NCS corn starches. High amylose NCS corn starch shows various granules shaping such as round, rod shaped and polygonal shapes. In contrast to the NCS starch granules, the SACS and COCS treated starch granules have irregular forms and rougher surfaces, while the granules' surface is smooth and free of pores and visible fissures. It might be because the combination of corn oil and stearic acid alters the surfaces of the starch granules. Significant fragmentation and a drastic change in granule form indicate that the starch granules' structural integrity was disrupted during the esterification process (Hoover, 2000). After the amylose migrated during the gelatinization process, the starch granule structures collapsed, leaving behind these irregular particles. Improved contact between the granules and the polymer matrix is made possible by the porous structure. Furthermore, as noted by Doi *et al*. (2002), the opened-up structure of starch following modification permits the starch's inherent high functionality to undergo a range of chemical modification. The properties of starch granules are affected by the presence of these proteins and lipids in or on the surface of the granules. For example, a high amount of lipids in cereals starches decreases the capacity of starch granules to bind with water, thus reducing the swelling and solubilisation of starch granules (Swinkels, 1985).

Esterification of starch may affect the morphology of the material (Steeneken, 2008). The esterification or acid treatment leads to morphological changes of starch granules including the appearance of pores and cracks on the granular surface as reported by the (Jayakody and R. Hoover, 2004). The scanning electron microscopy supported that findings of the SEM images revealed the disrupted structure of the granules after esterification (SACS and COCS) when compared with NCS starches.

Light Micrograph of Native and Treated Corn Starch

The light micrograph of NCS, SACS and CACS treated corn starch, iodinated were given in Figure (2 - 6), (7-8), (9-10). The granular structure of NCS, SACS and COCS corn starches were viewed under light microscope. The high amylose normal corn starch shows round and polygonal shaped starch granules. The high amylose normal corn starch observes the iodine solution which it turns the starch granules into light blue in colour. The shapes of the high amylose normal corn starch granules were round, polygonal and smooth particles were observed. The stearic acid treated corn starch (SACS) and corn oil treated corn starch (COCS) revealed irregular starch bodies. These irregular shapes of SACS and COCS corn starches were due to the addition of pullulunase and gelatinization treatments which either erodes or modify the shape of the starch granules or digest channels from selected points on the surface toward the center of the granule (Matsubara *et al.,* 2004). Five patterns of attack have been identified in the earlier studies by Evers (1979) who stated that the attacks were pin-holes/pepper-potting, sponge-like erosion, many medium-sized holes, distinct loci leading to single holes in individual granules and surface erosion. The iodinated starch granules of SACS and COCS were stained with iodine solution. The treated starch granules observes the iodine solution which it turn into dark blue and slightly pink in colour with irregular shaped starch granules.

SUMMARY AND CONCLUSION

Hence it was concluded that high amylose corn starch treated with corn oil was better than the NCS and SACS starches in terms of it, Chemical composition, functional and structural features. Thermal Characteristics. Compared to the B- and C-polymorphic starch granules, the RS-5 V type was much more heat stable. It was proved in this study that the treated SACS and COCS corn starches are highly heat stable than the NCS starches. The morphological alterations of starch granules, such as the emergence of pores and fissures on the granular surface, are caused by esterification or acid treatment. Scanning electron microscopy confirmed that, in comparison to NCS starches, 48 SEM pictures showed the granules' altered structure during esterification (SACS and COCS). High-amylose maize starch might be processed with FFAs to create a novel Type 5 resistant starch (RS5), which would then be employed in food formulation.

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Table. 1 : Chemical composition of Native and Treated Corn Starch

Chemical Composition	Native Corn Starch (NCS)	Stearic acid treated Corn Starch (SACS)	Corn Oil treated Corn Starch (COCS)
Starch Complex Yield (%)	ND	44.4±0.01^{a}	51.1±0.20^{b}
Moisture (%)	8.62±0.20^{a}	7.56±0.54^{b}	7.63±0.49^{b}
Ash (%)	0.09±0.00^{a}	0.11±0.01^{a}	0.16±0.01^{b}
Crude fibre (%)	0.73±0.20^{b}	0.48±0.00^{a}	0.83±0.02^{c}
Crude Fat (%)	0.26±0.11^{a}	0.26±0.11^{a}	0.53±0.11^{b}
Ph	6.19±0.01^{a}	6.17±0.02^{a}	6.20±0.03^{a}
Amylose (%)	33.6	35.2	36.8
Complex index (%)	ND	66.7	83.4

ND- Not Determined. All values are means of triplicates ± standard deviation. Means in the same row with different letters are significant different ($p<0.05$).

Table 2: Functional Properties of Native and Treated Corn Starch

Functional Properties	**Native Corn Starch (NCS)**	**Stearic acid treated Corn Starch (SACS)**	**Corn Oil treated Corn Starch (COCS)**
Water Absorption Capacity (g/g)	1.61±0.09^{a}	1.37±0.20^{a}	2.01±0.05^{b}
Oil Absorption Capacity (g/g)	1.64±0.09^{a}	1.28±0.06^{b}	1.39±0.02^{b}

Table 3: Swelling and Solubility of Native and Treated Corn Starch

Functional properties g/g	**Native Corn Starch (NCS)**	**Stearic acid treated Corn Starch (SACS)**	**Corn Oil treated Corn Starch (COCS)**
40°C	2.00±0.05^{a}	2.06±0.05^{a}	2.12±0.05^{a}
50°C	4.01±0.01^{a}	4.06±0.00^{b}	4.21±0.05^{c}
60°C	5.00±0.05^{a}	5.02±0.00^{a}	5.14±0.02^{b}
70°C	7.11±0.01^{a}	7.16±0.04^{b}	7.22±0.01^{c}
80°C	8.10±0.00^{a}	8.14±0.00^{b}	8.22±0.00^{c}
90°C	9.01±0.00^{a}	9.05±0.00^{b}	9.12±0.05^{c}
Solubility (%)			
40°C	2.01±0.00^{a}	2.03±0.00^{a}	2.13±0.00^{b}
50°C	4.01±0.00^{a}	4.02±0.00^{a}	4.15±0.01^{b}
60°C	6.08±0.00^{a}	6.09±0.00^{a}	6.15±0.02^{b}
70°C	5.01±0.01^{a}	5.13±0.02^{b}	6.06±0.00^{b}
80°C	4.01±0.00^{a}	4.03±0.00^{a}	4.12±0.01^{b}
90°C	3.01±0.00^{a}	3.02±0.00^{a}	3.16±0.00^{b}

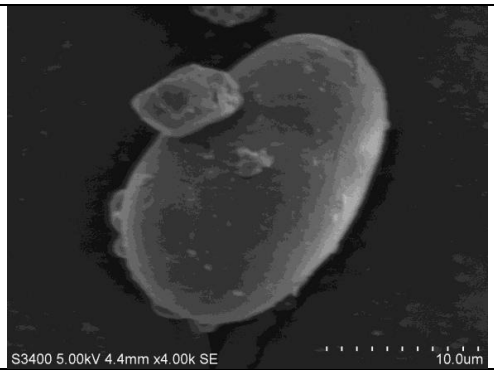


Fig. 1 Normal Corn Starch (NCS) at Starch (NCS) at 1000 X magnification

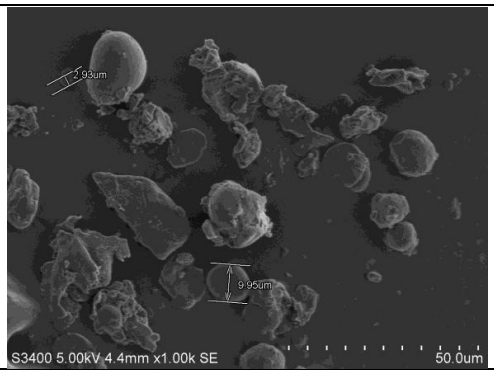


Fig. 2. Normal Corn 6000 X magnification

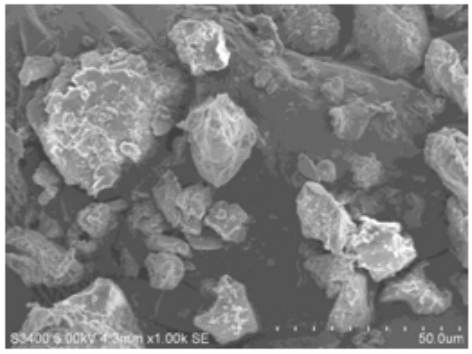


Fig. 3. Stearic acid treated (SACS) at 1000 X magnification

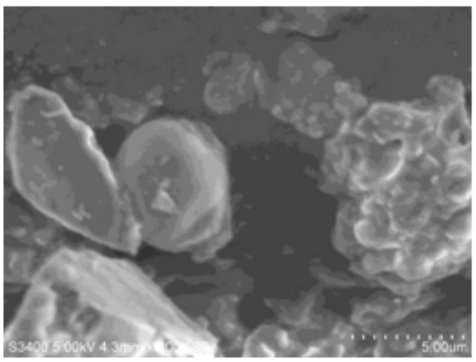


Fig. 4. Stearic acid treated Corn Corn Starch Starch (SACS) at 3000 X magnification

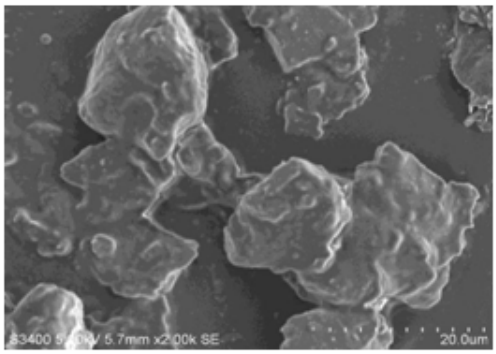


Fig. 5. Corn Oil treated Corn treated Corn Starch (COCS) at 1000 X magnification

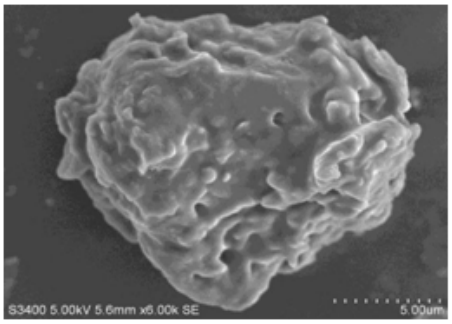


Fig. 6. Corn Oil Starch (COCS) at 6000 X magnification

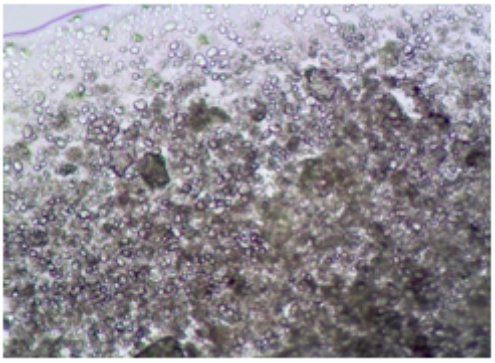


Fig. 7. Normal Corn Starch (NCS) at 10X Corn Starch Magnification

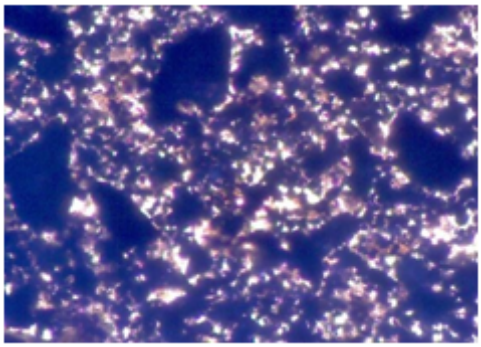


Fig. 8. Iodinated Normal (NCS) at 10X

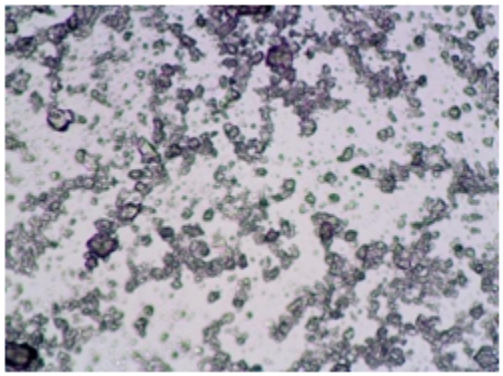


Fig. 9. Stearic Acid Treated Corn Starch Acid Treated (SACS) at 10X Magnification

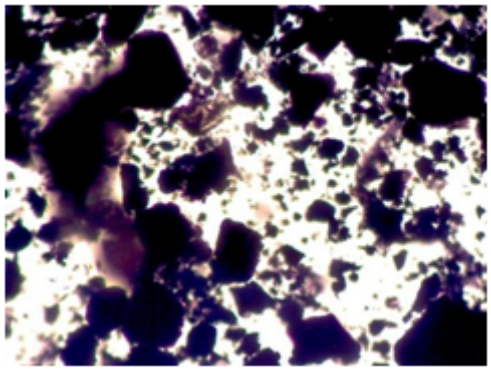


Fig. 10. Iodinated Stearic Corn Starch (SACS) at 10X Magnification

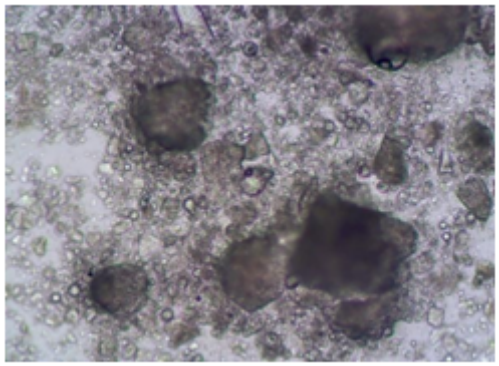


Fig. 11. Corn Oil Treated Corn Starch Oil Treated (CACS) at 10X Magnification

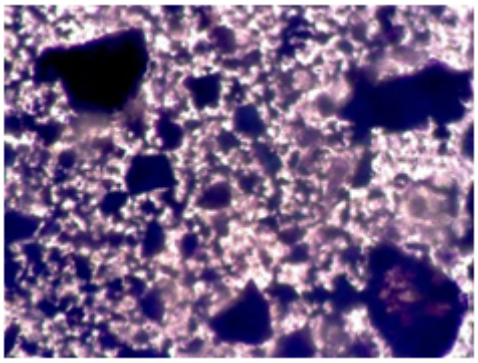


Fig. 12. Iodinated Corn
Corn Starch (CACS) at10X Magnification

CASE STUDY ARTICLE

An Integrated Novel Approach to Management of Huge Thrombosed Hemorrhoids - A Successful Case Report

Poornima Jalawadi[1], Priyadarshini Karanji[2]* and Chaitra. S[3]

[1]Associate Professor, Department of Shalya Tantra, JSS Ayurveda Medical College, Alanahalli, Mysuru, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru) Karnataka, India.
[2]Second Year PG Scholar, Department of Shalya Tantra, JSS Ayurveda Medical College, Alanahalli, Mysuru, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru) Karnataka, India.
[3]Associate professor, Department of Rachana Sharir, JSS Ayurveda Medical College, Alanahalli, Mysuru, (Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru) Karnataka, India.

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***Address for Correspondence**
Chaitra. S
Second Year PG Scholar,
Department of Shalya Tantra,
JSS Ayurveda Medical College,
Alanahalli, Mysuru,
(Affiliated to Rajiv Gandhi University of Health Sciences, Bengaluru)
Karnataka, India.
E.Mail: priyadarshinikaranji1998@gmail.com

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ABSTRACT

Ksharasutra is the contribution of Acharya Sushruta and has mentioned its application in Nadivrana, Arbuda, and Arshas in Sushruta Samhita. It is a safe, cost-effective, minimal hospital stay, and the chance of recurrence rate is less for treating arshas with fewer side effects. In this study, a patient suffered from 4th-degree interno-external thrombosed pile masses at 3,5,7 o'clock and 3rd-degree interno-external pile mass at the 11 o'clock position. Under spinal anesthesia , all the pile masses were ligated with Ksharasutra except at 7 o'clock internal hemorrhoid which was treated by Ksharalepa. The post-operative assessment was done daily; all pile masses had fallen by the 10th postoperative day, and the wound was healed within a month.

Keywords: Integrated Medicine, Ksharasutra, Thrombosed haemorrhoids Transfixed. Apamarga

INTRODUCTION

Arshas (hemorrhoids) are dilated veins within the anal canal in the sub-epithelial region formed by radicals of superior, middle, and inferior rectal veins and often described as varicose veins of the anus and rectum which is

considered a common disease mostly due to life style disorders, especially during advancing age [1]. Arshas is included in the eight morbidities that pose significant treatment challenges [2]. It occurs in Guda Pradesha (Anal canal). Guda is included in Sadyopranahara Marma which requires delicate management. At present, different types of treatment modalities like rubber band ligation, sclerosing injection therapy, infra-red coagulation therapy, haemorrhoidectomy, cryo-surgery, etc. are available [3]. There may associate with complications like incontinence, haemorrhage, and anal stricture [4]. Acharya Sushruta has described the ligation of Ksharasutra in Nadivrana (sinus), Arbuda, and Pratisaraniya Kshara (local application of Kshara) in the treatment of Arshas.

Case history

A 31-year-old married male, sale man by occupation, with IP no and OP no 184497/54591 visited our OPD on 24/08/2024 with complaints of previously bulged multiple masses per rectum that have turned into hard, big, painful masses which were not going inside even after pushing, following an episode of constipation associated with bleeding per rectum since yesterday.

History of Present Illness

As per the statement of the patient, he was healthy 1 year ago. He gradually noticed painless masses that used to go inside on their own after defecation, associated with occasional bleeding per rectum. Over the year, the masses had gradually grown in size and required manual pushing by the patient which he neglected and did not seek any medical advice. Since yesterday the masses had increased in size with severe pain and bleeding per rectum following an episode of constipation. Masses were not going inside even after pushing, hence he approached the Shalya Tantra department, JSS Ayurveda Hospital for further management. On enquiring, he said that his job demanded traveling on a two-wheeler up to 60km per day. On further interrogation, the patient revealed that he was not a known case of Diabetes or hypertension, but gave a history of addiction to alcohol and smoking and he had consumed non vegetarian food with alcohol on 22/08/2024 and parties till late night.

History of Past Illness

Medical history: N/K/C/O T2 DM and HTN.
Surgical history: Nothing contributory. s
Family history: All are said to be healthy.

Personal history

Diet: Mixed diet.
Sleep: 6-7 hours–Sound sleep.
Bowel: One time a day (constipated).
Micturition: 4 – 5 times a day.
Habit: Occasionally alcohol and smoking since 4 year.

Ashta sthanapariksha

Nadi - 76 bpm (Pitta-kapha)
Mala – 1 time a day.
Mutra - 4 -5 times a day.
Jihwa – Alipta.
Shabda – Prakruta.
Sparsha – Anushnasheeta.
Drik– Prakruta.
Akriti – Madhyama.

General examination

Pallor: Absent
Icterus: Absent

Cyanosis: Absent
Koilonychia: Absent
Lymphedema: Not palpable
Edema: Absent
Blood pressure: 130/90mmHg
Respiratory cycle: 16 cycles/min
Heart rate: 74 BPM

Systemic Examination
CNS
Higher mental function test: Conscious well oriented with time place person.
Memory: Recent and remote: intact
Intelligence: Intact
Hallucination/delusion/speech disturbance: Absent
Cranial nerve / sensory nerve/motor system: Normal
CVS
Auscultation: S1 and S2 heard, no murmur/added sound
RS
Inspection: B/L symmetrical
Palpation: Trachea is centrally placed and non-tender
Auscultation: B/L NVBS heard
GIT
Inspection: No scar marks, No discoloration
Palpation: Soft, non-tender.
Auscultation: Normal peristaltic sound heard
Percussion: Normal resonant sound heard over the abdomen

LOCAL EXAMINATION
On inspection
Pile mass at 3,5,7,11
Discoloration – no
Any scar mark- no
On palpation
a) Per rectal examination
Sphincter tone- Slightly laxed
Induration – absent

Pre-op
The informed written consent was taken from the patient.
The patient was advised nil by mouth from 7:30 am on the day of surgery.
Part preparation was done in the morning on the day of surgery.
A Proctoclysis enema was given in the morning on the day of surgery.
Inj. Tetanus Toxoid,0.5ml IM was given as a prophylactic measure
A sensitivity test was done with 2% Inj. Lignocaine 0.5cc subcutaneously in the morning on the day of operation.

Operative procedure
Under all aseptic precautions, the patient was made to lie in a lithotomy position after spinal anaesthesia. The operative part was painted with povidone iodine and drapped. Digital rectal examination and two-finger anal dilation was done using Lignocaine jelly (2%). The proctoscopy was done to visualize and re-assess the positions of

haemorrhoids. Apamarga ksharasutra was transfixed simultaneously at interno-external thrombosed haemorrhoids extending from 3 to 5 o'clock positions along with thrombosed external haemorrhoids at 7 o'clock position. This huge masses re-secured by tying another Ksharasutra. The distal part of the transfixed haemorrhoidal mass was excised and sent for histopathological examination. The ksharalepa was done at an internal pile mass of 7 'o'clock position. The 11 o'clock interno-external pile mass was also ligated with ksharasutra. A Diclofenac suppository was inserted followed by anal packing with Mahanarayana Taila and dressing was done.

Post-operative

The patient was kept nil by mouth for six hours and intravenous fluid of Ringer Lactate and Dextrose Normal Saline was given.
A sip of water was allowed followed by a glass of ganji after an appropriate bowel sound was heard.
Intravenous injection of metrogyl 100 ml 8 hourly for 5 days was given.
Inj. Tramadol IM was given as per the need of the patient.
Inj. Fortwin 1ml and Inj. Phenargan1ml IM were given at night on the day of surgery.

Differential Diagnosis

1.CA of Rectum
2.Rectal Prolapse
3.Proctitis
4.Haemorrhoids

Ayurvedic medication

Syp. Abhayarista 10 ml BD with 20 ml of water after food.
Syp. Drakshasava 10 ml BD with 20 ml of water after food.
Tab. Pilocid 1-0-1 ater food.
Tab. Styplon 2-2-2 after food.
Tab. Manasa mitravati 0-0-1 after food.
Sitz bath with PVK lotin with lume warm water twice a day.
Taila Purana with 10ml of jatyaditaila twice a day.

DISCUSSION

Acharya Sushruta had mentioned chaturvidha chikistha for Arshas, namely Aushadhi, Kshara, Agni, and Shastra [5]. Among these procedures, we adopted the Ksharasutra procedure because of huge thrombosed haemorrhoidal masses. Generally, the external and internal haemorrhoids are ligated all together to prevent the post operative bleeding. But in this case the mass was huge and congruent from 3 to 7 'o clock position externally where as the internal component was well demarcated between 3 to 5 ' o clock position and separately at 7 'o clock position. Hence, Apamarga ksharasutra was transfixed simultaneously at interno-external thrombosed haemorrhoids extending from 3 to 5 o'clock positions along with thrombosed external haemorrhoids at 7 o'clock position. The ksharalepa was done for internal pile mass at the 7 o'clock position. The 11 o'clock interno-external pile mass was also ligated with ksharasutra. Apamarga Ksharasutra blocks the blood flow to pile masses, leading to pressure necrosis, mechanical strangulation, then the death of pile mass, and kshara to better fibrosis of pile mass.

The masses fell on their own accord by the 10th postoperative day. The applied Apamarga Kshara on the thread acts as an anti-microbial, anti-inflammatory and promotes healing after sloughing out of pile masses. The pH of Ksharasutra is alkaline (pH-10.3); hence, it does not allow the growth of bacteria at the site of ligation. There are no postoperative complications like pain, bleeding, delayed healing, stricture formation, etc. In comparison to haemorrhoidectomy, the Ksharasutra procedure is said to be better as it has minimum post-operative complications,

minimal hospital stay, and a lesser rate of recurrence. Delayed complications like anal stricture and fecal incontinence are not found. The Ksharasutra procedure is a boon to patients suffering from Anorectal diseases because of its remarkable results when compared to the conventional methods.

Patient Perspective

The patient gave consent for the procedure and withstood the procedure well. The patient had pain and burning sensation in the post-operative area for around 5 days which had a declining trend in the Pain scale and gradually coped up well. Hemorrhoidal Masses had fallen by the 10th post-operativeday automatically. Bleeding, Pain, and burning sensation had reduced by almost 80% as per the patient's own statement. He had also quit smoking and alcohol and changed his lifestyle after the surgery & followed Pathya-Apathya as per the instructions given. The patient has recovered well after the procedure & was satisfied with the treatment provided.

CONCLUSION

The main benefits of the ksharasutra procedure include minimal tissue loss, cost-effectiveness, a shorter hospital stay, and a lower chance of recurrence. This case report discusses the successful integrated treatment of huge thrombosed internal and external haemorrhoids using Apamarga ksharasutra transfixation in a novel method where the pile masses were ligated individually. The result observed was encouraging without any complications. Further study can be carried out on the safety and efficacy of this novel technique.

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Table 1. Diagnostics

Type	Value	Unit
2024-08-24		
Albumin	3.6	g/dL
ALP(alkaline phosphatase)	110	IU/L
ALT(alanine aminotransferase)	43	U/L
AST(aspartate aminotransferase)	25	U/L
Bilirubin, conjugated(direct)	0.2	mg/dL
Bilirubin, total	0.2	mg/dL
BUN(blood urea nitrogen)	12.7	mg/dL

Creatinine	1.1	mg/dL
Eosinophils	2	%
ESR(erythrocytes sedimentation rate)	12	mm/1st hour
GGT(gamma-glutamyl transferase)	41	U/L
Hb(Haemoglobin)	15.8	g/Dl
HbA1C(haemoglobin A1C)	5.9	%
HBsAg(hepatitis B surface antigen)	Negative	
Lymphocytes	30	%
Monocytes	5	%
Neutrophils	63	%
Platelet count	2.57	thousand cells/cubic mm
Protein, total	8.5	g/Dl
WBC (total white blood cells)	7500	cells/mm^3





Fig. 1. Hemorrhoidal masses at 3, 5, 7, and 11 o'clock positions.

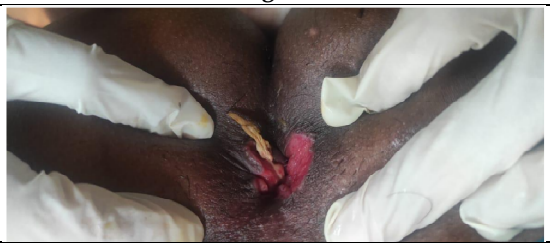


Fig. 2. On the 7th post-operative.



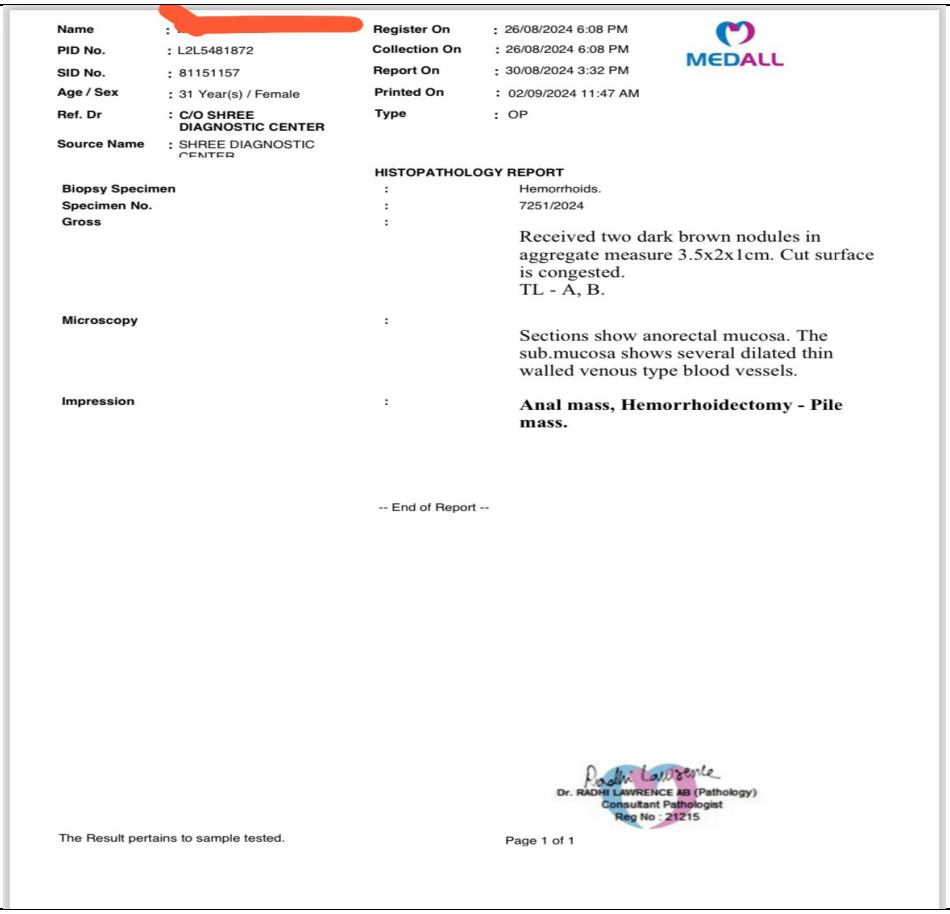
Fig. 2. On 10th post operative day



Fig. 3. 5th post operative day



Fig. 4. 30th post operative day



Name	: [redacted]	**Register On**	: 26/08/2024 6:08 PM
PID No.	: L2L5481872	**Collection On**	: 26/08/2024 6:08 PM
SID No.	: 81151157	**Report On**	: 30/08/2024 3:32 PM
Age / Sex	: 31 Year(s) / Female	**Printed On**	: 02/09/2024 11:47 AM
Ref. Dr	: **C/O SHREE DIAGNOSTIC CENTER**	**Type**	: OP
Source Name	: SHREE DIAGNOSTIC CENTER		

MEDALL

HISTOPATHOLOGY REPORT

Biopsy Specimen	:	Hemorrhoids.
Specimen No.	:	7251/2024
Gross	:	Received two dark brown nodules in aggregate measure 3.5x2x1cm. Cut surface is congested. TL - A, B.
Microscopy	:	Sections show anorectal mucosa. The sub.mucosa shows several dilated thin walled venous type blood vessels.
Impression	:	**Anal mass, Hemorrhoidectomy - Pile mass.**

-- End of Report --

Dr. RADHI LAWRENCE MB (Pathology)
Consultant Pathologist
Reg No : 21215

The Result pertains to sample tested.

Page 1 of 1

Fig. 5. Histopathology reports.

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RESEARCH ARTICLE

Benchmarking ML Models for Software Defect Detection using NASA Data

S.S. Prasanna Vengatesan[1]* and N. Balajiraja[2]

[1]Research Scholar, Department of Computer Science, J.J College of Arts and Science (Autonomous), Pudukkottai, (Affiliated to Bharathidasan University, Tiruchirappalli), Pudukkottai, India.
[2]Assistant Professor, Department of Computer Science, J.J College of Arts and Science (Autonomous), Pudukkottai, (Affiliated to Bharathidasan University, Tiruchirappalli), Pudukkottai, India.

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***Address for Correspondence**
S.S. Prasanna Vengatesan
Research Scholar,
Department of Computer Science,
J.J College of Arts and Science (Autonomous), Pudukkottai,
(Affiliated to Bharathidasan University, Tiruchirappalli),
Pudukkottai, India.
E.Mail: ssprasanna2020@gmail.com

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ABSTRACT

Identifying software defects early is a fundamental aspect of software engineering that helps improve product quality and reduce the effort required for maintenance. This research evaluates the performance of several machine learning models for predicting defects, using datasets from NASA. Six widely used classifiers Logistic Regression, Decision Tree, Support Vector Machine (SVM), Random Forest, Gradient Boosting, and k-Nearest Neighbors (k-NN) are tested on various NASA software datasets. The study measures their effectiveness through metrics including precision, recall, F-measure, accuracy, Matthews Correlation Coefficient (MCC), and the Receiver Operating Characteristic (ROC) curve area. Findings reveal that ensemble-based algorithms, particularly Random Forest and Gradient Boosting, achieve better predictive accuracy than the other models. These results highlight the promise of machine learning techniques for enhancing defect prediction and provide valuable guidance for choosing the right algorithms in software quality assurance. Feature selection methods are also analyzed to determine their role in optimizing model accuracy. The comparative analysis offers practical insights for developers aiming to integrate defect prediction tools into their workflow. Overall, this research underscores the growing relevance of AI-driven approaches in maintaining high software reliability.

Keywords: Artificial Intelligence, Explainable AI, Fairness in AI, Clustering Ensemble, BigData, Model-Agnostic Interpretability, Bias Detection, Scalable AI Systems, Ethical AI, Data Transparency.

INTRODUCTION

In modern software development, ensuring software quality is critical to meeting user expectations and maintaining system reliability. One of the key challenges in this domain is the early detection of software defects, which helps reduce debugging time, lowers maintenance costs, and improves overall software performance. Traditional defect detection methods such as manual code reviews and extensive testing are often time-consuming, expensive, and sometimes insufficient for complex software systems. As a result, researchers and practitioners increasingly rely on automated methods to predict software defects effectively. Machine learning approaches have gained prominence in software defect prediction due to their capability to learn from historical data and apply discovered patterns to new software instances. By analyzing software metrics together with past defect information, ML models can classify components as defective or defect-free, supporting timely quality assurance efforts. Various algorithms such as Logistic Regression, Decision Trees, Support Vector Machines, Random Forest, Gradient Boosting, and k-Nearest Neighbors have been applied to this problem, each demonstrating different levels of performance. NASA datasets are extensively used as benchmark resources in software defect prediction research. They contain software modules characterized by multiple metrics, along with defect labels collected from NASA software projects. These publicly available datasets offer a practical foundation for evaluating and comparing the effectiveness and robustness of various machine learning algorithms. Due to the diversity of NASA datasets each representing different projects and complexity levels they offer an excellent platform for comparative analysis of predictive algorithms. This research conducts a thorough assessment of several machine learning classifiers applied to NASA datasets to identify the most accurate and reliable approaches for software defect prediction. The evaluation compares the models using performance metrics such as precision, recall, F-measure, accuracy, Matthews Correlation Coefficient (MCC), and the area under the ROC curve. By understanding the strengths and limitations of these algorithms on NASA datasets, this study aims to guide software engineers in selecting appropriate machine learning approaches tailored to their defect prediction needs. The results of this analysis are expected to contribute valuable insights into improving defect prediction systems, ultimately leading to better software quality management practices. Through efficient defect prediction, organizations can optimize their testing efforts, reduce software failures in production, and deliver higher-quality software products within budget and time constraints.

RELATED WORK

Predicting software defects is a key factor in enhancing the reliability of software systems and reducing the workload involved in testing and maintenance. Over the years, extensive research has focused on leveraging machine learning techniques for defect identification. These techniques analyze historical defect data to categorize software modules as defective or non-defective. Such classification aids in the effective distribution of testing resources and facilitates the early recognition of potential problems in the software development process. Conventional machine learning algorithms like Decision Trees, Naive Bayes, Support Vector Machines (SVM), Random Forest, and K-Nearest Neighbors (KNN) have been widely used in software defect prediction. Decision Trees are known for their simplicity and interpretability, though they may struggle with noisy datasets. Naive Bayes offers fast computation but relies on the assumption of feature independence, which may not accurately reflect real-world software data. SVMs are well-suited for classification problems. Random Forest, an ensemble-based approach, has consistently outperformed several models in past research due to its capacity to mitigate overfitting and accuracy. NASA's PROMISE repository, containing datasets like CM1, JM1, KC1, and PC1, has become a standard benchmark for evaluating software defect prediction models are commonly evaluated using labeled datasets derived from real-world NASA software projects, which serve as reliable resources for empirical research. In a comparative study by Lessmann*et.al.,*(2024), several classification techniques were applied to NASA datasets. The findings revealed that ensemble approaches, especially Random Forest, consistently outperformed other classifiers in terms of both accuracy and stability. Similarly, Khoshgoftaar*et.al.,*(2022) noted that class imbalance and noise in software metrics could significantly affect the prediction capabilities of conventional models. Recent research has focused on enhancing prediction performance through the integration of feature selection, data preprocessing, and ensemble

learning. Malhotra (2022) reviewed empirical studies on defect prediction and emphasized the critical role of data preprocessing and algorithm tuning. Feature selection techniques, such as correlation-based and wrapper-based methods, have been widely adopted to remove irrelevant or redundant features, which often lead to improved model generalization and reduced computational complexity. Hybrid and ensemble learning methods have demonstrated strong capabilities in dealing with complex defect patterns and imbalanced data distributions. These approaches integrate multiple classifiers to capitalize on their individual advantages, resulting in improved model effectiveness. For instance, combining Support Vector Machines (SVM) with Random Forest has yielded better predictive outcomes, especially in scenarios involving noisy or high-dimensional datasets. Such techniques not only enhance classification accuracy but also improve the identification of rare defective cases, which is crucial for real-world software applications. Studies have also explored the use of clustering techniques in conjunction with supervised learning to further enhance defect prediction. Clustering helps in grouping similar software modules, which can then be analyzed using classification algorithms. This semi-supervised approach has proven useful in reducing the impact of data noise and improving overall model stability. In addition, resampling methods like SMOTE are frequently employed to address the issue of class imbalance, which is a common characteristic of software defect datasets. In summary, existing literature indicates that no single machine learning model is universally superior for software defect prediction. However, ensemble and hybrid approaches generally yield better and more reliable results, particularly when applied to real-world datasets like those from NASA. The continuous evolution of preprocessing techniques, algorithmic innovations, and benchmark evaluations is essential for improving the performance and reliability of software defect prediction systems.

METHODOLOGY

The objective of this study is to evaluate the performance of various machine learning algorithms in software defect prediction, using NASA datasets as the foundation. The research follows a structured approach, including data collection, preprocessing, feature selection, model development, and performance assessment. Each step is methodically implemented to ensure accuracy, consistency, and reproducibility of the results.

Data Preprocessing

Before training the models, the data undergoes preprocessing to ensure quality and consistency. Missing values are managed by replacing them with the mean for continuous data or the mode for categorical data, and categorical variables are encoded when necessary. Numerical features are scaled through normalization or standardization, which is particularly important for models like SVM that are sensitive to the range of input values. Additionally, to address the issue of imbalanced classes where defective modules are fewer SMOTE (Synthetic Minority Over-sampling Technique) is applied to generate synthetic samples and balance the dataset.

Feature Selection

Feature selection is performed to reduce dimensionality and improve model performance. Correlation-based feature selection and mutual information are used to retain only the most relevant features. This step helps to eliminate noise and redundant data, enhancing the model's generalization ability.

ML Models

The study evaluates several machine learning models including DT, Naive Bayes, SVM, RF, and K-Nearest Neighbors. A hybrid model combining Random Forest and SVM is also developed to leverage the strengths of both algorithms. All models are implemented using Python libraries such as scikit-learn. Model performance is assessed using metrics including Accuracy, Precision, Recall, F1-Score, and ROC-AUC, with evaluation conducted through 10-fold cross-validation technique is employed to ensure statistical reliability and to avoid overfitting. Performance metrics are averaged across all folds to provide a robust comparison among the models.

Tools and Environment

The experiments are the implementation was carried out in Python utilizing libraries such as Pandas, NumPy, scikit-learn, and imbalanced-learn for data preprocessing and model development. The computations are performed on a standard computing system with adequate processing power and memory to handle the datasets efficiently. This methodology ensures a comprehensive analysis of how different ML techniques perform on real-world software defect data, offering insights into their effectiveness and practical utility. NASA Cleaned Datasets (Table.1)

RESULTS AND DISCUSSION

This section presents the evaluation outcomes of various machine learning models applied to software defect prediction using cleaned NASA datasets. Model performance was assessed through metrics such as accuracy, precision, recall, F1-score, and AUC-ROC, offering a comprehensive view of their capability to differentiate defective from non-defective software modules. CM1 Dataset Results (Table 2) JM1 Dataset Results (Table 3). The classification outcomes of several machine learning algorithms applied to the KC1 and KC3 datasets, respectively. In both datasets, Random Forest emerged as the top-performing model, achieving the highest F-measure values for both defective and non-defective classes. For the KC1 dataset, Random Forest attained F-measure scores of 92.6% for non-defective and 68.6% for defective instances, while in the KC3 dataset; these scores were 93.8% and 70.4%, respectively. Gradient Boosting also delivered robust performance, maintaining strong precision and recall across both classes and closely trailing Random Forest. The Support Vector Machine (SVM) demonstrated balanced results with solid F-measure values, especially for defective modules. In contrast, Logistic Regression and k-Nearest Neighbors (k-NN) showed weaker. These results emphasize the effectiveness of ensemble approaches, particularly Random Forest and Gradient Boosting, in handling class imbalance and improving defect prediction accuracy on NASA's KC1 and KC3 datasets. This contrast further supports the suitability of advanced ensemble techniques, which leverage multiple learners to achieve higher generalization and resilience against noise. The superior performance of Random Forest and Gradient Boosting thus not only reflects their algorithmic strengths but also their adaptability to real-world software defect datasets such as NASA's KC1 and KC3. KC1 Dataset Results (Table 4), KC3 Dataset Results (Table 5). Table 5: KC2 Dataset Results. Table 7 summarizes the classification outcomes of various ML algorithms applied to the MC1 dataset, with a focus on both non-defective and defective software modules. Among the models evaluated, Random Forest yielded the most accurate predictions, achieving a high F-measure of 94.5% for non-defective instances and 71.8% for defective ones, indicating its robustness in handling both classes effectively. Gradient Boosting followed closely, also performing well with strong precision and recall values across both classes. SVM maintained a solid balance, particularly for the defective class, outperforming Logistic Regression and k-NN in terms of F-measure. Decision Tree showed moderate performance, while k-NN and Logistic Regression were less effective in identifying defective modules, exhibiting lower recall and F-measure scores. Overall, the ensemble methods especially Random Forest stood out for their superior ability to predict software defects in the MC1 dataset. Random Forest emerged as the top performer, achieving an F-measure of 93.4% for non-defective and 69.8% for defective instances, demonstrating its strong predictive strength. Gradient Boosting followed closely, showing high precision and recall for both classes, with a 68.2% F-measure for defective samples. SVM delivered reasonable results, balancing precision and recall with a 63.6% F-measure for the defective category. Decision Tree and Logistic Regression showed average performance, while k-Nearest Neighbors (k-NN) had the poorest results, especially for defective modules, with the lowest F-measure of 55.3%. These outcomes reinforce the effectiveness of ensemble methods, particularly Random Forest, in managing class imbalance and accurately predicting defects in the MC2 dataset.

CONCLUSION

This study investigated the performance of various machine learning algorithms for predicting software defects using multiple cleaned NASA datasets. The analysis involved applying classifiers such as Logistic Regression, Decision Tree, Support Vector Machine (SVM), Random Forest, Gradient Boosting, and k-Nearest Neighbors (k-NN) across well-known datasets including CM1, JM1, KC1, KC3, MC1, MC2, MW1, and PC1–PC5. Results demonstrated

that ensemble-based methods, particularly Random Forest and Gradient Boosting, consistently delivered superior results across evaluation metrics like accuracy, F-measure, Matthews Correlation Coefficient (MCC), and ROC-AUC. These models effectively addressed class imbalance issues, improving defect detection accuracy and overall model stability. SVM also showed strong performance, especially in terms of ROC-AUC and F-measure, while k-NN performed less effectively, likely due to its sensitivity to noise and imbalanced data. These findings highlight the advantages of ensemble learning for software defect prediction, offering valuable insights for software quality assurance teams aiming to enhance early defect detection and optimize testing efforts. Future research may explore hybrid methods, deep learning architectures, and adaptive sampling techniques to further improve prediction accuracy in highly imbalanced or evolving software environments.

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Table 1: NASA Cleaned Datasets

Dataset	Attributes	Modules	Defective	Non-Defective	Defective (%)
CM1	22	505	48	457	9.50%
JM1	22	10,878	2,102	8,776	19.32%
KC1	22	2,108	325	1,783	15.42%
KC2	22	522	107	415	20.50%
KC3	40	458	52	406	11.35%
MC1	38	9,439	68	9,371	0.72%
MC2	40	161	44	117	27.33%

Table 2: CM1 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	88.6	94.1	91.3
	Defective	52.3	35.4	42.0
Decision Tree	Non-Defective	90.2	92.6	91.4
	Defective	60.8	50.0	55.0
SVM	Non-Defective	91.4	93.0	92.2
	Defective	64.3	54.2	58.8
Random Forest	Non-Defective	93.1	95.2	94.1
	Defective	70.4	60.4	65.0
Gradient Boosting	Non-Defective	92.0	94.5	93.2
	Defective	68.1	58.3	62.8
k-NN	Non-Defective	87.2	91.0	89.0
	Defective	48.7	38.5	43.0

Table 3: JM1 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	89.4	93.5	91.4
	Defective	62.0	50.3	55.5
Decision Tree	Non-Defective	90.1	91.8	90.9
	Defective	65.7	60.2	62.8
SVM	Non-Defective	91.0	92.7	91.8
	Defective	66.8	61.1	63.8
Random Forest	Non-Defective	93.3	94.6	93.9
	Defective	73.2	68.4	70.7
Gradient Boosting	Non-Defective	92.5	93.9	93.2

	Defective	71.5	66.3	68.8
k-NN	Non-Defective	88.0	90.2	89.1
	Defective	58.4	52.6	55.3

Table 4 KC1 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	87.6	91.0	89.3
	Defective	60.2	52.8	56.3
Decision Tree	Non-Defective	88.5	89.8	89.1
	Defective	63.0	59.1	61.0
SVM	Non-Defective	89.7	91.5	90.6
	Defective	65.2	61.0	63.0
Random Forest	Non-Defective	92.0	93.3	92.6
	Defective	70.8	66.5	68.6
Gradient Boosting	Non-Defective	91.4	92.8	92.1
	Defective	69.3	64.7	66.9
k-NN	Non-Defective	86.1	88.7	87.4
	Defective	56.0	50.5	53.1

Table 5: KC3 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	89.2	91.8	90.5
	Defective	64.1	58.3	61.0
Decision Tree	Non-Defective	90.0	92.1	91.0
	Defective	66.2	60.9	63.4
SVM	Non-Defective	91.1	93.0	92.0
	Defective	67.4	62.0	64.6
Random Forest	Non-Defective	93.0	94.7	93.8
	Defective	72.8	68.1	70.4
Gradient Boosting	Non-Defective	92.5	94.2	93.3
	Defective	71.6	67.0	69.2
k-NN	Non-Defective	87.8	90.5	89.1
	Defective	59.7	54.2	56.8

Table 6: KC2 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	85.2	88.1	86.6
	Defective	58.7	52.3	55.3
Decision Tree	Non-Defective	86.4	87.6	87.0
	Defective	61.2	57.4	59.2
SVM	Non-Defective	87.8	89.0	88.4
	Defective	63.0	59.2	61.0
Random Forest	Non-Defective	90.3	91.7	91.0
	Defective	68.5	64.1	66.2
Gradient Boosting	Non-Defective	89.7	91.0	90.3
	Defective	67.3	62.8	65.0
k-NN	Non-Defective	84.1	86.9	85.5
	Defective	55.4	49.8	52.4

Table 7: MC1 Dataset Results

Classifier	Class	Precision (%)	Recall (%)	F-Measure (%)
Logistic Regression	Non-Defective	90.5	92.4	91.4
	Defective	63.2	56.7	59.7
Decision Tree	Non-Defective	91.2	92.8	92.0
	Defective	65.4	59.5	62.3
SVM	Non-Defective	92.3	93.5	92.9
	Defective	67.9	62.8	65.2
Random Forest	Non-Defective	94.0	95.1	94.5
	Defective	74.5	69.3	71.8
Gradient Boosting	Non-Defective	93.4	94.7	94.0
	Defective	72.8	67.5	70.0
k-NN	Non-Defective	88.9	90.8	89.8
	Defective	57.1	51.4	54.1

RESEARCH ARTICLE

Preparation and Evaluation of Nimesulide - Loaded Proniosomal Gel for Targeted Topical Anti-Inflammatory Applications

Akash Gowda V[1], Suma U.S[2]*, R. Zohmingliani[1], Maria Sanjana .A[2] and Sumant[1]

[1]Research Scholar, Department of Pharmaceutics, Mallige College of Pharmacy, Silvepura, Chikkabanavara, (Affiliated to Rajiv Gandhi University of Health Sciences, Jayanagar), Bengaluru, Karnataka India.
[2]Associate Professor, Department of Pharmaceutics, Mallige College of Pharmacy, Silvepura, Chikkabanavara, (Affiliated to Rajiv Gandhi University of Health Sciences, Jayanagar), Bengaluru, Karnataka India.

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***Address for Correspondence**
Suma U.S
Associate Professor,
Department of Pharmaceutics,
Mallige College of Pharmacy, Silvepura, Chikkabanavara,
(Affiliated to Rajiv Gandhi University of Health Sciences, Jayanagar),
Bengaluru, Karnataka India.
E.Mail: sumau316@gmail.com

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ABSTRACT

The present study seeks to formulate and evaluate a proniosomal gel for the topical delivery of nimesulide as a potential therapeutic intervention. Nimesulide, classified as a BCS class II drug, is employed in the treatment of osteoarthritis and inflammation. Nimesulide proniosomes were developed using the coacervation phase separation technique, which involved the combination of lecithin, cholesterol, and various grades of non-ionic surfactants. For each proniosomal formulation prepared, physicochemical properties such as size, shape, encapsulation efficiency, and drug release were evaluated. The objective of the present study was to formulate six topical proniosomal gel formulations with varying concentrations of cholesterol and surfactants. All formulations were assessed for parameters including percentage entrapment efficiency, pH, vesicle size, and in vitro drug release. The vesicles were observed to be spherical and distinct. After nine hours, the in vitro drug release was determined to be 93.59%, with formulation F6 demonstrating superior performance across all evaluation tests. When the drug release data were fitted to various kinetic models, the release from each formulation adhered to Higuchi kinetics. This study illustrates the controlled release of medication following nimesulide encapsulation in proniosomal gel and suggests that nimesulide proniosomal formulations may be utilized for topical drug delivery systems in the treatment of skin infections.

Keywords: Nimesulide, Osteoarthritis, co-acervation phase separation method, Non-ionic surfactants, soya lecithin, cholesterol.

INTRODUCTION

Skin serves as the primary barrier for the delivery of topical medications, and the stratum corneum plays a significant part in this barrier function[1]. Topical medication distribution is limited by the stratum corneum's low permeability. To get around these restrictions and provide control over the distribution of the medicine into the body, a unique drug delivery mechanism is employed[2]. Novel drug delivery methods enable sustained drug release at a predetermined rate, maintaining a comparatively constant effective medication level while minimising unwanted side effects. These cutting-edge drug delivery systems include niosomes, liposomes, lipoproteins, and microcapsules, which can break down gradually to target particular locations[3]. Topical drug administration refers to a localised method of delivering medication through the skin, vaginal, rectal, and ophthalmic routes to any part of the body. Topical drug distribution occurs via the skin, which is one of the human body's most accessible organs for topical administration[4]. Proniosomes are amorphous, free-flowing pro-vesicles that easily transform into niosomes upon hydration. They have a liquid crystalline consistency[5]. The components found in proniosomes mainly phospholipids and non-ionic surfactants have the ability to diffuse into the stratum corneum, disturbing the fluidity of the lipid bilayers producing faulty permeability barrier function to act as penetration enhancers[6]. The ratio of cholesterol to non-ionic surfactant may have an impact on the integrated drug's release properties and entrapment efficiency[7]. When applied to skin in an occlusive situation, proniosomal gel becomes hydrated with the skin's moisture and transforms into niosomes. Proniosomal gel has a lot of promise to lessen medication side effects and boost transdermal drug delivery's therapeutic efficacy[8]. One of the most frequent types of arthritis is osteoarthritis. It can affect almost any joint in the body and is linked to a deterioration of the cartilage in the joints. It commonly affects the weight-bearing joints in the hips, knees, and spine[9]. Inflammation and pain are treated with NSAIDs; however, because they increase the risk of adverse effects in the upper gastrointestinal tract and do not address the underlying pathophysiology of articular disease, they are not very effective in altering the course of the disease or improving quality of life[10]. The purpose of present study to formulate proniosomes as transporters for topical delivery of nimesulide. Proniosomes converts into niosomes upon hydration which can prove as substitute to increase poor skin penetration and residence time of the topical anti- inflammatory drugs.

MATERIALS AND METHODS

Materials

Nimesulide was obtained as a gift sample from G. R. Traders, Hyderabad, span 20, 60, 80 and Tween 80 were purchased from Karnataka Fine Chemicals, Bangalore. Phosphate buffer pH
7.4 were prepared as described in the Indian pharmacopoeia (1996).

Method of preparation

The coacervation phase-separation approach was used to create proniosomes. The following steps were included in the method, which will be carried out in dry, wide-mouthed glass containers
(i) weighing the necessary quantities of cholesterol, soya lecithin, and lipophilic surfactant; (ii) adding the drugs ingredient nimesulide, which is soluble in 100% ethanol; (iii) sealing glass containers to stop solvent loss; (iv) heating the resulting mixture in a water bath at 60–70 °C for approximately 5 minutes while shaking to completely dissolve all of the solid ingredients, particularly the cholesterol and surfactant; (v) adding distilled water that has been preheated to 60 °C (to create a clear liquid); (vi) continuing to warm the mixture at the same temperature for approximately 3 minutes while shaking moderately; and (vii) cooling the liquid mixture at to room temperature and solidifying into a white, creamy proniosomal gel, the liquid combination will be stored in the dark for characterization, getting the proniosomal gel ready.

Preparation of proniosomal gel

Carbopol 934 (1g) was soaked in 100 ml of distilled water for three hours to produce Carbopol gel 1% w/v. The mixture was then put on a mechanical stirrer set at 100 rpm to create a homogenous viscous solution.

Triethanolamine was added in little amounts (two to four drops) until the gel formed. Since 1%w/w of nimesulide is present in the proniosomal gel preparation. Preservatives such as propyl and methyl paraben were utilized.

Characterization of proniosomes and proniosomal gel

The coacervation phase separation method was successfully used to create the proniosomal gel. Several parameters were evaluated for the proniosomes that were prepared. Experimental preformulation was done to maximise the product's acceptance, safety, and stability. Investigations into drug-excipient interactions using FT-IR incompatibility were carried out.

Vesicle size analysis

Proniosome vesicle size was assessed using a SEM and an optical microscope. Under a microscope, the size of each prepared batch of proniosomes was examined[12]. Each batch's proniosomal vesicle size was measured at several locations on the slide using a little drop of proniosomal dispersion, and the average size of the proniosomal vesicles was calculated[13].

Determination of Entrapment Efficiency (EE %)

In a test tube, 0.2g of the proniosomal suspension was collected and reconstituted using 10 ml of pH 7.4 isotonic phosphate buffer. In a sonicator bath, this aqueous solution was subjected to sonication Technique[14]. Centrifugation at 3000 rpm for 30 minutes at 20 °C was used to separate drugs containing niosomes from the mixture. The UV technique was used to measure the amount of unentrapped nimesulide at 205 nm in the 1 ml of supernatant. The percentage of entrapment efficiency was calculated by the following equation[15].

$$\% EE = \frac{\text{Total drug} - \text{Unentrapped drug}}{\text{Total drug}} \times 100$$

Particle size distribution and zeta potential

Using Zeta sizer and Malvern software, the optimised formulation's zeta potential was examined at 25 °C[16]. The zeta potential was measured by dispersing electrodes in a proniosomal solution that had been diluted 100 times with double-distilled water, and setting the voltage at 1.4 V. Each sample was run 3 times and analysis was continued at 25 °C[17].

Drug content analysis

A particular amount of proniosomal dispersion (100 mg) was taken and dissolved in 100 ml of pH 7.4 phosphate buffer. For three hours, the volumetric flask containing the dispersion was shaken on a mechanical stirrer to ensure that the drug was completely soluble[18]. This solution was filtered, and a blank phosphate buffer (pH 7.4) was used for spectrophotometric estimation at 205 nm[19].

% Drug content= practical drug content/theoretical drug content ×100

Evaluation of proniosomal gel

Proniosomal gel formulations that were developed were evaluated using a number of parameters.

Physical parameters

Appearance

The produced gels' colour, homogeneity, and foreign particle presence were assessed.

pH

A digital pH meter was used to measure the pH after 2.5 g of gel were dissolved in 25 milliliters of distilled water.[20]

Spreadability studies

By pressing one gram of proniosomal gel between two clear circular glass slides, the spreadability of each batch of gel was assessed. Leaving them for five minutes allowed for maximum spreading. To express the spreadability, the diameter of the produced circle was measured[21].

Spreadability (g cm/s) (S) = M×L/T

Where, M = weight tied to upper slide, L= length moved on the glass slide, T= time taken[22].

Viscosity and rheological studies

Viscosity and rheological characteristics of proniosomal gel were determined using a spindle 64 in a Brookfield digital viscometer (Model LVDV-E, USA) [23]. The spindle was placed in a beaker containing 100 gram of the gel. At 25°C, the viscosity of the gel was determined at various angular velocities. An average run involved varying the angular velocity between 5 and 25 rpm. The viscosity was determined by averaging two observations[24].

***In-vitro* diffusion studies**

The drug diffusion test is conducted *In vitro* using a Franz diffusion cell with an egg membrane. 25 ml of 7.4 pH PBS were taken in the receiver compartment, and one gram of nimesulide- loaded proniosomal gel was taken in the donor compartment[25]. To keep the volume of the release media constant throughout the experiment, aliquots (1 ml) of the medium were taken out at 1, 2, 3, 4, 5, and 9 hours and replaced with an equivalent volume of the fresh medium PBS 7.4 pH. After passing through what-man filter paper, all samples were examined at 205 nm using a Shimadzu -1800 UV spectrophotometer (Japan)[26].

Release kinetics

The kinetics of the drug release process in each formulation were described using a variety of equations, including the Higuchi's model, the Peppas model, the zero-order rate equation, and the first order rate equation[27 28].

Stability studies

According to accelerated stability, three months of stability testing were performed on the optimized batches of nimesulide proniosomal gel. In the present research, optimized formulations were assessed for pH, drug content, and *In-vitro* drug diffusion investigations, as well as stability studies at 40°C+2°C/75% RH for the first three months[29 30].

RESULTS AND DISCUSSSION

The spectra for a 10 μg/ml solution are illustrated in Figure 1, with the maximum wavelength (λ max) of nimesulide in a pH 7.4 environment identified at 205 nm. Nimesulide adheres to Beer's law within a concentration range of 0.4 - 5 μg/ml in a pH 7.4 solution, exhibiting a regression coefficient (r2) of 0.9973 and a slope (m) of 0.1909. Figure 2 depicts the calibration curve's construction. The melting point of the drug sample was found to be 140°C using the Thiels tube method and 142.22°C via the DSC method. The results confirmed the drug's purity, meeting BP standards within the reported range of 143°C. Figure 5 presented the DSC thermograph of nimesulide. The thermogram for both the optimized formulation and the pure drug was obtained, showing an endothermic peak at 142.22°C for nimesulide. The encapsulated proniosomes optimized formulation F6 exhibited a peak at 127.50°C, lower than the pure drug's endothermic peak, indicating successful encapsulation with a carrier. The pH of nimesulide proniosomal gel formulations was found to range from 5.3 to 6.8, aligning with the typical skin pH range. The viscosities of all gel formulations were within an acceptable range. Spreadability is a crucial aspect of gel drug delivery systems, reflecting how easily the gel can be applied or spread on the skin with minimal shear stress. The spreadability values of the created gel ranged from 6.65±0.65 gm.cm/sec to 11.41±0.95 gm.cm/sec, indicating that all formulations spread easily with minimal shear stress. All six formulations had viscosities between 13940 and 21290 cps at 25 rpm. In comparison to other formulations, the F6 (21290cps) formulation was determined to have superior viscosity. In comparison to the other five formulations, the F6 formulation exhibited the highest drug content, of 94.5% , in the

developed proniosomal gel. FTIR was used to determine the compatibility of the drug excipient. The drug and polymer were the subjects of the FTIR. The polymer FTIR is shown in fig:4, while the FTIR spectra of the pure drug nimesulide revealed distinct peaks at 3277, 3085, 1587, 1515, 740, 1216, and 1315 cm^{-1}. The proniosomal formulation's FTIR spectra show the typical peaks of pure drugs, suggested that there has been no alteration in drug-polymer interaction. This suggested that the drug and the polymer utilized in the proniosome formulation are not potentially incompatible. In Figure 7, the zeta potential of the nimesulide proniosome formulation F6 was measured at –26.3 mV, suggesting enhanced stability. The surface morphology was analysed using scanning electron microscopy (SEM), and Figure 7 presents the SEM images of the proniosome formulation F6. The Malvern particle size analyzer determined the average particle size to be 267.8 nm, with a polydispersion index of 0.375 for the optimized formulation F6. Table 3 details the entrapment efficiency of each ingredient, with F1, F2, F3, F4, F5, and F6 exhibiting efficiencies of 65.86%, 68.97%, 71.80%, 61.86%, 83.25%, and 84.41%, respectively. Formulation F6 demonstrated the highest entrapment efficiency at 84.41%. This high efficiency is attributed to its elevated phase transition temperature, which facilitated the formation of a homogeneous bilayer structure, allowing for complete entrapment of the lipophilic drug. Span 20 and Tween 80 showed relatively lower entrapment efficiencies, characterized by a low phase transition temperature and a liquid state at ambient temperature. In vitro drug release studies using a Franz diffusion cell, as depicted in Figure 9, indicated the cumulative drug penetration for each formulation. Based on these results, Formulation F6 was identified as the optimal formulation, achieving a drug release of 93.59% over 9 hours. Several models, including zero order, first order, and the Higuchi and Korsemeyer Pappas model, were used to fit the results from *In-vitro* diffusion tests. The Higuchi model was determined to have the highest correlation of the optimal formulation F6 (R2=0.9813). The drug release would be caused by the super case-II transport mechanism, as shown by the n value being more than 0.89. The generated optimized formulation (F6) was subjected to accelerated stability studies for three months while being kept at 40 ± 2°C and 75 ± 5% relative humidity. Proniosomal gel formulation F6 stability studies demonstrated that the formulation is stable throughout storage, with minimal changes in pH, drug content, viscosity, and percentage cumulative drug release. The outcome of this research indicates that Proniosomal nimesulide gel formulation is applicable for treating bacterial infections locally and systematically for higher medical aid. Over all conclusion, Nimesulide loaded proniosomal gel formulations were successfully prepared by coacervation phase separation method using surfactant and cholesterol. In order to create the anti-inflammatory proniosomal for topical administration, nimesulide was employed as an anti-inflammatory medication. The six batches of proniosomal gel that were made (F1–F6) were assessed based on a number of factors, including stability, *In-vitro* release, viscosity, entrapment efficiency, and physical assessment. Out of the six proniosomal gel formulations (F1–F6), the F6 formulation was determined to be the best based on the assessment parameters' results. Indicating the extended drug release pattern of formulated proniosomal gel, the F6 formulation of proniosomal exhibits the highest percentage entrapment efficiency (84.41%) and highest drug release in 9 hours (93.59%). After three months, the proniosomal gel formulation F6 remained stable and upon hydration, formed spherical niosome vesicles.

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Table 1: Formulation Table

Sl. no	Formulation	F1	F2	F3	F4	F5	F6
1	Nimesulide (mg)	100	100	100	100	100	100
2	Span 60 (mg)	1600	1800	-	-	-	-
3	Span 80 (mg)	-	-	1600	-	1800	-
4	Span 20 (mg)	-	-	-	-	-	1600
5	Tween 80(mg)	-	-	-	1600	-	-
6	Cholesterol(mg)	200	200	200	200	200	200
7	Soya Lecithin(mg)	100	100	100	100	100	100
8	Ethanol(ml)	1	1	1	1	1	1
9	Phosphate buffer(ml)	1	1	1	1	1	1
10	Carbopol 934(g)	1	1	1	1	1	1
11	Methyl Paraben(mg)	20	20	20	20	20	20
12	Distilled Water(ml)	100	100	100	100	100	100

Table 2: All the prepared batches (Fl to F6) were evaluated visually for, colour, homogeneity.

Batches	Colour	Homogeneity
F1	turbid	Good
F2	turbid	Good
F3	turbid	Good
F4	turbid	Good
F5	turbid	Good
F6	turbid	Good

Table 3: Characterization of proniosomal gel formulations for pH, Spreadability, entrapment efficiency, drug content

Formulation code	pH	Spreadability	% Entrapment efficiency	Drug content (%)
F1	5.6±0.20	6.65±0.65	65.86±1.25	84.53±1.64
F2	5.3±0.15	6.93±0.30	68.97±1.52	87.86±1.95
F3	6.8±0.30	8.25±0.98	71.80±1.73	89.5±1.05
F4	5.9±0.20	6.67±0.76	61.86±1.04	82±1.99
F5	6.7±0.25	7.66±0.68	83.25±1.07	91.1±1.31
F6	6.8±0.36	11.41±0.95	84.41±1.83	94.5±1.38

Table 4: Viscosity studies

Batches	F1	F2	F3	F4	F5	F6
Viscosity (cps) 25rpm	13940	16200	17980	20340	20500	21290

Table 5: Release kinetics optimized formulation F6

Formulation code	Kinetic model(R^2)				n values	Best fit model
	Zero order	First order	Higuchi model	Peppas model		
F6	0.9849	0.6623	0.9813	0.9761	1.010	Zero order/Higuchi model

Table 6: Accelerated stability studies for the optimized formulation F6 at 40±2°C and 75±5%

Months	40°C/75%RH				
	Appearance	pH	Viscosity (cps)	% Drug content	% CDR
Initial	turbid	6.8 ±0.36	21920	94.5±1.38	93.59±0.82
90 days	turbid	6.8±0.23	21850	94.2±1.03	93.05±0.99

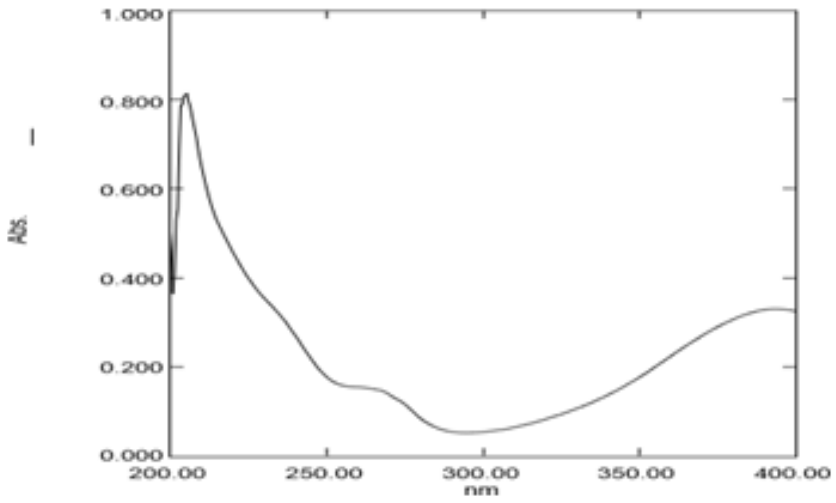


Figure 1: UV Spectrum of nimesulide of 10 µg/ml concentration at pH 7.

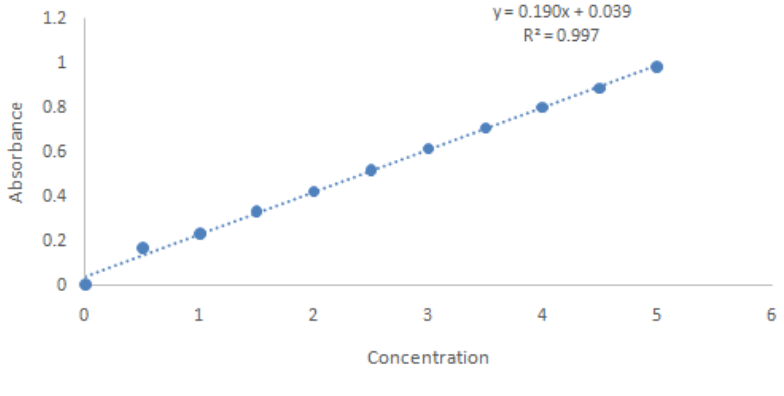


Fig. 2: Standard Calibration curve of nimesulide in buffer pH 7.4

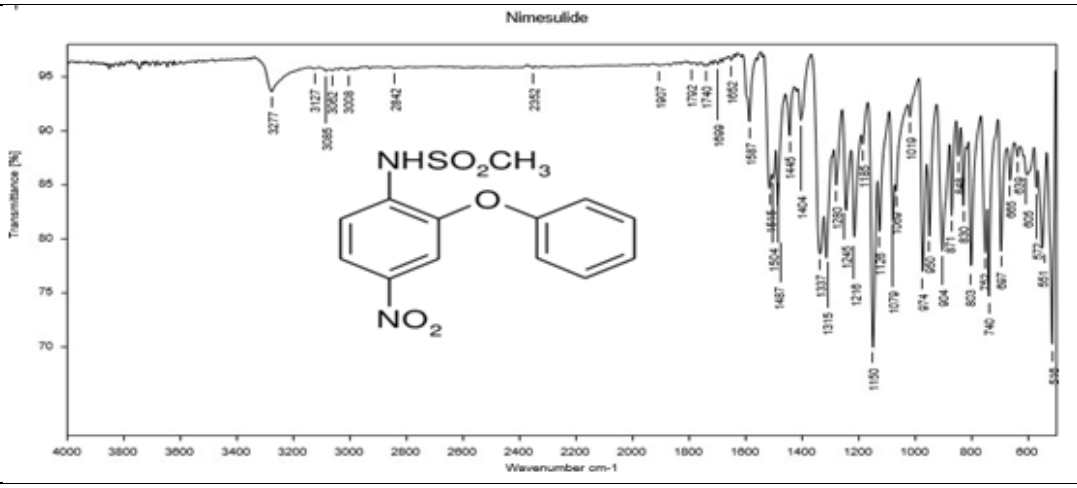


Fig.3: FTIR Spectra of pure Nimesulide

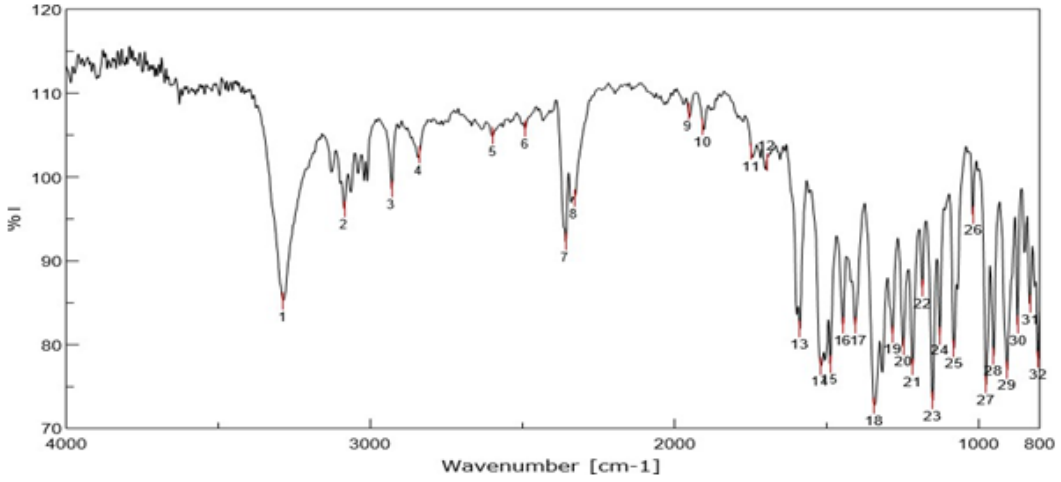


Fig.4: FTIR Spectra of polymer

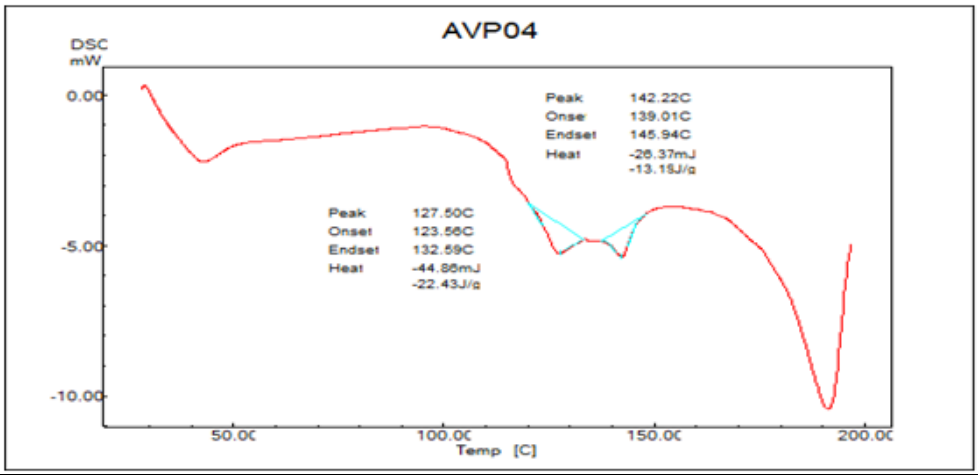


Fig. 5: DSC Thermogram of optimized formulation of proniosomes

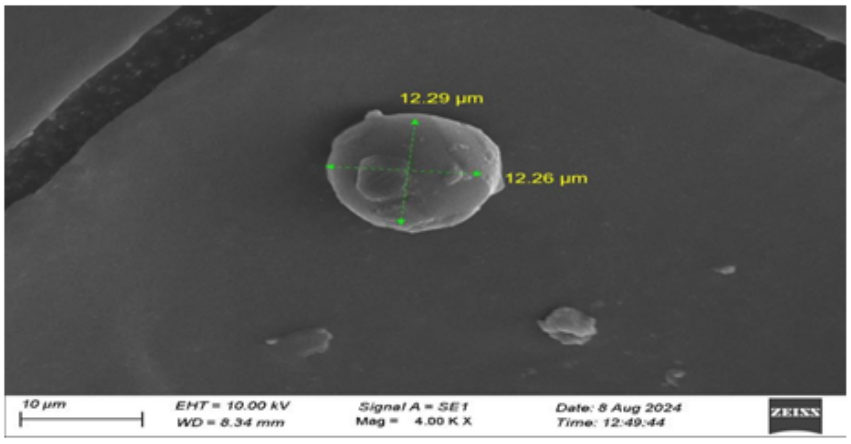


Fig.6: Scanning electron microscopy of optimized formulation F6

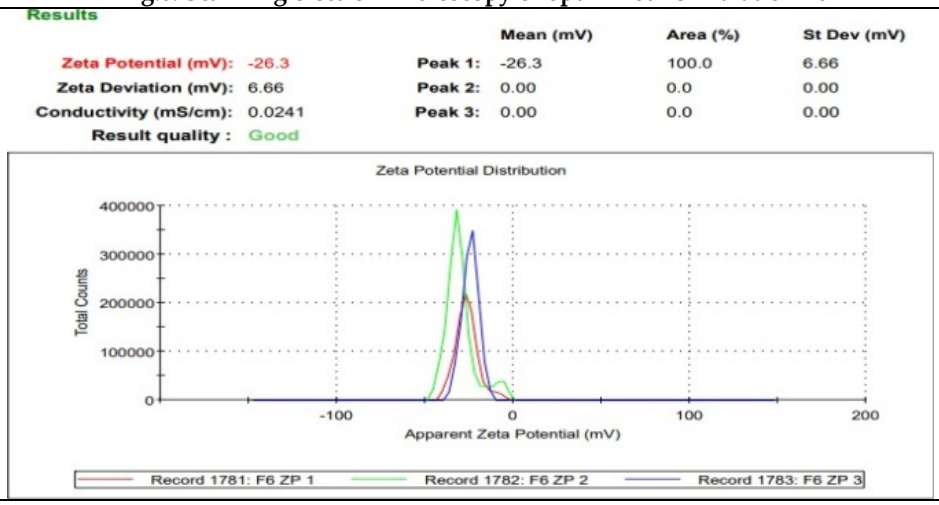


Fig.7: Zeta potential analysis of formulation F6

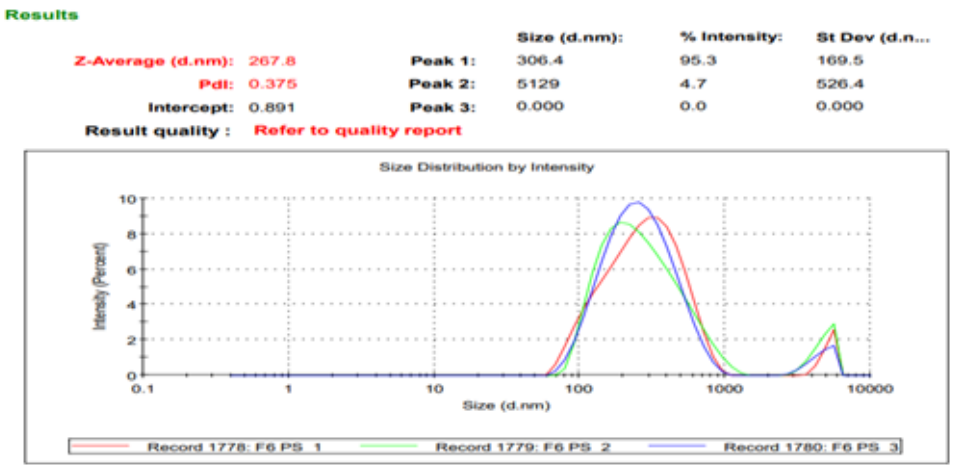


Fig.8: Particle size distribution analysis of formulation F6

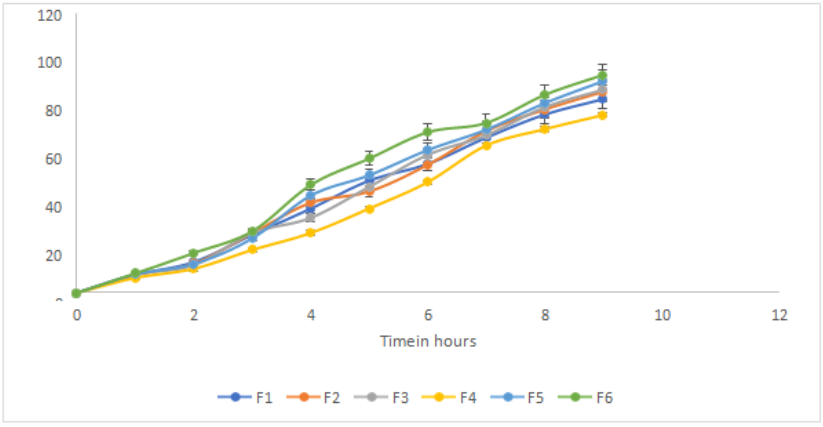


Figure 9: *In-vitro* release profile of nimesulide-loaded proniosomal gel

RESEARCH ARTICLE

Regulatory Issues for the Import of Pharmaceutical Products into India and Brazil

Shaik Haseena Begum*[1] and G. Kavya Latha[2]

[1]Student, Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.
[2]Assistant Professor, Department of Pharmaceutical Analysis, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.

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***Address for Correspondence**
Shaik Haseena Begum
Student, Department of Regulatory Affairs,
Chalapathi Institute of Pharmaceutical Sciences,
(Affiliated to Acharya Nagarjuna University),
Guntur, Andhra Pradesh, India.
E.Mail: hasishaik9030@gmail.com

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ABSTRACT

The importation of pharmaceutical drugs is a critical aspect of ensuring access to high-quality medicines in global markets. However, regulatory frameworks governing drug imports vary significantly across countries, impacting market entry, compliance, and patient safety. This study explores the regulatory challenges associated with the import of pharmaceutical drugs into India and Brazil, two emerging economies with distinct yet complex regulatory landscapes. It provides a comparative analysis of the regulatory frameworks established by the Central Drugs Standard Control Organization (CDSCO) in India and the Brazilian Health Regulatory Agency (ANVISA) in Brazil. Key aspects covered include drug registration, Good Manufacturing Practices (GMP) compliance, dossier requirements, pricing regulations, timelines, and post-market surveillance. The research identifies critical bottlenecks such as lengthy approval processes, stringent documentation requirements, and evolving regulatory policies that impact international pharmaceutical trade. Additionally, the study examines recent regulatory reforms in both countries and their implications for foreign pharmaceutical companies seeking market entry. By highlighting the challenges and proposing strategic solutions, this study aims to aid pharmaceutical manufacturers, regulatory professionals, and policymakers in navigating the complexities of drug importation into India and Brazil. The findings emphasize the need for greater harmonization and streamlined regulatory processes to enhance global pharmaceutical trade and improve patient access to essential medicines.

Keywords: Regulatory Issues; India; CSDCO; ANVISA; India; Brazil; Import; Pharmaceutical products.

INTRODUCTION

The global pharmaceutical industry plays a critical role in ensuring public health and well-being by providing access to essential medications. As markets become increasingly interconnected, the import of pharmaceutical drugs has gained prominence, offering opportunities for countries to access a wider range of treatments and innovations. However, the process of importing pharmaceutical drugs is complex and subject to stringent regulatory frameworks, which vary significantly across different regions. This thesis aims to explore the regulatory issues associated with the import of pharmaceutical drugs in two major emerging markets: India and Brazil. Both countries have substantial pharmaceutical industries and are key players in the global market. Nonetheless, they face distinct challenges in regulating the import of pharmaceutical drugs, which can impact drug availability, quality, and public health outcomes. In this study, we will examine the regulatory frameworks in India and Brazil, focusing on the key regulatory bodies, import approval processes, and documentation requirements. By conducting a comparative analysis of the regulatory issues in India and Brazil, this thesis aims to identify similarities and differences in their approaches, as well as the implications for pharmaceutical companies and public health.

Historical Background

The origins of the pharmaceutical industry trace back to the early 19th century. In the United States, between 1818 and 1822, fine chemical manufacturers established factories in Philadelphia, marking the inception of large-scale drug production. Simultaneously, European companies like Merck, Eli Lilly, and Roche transitioned from supplying natural products to manufacturing drugs. Additionally, chemical companies such as Bayer and Pfizer began exploring medical applications for their products during this period.

Significance in Modern Healthcare

The pharmaceutical industry has been instrumental in medical advancements, leading to the discovery and production of numerous new drugs in industrial laboratories. These developments have significantly improved disease control and elimination, contributing to increased life expectancy and better health outcomes globally.

Economic Impact

Beyond healthcare, the pharmaceutical sector substantially influences the global economy. It drives economic growth through job creation, research and development investments, and the generation of substantial revenue. The industry's commitment to innovation not only leads to new treatments and cures but also fosters technological advancements and economic development.

Challenges and Future Outlook

Despite its achievements, the pharmaceutical industry faces challenges such as identifying new drug targets, obtaining regulatory approvals, and refining drug discovery processes. Addressing these issues is crucial for the continued advancement of disease control and elimination. In summary, the pharmaceutical industry's evolution from early drug production to its current role as a cornerstone of modern medicine underscores its importance. Its contributions to medical progress and economic growth highlight the need for ongoing innovation and adaptation to meet future healthcare challenges.

Importance of Import Regulation

Effective import regulations are crucial in ensuring the safety, efficacy, and quality of pharmaceutical drugs entering a country. For nations like India and Brazil, which have burgeoning healthcare sectors and significant pharmaceutical markets, robust import regulatory frameworks are essential to protect public health and maintain market integrity.

India's Pharmaceutical Import Regulations

The Central Drugs Standard Control Organization (CDSCO) emerged as a result of India's evolving pharmaceutical regulatory landscape. The organization's foundations were laid with the enactment of the Drugs and Cosmetics Act

of 1940, a landmark legislation introduced during British colonial rule to regulate the import, manufacture, distribution, and sale of drugs in India. Following India's independence, the Drugs and Cosmetics Rules of 1945 were formulated to provide the operational framework for implementing the Act. The organization has evolved significantly since its inception, particularly after the 1962 amendments to the Drugs and Cosmetics Act that strengthened regulatory controls following global concerns raised by the thalidomide tragedy. In the decades since, CDSCO has continually expanded its scope and authority, with major revisions to its structure and mandate occurring in the 1980s, 2000s, and most recently through the New Drugs and Clinical Trials Rules, 2019. [1]

Organizational Structure of CDSCO

CDSCO has a hierarchical structure comprising various departments and regulatory bodies:

1. Drugs Controller General of India (DCGI)
2. Headquarters (HQ) – New Delhi
3. Zonal and Sub-Zonal Offices
4. Central Drug Testing Laboratories (CDTLs)
5. Subject Expert Committees (SECs) & New Drug Advisory Committees (NDACs)
6. State Drug Regulatory Authorities
7. Pharmacovigilance Programme of India (PvPI)

India's pharmaceutical industry is one of the largest globally, both in terms of domestic consumption and export. To safeguard public health, the Central Drugs Standard Control Organization (CDSCO) oversees the import of pharmaceutical products. Key regulatory aspects include

Import Licensing: A valid import license issued by CDSCO is mandatory for importing pharmaceutical products into India. The license is granted under Form 10 of the Drugs and Cosmetics Rules, 1945.

Registration: Imported drugs must be registered with CDSCO under Form 41, serving as authorization for the importation of specific pharmaceutical products.

Brazil's Pharmaceutical Import Regulations

The Agência Nacional de Vigilância Sanitária (ANVISA), or the Brazilian Health Regulatory Agency, was established on January 26, 1999, under Law No. 9,782. This creation aimed to enhance the safety of products and services consumed by the Brazilian population, marking a significant advancement in the country's public health infrastructure.

Historical Context and Establishment

Before ANVISA's inception, Brazil's health surveillance was managed by various fragmented entities, leading to inconsistencies in regulatory practices. The 1990s saw a growing public demand for more stringent health regulations, particularly concerning the safety of consumable goods and services. In response, the Brazilian government consolidated these functions into a single autonomous agency—ANVISA—to ensure a more coordinated and effective health surveillance system.

Organizational Structure of ANVISA

ANVISA's organizational structure consists of several key components that facilitate its regulatory functions

1. Collegiate Board of Directors (DICOL - Diretoria Colegiada)
2. President-Director
3. Thematic Regulatory Departments
4. Technical Advisory Committees
5. Regional and International Cooperation Units
6. Administrative and Support Departments

Evolution and Achievements

Over the years, ANVISA has evolved into one of the most respected regulatory agencies globally. Its rigorous standards and comprehensive regulatory practices have significantly contributed to the improvement of public health in Brazil. The agency's commitment to transparency and efficiency has bolstered its reputation both domestically and internationally. Brazil, as the largest pharmaceutical market in South America, relies significantly on imported medicines to meet its healthcare needs. The country's regulatory framework, managed by the Brazilian Health Regulatory Agency (ANVISA), includes

Local Representation: Foreign pharmaceutical companies must establish a local manufacturing unit or office, or appoint a Brazilian distributor authorized to import and distribute medical products.

Product Registration: All imported pharmaceutical products must be registered with ANVISA to ensure compliance with Brazilian health standards.

These measures are designed to ensure that imported pharmaceuticals meet the country's safety and efficacy standards, protecting public health.

Challenges and Considerations

Both India and Brazil face challenges in their pharmaceutical import processes:

Complex Approval Processes: The regulatory systems in these countries can be intricate, with new drugs taking a year or more to be approved for marketing.

Tariff Implications: Import tariffs can significantly impact the cost of pharmaceuticals. For instance, in Brazil, bound tariffs on Active Pharmaceutical Ingredients (APIs) can reach up to 34%, while in India, they can be as high as 70%.

Understanding and navigating these regulatory landscapes are essential for pharmaceutical companies aiming to operate in these markets.

MATERIALS AND METHODS

The materials and methods used in this study involved a comprehensive qualitative and comparative analysis of the regulatory frameworks governing the import of pharmaceutical products into India and Brazil. Data were collected from official regulatory authority websites such as the Central Drugs Standard Control Organization (CDSCO) in India and the National Health Surveillance Agency (ANVISA) in Brazil, as well as relevant international regulatory sources like WHO and ICH guidelines. Key regulatory documents, including guidelines, acts, notifications, and import licensing procedures, were reviewed and analyzed. A systematic literature review was conducted using peer-reviewed journals, regulatory databases, and industry reports to identify common regulatory challenges, approval processes, and documentation requirements. The study employed a comparative methodology to identify similarities, differences, and potential bottlenecks in the import processes of both countries. Expert opinions from regulatory professionals and published case studies were also considered to support the findings and provide contextual insights.

RESULTS

Procedure for Import and Registration of Drugs in India

Import License

Before applying for an import license, it is essential to determine the specific regulatory requirements applicable to the product category. The import license is issued by different regulatory bodies based on the product category. For example, pharmaceuticals and medical devices require approval from the Central Drugs Standard Control Organization (CDSCO), while food products need clearance from the Food Safety and Standards Authority of India

(FSSAI). Other items, such as electronics, chemicals, and hazardous substances, may require approval from the Bureau of Indian Standards (BIS), the Directorate General of Foreign Trade (DGFT), or the Ministry of Environment, Forest and Climate Change (MoEFCC). To obtain an import license for drugs, an application must be submitted to the licensing authority using Form 8 for drugs other than those listed in Schedule X and Form 8-A for Schedule X drugs. This application can be made by either the manufacturer or their authorized agent in India, provided the agent holds a wholesale license for the sale or distribution of drugs. The application must be accompanied by a license fee of ₹1,000 for a single drug and ₹100 for each additional drug, along with an undertaking in Form 9, duly signed by or on behalf of the manufacturer. Additionally, the application must include a copy of the Registration Certificate issued in Form 41 under Rule 27-A. However, in urgent situations, the central government may grant an import license using Form 10 or 10-A without requiring a Registration Certificate under Rule 27-A, provided the reasons are documented in writing. If the original license is lost, damaged, or defaced, a duplicate copy can be obtained by paying a fee of ₹250. Once submitted, the licensing authority reviews the application to ensure compliance with regulatory standards. This may involve document verification, inspection of manufacturing facilities, and adherence to safety norms. In certain emergencies, the central government may grant an import license without requiring a Registration Certificate under Rule 27-A, provided reasons are recorded in writing. Upon receiving an application for an import license in the format specified under Rule 24, the licensing authority, once satisfied that the applicant will comply with the license conditions, may issue the import license in either Form 10 or Form 10-A, depending on the case. Unless it is suspended or cancelled earlier, the license remains valid for three years from the date of issue. To obtain a renewed license, a fresh application must be submitted at least three months prior to the license's expiry date**.** The license allows the applicant to import the specified products into India, subject to ongoing regulatory compliance and periodic renewals as required. The validity of an import license varies depending on the product and regulatory body. In most cases, the license is issued for a specified period, after which it must be renewed to continue importing the product. Renewal applications should be submitted well in advance to avoid disruptions in the supply chain. After obtaining the import license, importers must comply with all Indian import regulations, including product labelling, storage, and distribution requirements. Periodic audits and quality checks may be conducted to ensure compliance with national standards. Any violations may lead to suspension or cancellation of the import license**.**

Registration Certificate

In India, a Registration Certificate is required for certain categories of products—especially regulated goods like pharmaceuticals, medical devices, electronics, and cosmetics—before they can be imported, sold, or distributed. This certificate ensures that the product complies with Indian quality, safety, and labeling standards. Regulatory bodies such as the Central Drugs Standard Control Organization (CDSCO), Bureau of Indian Standards (BIS), and FSSAI issue these certificates depending on the product category. The first step in obtaining a registration certificate in India is to determine which regulatory authority governs your product.

- Pharmaceuticals and medical devices are regulated by the Central Drugs Standard Control Organization (CDSCO).
- Electronics and IT goods come under the Bureau of Indian Standards (BIS), particularly under the Compulsory Registration Scheme (CRS).
- Food products are regulated by the Food Safety and Standards Authority of India (FSSAI).

If you're a foreign manufacturer, you must appoint an Authorized Indian Representative (AIR) who will be responsible for communication with the regulatory body and submission of applications. The AIR must be based in India and have a valid license (e.g., wholesale license for pharmaceuticals). The application must be submitted in FORM 40.For CDSCO-regulated products, applications can be submitted through the SUGAM online portal. BIS applications are submitted via their own portal, especially for products under the Compulsory Registration Scheme (CRS)**.** Each regulatory body charges specific fees for registration and licensing. For instance

- CDSCO: ₹1,000 for a single drug and ₹100 for each additional drug (Form 8/8A), plus ₹1,500 for Form 41 (registration certificate)
- BIS: Fee depends on product type, testing, and number of variants
- FSSAI: Fee varies by license type (state/central/importer**)**

Once your application is submitted, the authority will scrutinize it. If needed, they may request additional documents or conduct an inspection.

- If everything is in order, a Registration Certificate (e.g., Form 41 under CDSCO, BIS CRS certificate, or FSSAI License) is issued.
- The registration is typically valid for 3 to 5 years, depending on the product and authority.
  Make sure to apply for renewal before expiry to avoid disruptions. Additionally, continue to comply with local labeling, safety, and distribution laws. Non-compliance may lead to suspension or cancellation of the registration.[2]

Procedure for Import and Registration of Drugs in Brazil

Importing pharmaceutical drugs into Brazil is a regulated process overseen by the National Health Surveillance Agency (ANVISA).Brazilian importers must register with the Foreign Trade Secretariat (SECEX), a branch of the Ministry of Development, Industry, Trade and Services (MDIC) via its Integrated System for Foreign Trade (Siscomex). Companies seeking to import goods into Brazil must obtain a RADAR licence. The RADAR licence grants a password to access the Brazilian Integrated Foreign Trade System (SISCOMEX). The SISCOMEX is an electronic integrated trade documentation system designed by the Brazilian Government to control and monitor the Brazilian Foreign Trade.

- Register with RADAR (Registro e Rastreamento da Atuação dos Intervenientes Aduaneiros) through Receita Federal to access SISCOMEX.
- Enables your company to submit import declarations and monitor the process.

Every pharmaceutical product must be registered before being imported, unless exempted under special regulations (like compassionate use or clinical trials).Submit the Dossier Técnico (Technical Dossier) detailing product composition, manufacturing process, safety, efficacy, and labelling.Products manufactured abroad must have Good Manufacturing Practices (GMP) approval from ANVISA, which may involve on-site inspection. Once the product is registered, an Import License (Licença de Importação - LI) is submitted via SISCOMEX. The LI includes, product details, supplier, importer, quantity, intended use, and classification. For pharmaceuticals, ANVISA must approve the LI before the goods are shipped or cleared. Pay the Sanitary Surveillance Inspection Fee associated with the importation process. The fee amount varies based on the nature of the importation and the size of the company. For instance, the consent to import samples for analysis may incur fees ranging from R$8.86 to R$177.29. Import licenses are typically valid for 90 days. Detailed fee structures can be found in ANVISA's Resolution RDC No. 222 of 2006. After submission, ANVISA will evaluate the application and supporting documents. The standard review process can take up to 365 days, while priority reviews may be completed in approximately 120 days. This evaluation includes a thorough assessment of the product's safety, efficacy, and compliance with Good Manufacturing Practices (GMP)**.** Upon approval, proceed with the customs clearance process. Ensure all import documentation is accurate and complete to facilitate the release of goods. Compliance with ANVISA's regulations is crucial to avoid delays or penalties.[3]

Registration Certificate

To market a pharmaceutical product in Brazil, a company must obtain aRegistro Sanitário(Sanitary Registration Certificate) from ANVISA (Agência Nacional de Vigilância Sanitária). The process begins with the appointment of **a** local Brazilian legal representative or subsidiary, as foreign companies are not permitted to apply directly. This local entity must possess a valid AFE (Autorização de Funcionamento de Empresa) — an authorization to operate issued by ANVISA. Once authorized, the applicant must prepare and submit a registration dossier in the CTD (Common Technical Document) format, under RDC No. 73/2016 and related ANVISA guidelines. The complete dossier includes administrative information, product composition, manufacturing data, quality control measures, stability data, and clinical or preclinical evidence depending on the product type (e.g., new drug, generic, or similar). The application is submitted electronically via the Peticionamento Eletrônico system on ANVISA's portal. Once submitted and the fee is paid, ANVISA begins the technical and regulatory review, which can take 12–24 months, depending on product complexity and regulatory backlog. If the application mets all requirements, ANVISA issues a 10-year valid

registration certificate that must be maintained and renewed. The complete dossier includes administrative information, product composition, manufacturing data, quality control measures, stability data, and clinical or preclinical evidence depending on the product type (e.g., new drug, generic, or similar). The application is submitted electronically via the Peticionamento Eletrônico system on ANVISA's portal. Once submitted and the fee is paid, ANVISA begins the technical and regulatory review, which can take 12–24 months, depending on product complexity and regulatory backlog. If the application meets all requirements, ANVISA issues a 10-year valid registration certificate that must be maintained and renewed**.** ANVISA charges fees based on the product classification and the size of the applying company. For example, the registration of a new synthetic or biological drug typically ranges from BRL 119,000 to BRL 160,000, while generics and similar medicines may cost between BRL 54,000 and BRL 80,000. Simpler products like OTC drugs have lower fees, around BRL 20,000 to BRL 40,000. Micro and small enterprises (classified as ME or EPP) are eligible for significant fee reductions, up to 90%, upon presenting proper documentation. A product's registration in Brazil can be canceled in several ways. The marketing authorization holder may request voluntary cancellation by submitting a petition via the Peticionamento Eletrônico system. Additionally, ANVISA may cancel a registration administratively if the product is not renewed within the 10-year period, is found to pose a public health risk, or if the company fails to comply with Good Manufacturing Practices (GMP), product safety regulations, or pharmacovigilance requirements. Another reason for cancellation is non-commercialization, i.e., the product is not marketed in Brazil for more than 12 consecutive months without justification.

Comparative Analysis of Import Regulations in India and Brazil

India and Brazil have established robust frameworks for pharmaceutical imports, overseen by their respective regulatory agencies, the Central Drugs Standard Control Organization (CDSCO) in India and ANVISA in Brazil. While the two countries share similarities in their focus on quality, safety, and efficacy, they differ significantly in their procedural specifics, timelines, and compliance standards. (Table 1)

CONCLUSION

Both India and Brazil have stringent pharmaceutical import regulations to ensure drug quality, safety, and efficacy. However, companies face significant challenges in navigating the complexities of each country's regulatory framework. Below are the key issues and challenges specific to both markets.

Key Issues and Challenges of Import Regulations in India and Brazil

INDIA

Regulatory Complexity

Multiple Approvals: Importers must obtain drug registration (Form 41) and an import license (Form 10). These processes are often perceived as duplicative and time-consuming. State vs. Central Authority Conflicts: Overlap in responsibilities between the CDSCO and state drug control authorities can lead to delays.

Delayed Timelines

Approval Delays: Import approvals often take 6-12 months, and delays can arise due to incomplete submissions or lack of coordination between stakeholders.

Insufficient Regulatory Harmonization

Non-Alignment with Global Standards: While India follows Schedule M for GMP compliance, it is not fully harmonized with ICH or WHO standards, leading to additional certification requirements for international companies.

Labelling Challenges

Language Restrictions: Labels must be in English, which may not align with exporting country norms.

Lack of Technological Integration

Paper-Based Systems: Despite efforts to digitize, much of the regulatory process remains manual, increasing the risk of errors and inefficiencies.

BRAZIL

Lengthy and Costly Approval Process

- ANVISA Inspections: ANVISA mandates its own GMP inspections for foreign facilities, adding costs and extending timelines. Approval may take up to 18 months.
- Stringent CTD Requirements: The Common Technical Document (CTD) format demands extensive documentation, making registration complex.

Limited Mutual Recognition

GMP Certification: Brazil's reluctance to accept GMP certifications from other regulatory authorities, unless under specific agreements, creates additional hurdles for manufacturers.

Trade Barriers

- High Import Duties: Pharmaceuticals are subject to substantial tariffs and taxes, increasing the cost of imports.
- Customs Delays: Bottlenecks at ports and extensive documentation requirements can delay market access.

Language and Labeling Issues

Portuguese Labels: All product labels and accompanying documentation must be in Portuguese, requiring translation and revalidation processes.

Complex Post-Marketing Requirements

Pharmacovigilance: Stringent post-market surveillance regulations increase compliance costs and resource allocation for the company.

COMMON CHALLENGES IN BOTH COUNTRIES

- Lack of Harmonization with Global Standards: Both countries' regulatory frameworks differ from ICH guidelines, leading to duplication in testing and documentation.
- High Regulatory Costs: Lengthy approval processes and compliance requirements raise the cost of market entry.
- Limited Transparency: Importers often face challenges in navigating opaque regulatory requirements, leading to delays and inconsistencies. (Table 2)

India and Brazil, as emerging pharmaceutical markets, present significant opportunities but also unique challenges for pharmaceutical imports. Both countries aim to protect public health through robust regulatory frameworks. However, the complexities, delays, and stringent requirements often impede timely access to essential medicines and increase costs for manufacturers.

Key Takeaways

1. Regulatory Diversity: India's reliance on the CDSCO and Brazil's dependence on ANVISA reflect differing approaches to drug safety and efficacy standards. India's fragmented system involves state and central coordination, whereas Brazil has centralized oversight but requires ANVISA's specific GMP compliance.
2. Harmonization Gaps: Both countries lack full alignment with global standards like ICH, WHO-GMP, and PIC/S, necessitating additional certifications for international companies.
3. Approval Timelines: Long timelines—6-12 months in India and 12-18 months in Brazil—contribute to delays in market entry.
4. High Regulatory Costs: Repeated inspections, dossier preparation, and compliance with unique local requirements increase operational costs**.**
5. Customs and Language Barriers: Inefficient customs clearance and mandatory labeling in local languages (English for India and Portuguese for Brazil) further complicate imports.

Recommendations

1. Harmonization: Aligning national standards with global frameworks like WHO, ICH, and PIC/S could reduce duplication and improve efficiency.
2. Digitalization: Both countries should invest in digitized systems for faster processing and greater transparency.
3. Collaboration: Strengthening collaborations between regulatory authorities and industry stakeholders can streamline procedures and reduce bottlenecks**.**

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[Table 1 : Comparison Table of Import Regulations in India and Brazil

Aspect	India	Brazil
Regulatory Agency	CDSCO	ANVISA
GMP Standards	Schedule M / WHO GMP	ANVISA-specific GMP
Registration Format	Form 41 (simpler than CTD)	CTD dossier
Inspection Requirements	Limited, accepts foreign certifications	Frequent, often ANVISA-led
Timelines	~6-12 months	~12-18 months
Labeling Language	English	Portuguese
Climatic zone	Zone IV	Zone IV

Table 2: Comparison Table of Challenges in Both India and Brazil

Challenge	India	Brazil	Remarks
Regulatory Complexity	Multiple approval steps involving CDSCO and state authorities	Stringent ANVISA registration and inspection requirements	Both countries have extensive and sometimes overlapping regulatory requirements.
Lengthy Approval Timelines	Drug import approvals take 6-12 months	Approvals can take 12-18 months, especially for biological products	Long timelines increase the cost and delay market access for manufacturers.
GMP Certification Issues	Requires alignment with Schedule M; limited international harmonization	ANVISA insists on its own inspections; limited recognition of foreign GMP certificates	Lack of mutual recognition adds to complexity and costs for global manufacturers.
Language and Labeling	Labels must be in English	Labels and documents must be in Portuguese	Language localization is mandatory, increasing compliance requirements.
High Regulatory Costs	Compliance costs due to manual processes and non-standardized regulations	Costs are higher due to ANVISA inspections and complex dossier requirements	Regulatory compliance is resource-intensive in both countries.
Customs and Import Delays	Delays due to manual procedures and lack of digital integration	Delays from customs bottlenecks and extensive documentation requirements	Inefficient customs processes hinder timely product availability.
Pharmacovigilance Compliance	Mandatory post-marketing surveillance and reporting	RDC No. 4/2009 enforces stringent pharmacovigilance systems	Both require robust post-market safety monitoring, increasing operational demands.
Trade Barriers	High tariffs and non-tariff barriers for some drugs	High import duties and taxes on pharmaceutical products	Both countries impose tariffs that raise the cost of imports.

RESEARCH ARTICLE

Nanosuspensions : A Technique to Deliver Herbal Products

Lagnajit Mahapatra*

Professor, Department of Pharmacy, Haldia Institute of Pharmacy, (Affiliated to The West Bengal University of Health Sciences, Kolkata), West Bengal, India.

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***Address for Correspondence**
Lagnajit Mahapatra
Professor, Department of Pharmacy,
Haldia Institute of Pharmacy,
(Affiliated to The West Bengal University of Health Sciences, Kolkata),
West Bengal, India.
E.Mail: lagnajit.mahapatra@gmail.com

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ABSTRACT

One of the most promising methods for enhancing the biopharmaceutical performance of weakly water soluble pharmacological substances, particularly for natural products, is the use of drug nanosuspensions, a universal formulation technique for better drug delivery of pharmaceuticals with hydrophobic properties. The purpose of this review was to provide an overview of the major technological challenges in the development of nanosuspensions, including the in vivo study of the systemic administered nanosuspensions, guidelines for stabilizer screening, and methods for achieving intravenous target delivery. Additionally, the difficulties of using nanosuspensions to transport natural goods were also covered and debated. As a result, it aimed to offer guidance and support for the creation of nanosuspensions, the use of stabilizers, the controllability of targeted delivery of the herbal products nanosuspensions, and the predictability of in vivo studies.

Keywords:

INTRODUCTION

Herbal products have long been utilized as alternative treatments for a range of illnesses with comparatively less side effects, and they are a significant source of therapeutically useful molecules worldwide[1].Recently, they have drawn interest for the identification of novel therapeutic agents.These natural compounds have considerable pharmacologic al activity, but because of their limited water solubility, they have low bioavailability, which results in subtherapeutic drug concentrations.Gris-PEG, a sustained-release tablet containing griseofulvanin nanosuspensions, was the first medicine to be commercialized using nanosuspensions in 1995.There are now around 20 products on the market for nanosuspensions, with parenteral and oral being the two main ways of drug administration. Analgesia, schizophrenia, antifungal, anti-inflammatory, and other areas are among the many indications[2]. The main benefits

of using nanosuspensions in pharmaceutical technology for herbal product delivery include improving skin surface permeation for transdermal drug delivery and increasing the rate of dissolution and saturated solubility, which improve bioavailability for oral administration. Two factors contribute to the increased penetration of the skin's surface. According to Fick's first law, the nanosuspensions' greater saturation solubility produced a greater concentration gradient and a greater diffusive flow into the skin as compared to the micronized drug powders. Additionally, because there are a lot of nanosuspensions particles, the medicine may be more equally spread across the skin's surface, which results in a more even concentration gradient distribution[3]. For the oral administration of herbal products, nanosuspensions may be used. Drugs in nanosuspensions are mostly administered orally to increase the bioavailability of insoluble drugs; individual differences have a significant impact on drug absorption[4]. First off, nanosuspensions have a comparatively strong adherence to the gastrointestinal mucosa because of their tiny particle size and large specific surface area, which prolongs their retention period in the gastrointestinal system. The absorption rate and degree are raised when the medication is released at the adsorption site, its saturation solubility rises, and the electrochemical gradient in the intestinal wall and plasma increases[5]. TriCor tablets made with fenofibrate nanosuspensions technology could be consumed either fed or fasted. Fenofibrate must be administered with food prior to TriCor's release in order to ensure adequate absorption. When fenofibrate is taken with food, its absorption differs by about 35% compared to when it is taken alone. Drugs can dissolve more quickly and completely in the gastrointestinal tract and be absorbed more readily by the body thanks to nanosuspensions dosage forms[6]. At a reduced dosage, the authorized 48 mg and 145 mg TriCor tablets can accomplish the same level of efficacy as the earlier 54 mg and 160 mg tablets, respectively. Additionally, it is more convenient for patients to take the TriCor tablets with or without food. The new pills are still taken once day. The initial dosage of 54 mg and 160 mg of TriCor tablets was replaced with 48 mg and 145 mg tablets at the same cost. Thus, the nanosuspensions technique abolished the dietary effect on fenofibrate bioavailability. Despite there aren't any herbal products available on the market yet, the nanosuspensions technology can undoubtedly remove the impact of fasting or non-fasting on the bioavailability of natural products[7]. Additionally, nanosuspensions could be used for the injectable administration of natural goods. In order to prevent the negative reactions brought on by organic solvents, the formulation of the nanosuspensions only requires a tiny amount of surfactants as stabilizers. Human capillaries have a diameter of roughly 2 μm, while nanosuspensions that are injected into blood typically have a size range of 0.1–1 μm, which helps prevent vascular embolism[8]. The ability of nanosuspensions to load drugs is comparatively high. For drugs whose efficacy is measured by AUC and adverse reactions by Cmax, the pH changes of the nanoparticles after they are phagocytosed by macrophages in the circulatory system could also be utilized to achieve slow and controlled release when the drug is administered subcutaneously or intramuscularly[9].

Approach for nanosuspensions preparation

Smart Crystal Method

A particular nanosuspension formulation intended for cutaneous applications is the main focus of smart crystal technique. The description emphasizes that this approach is distinct and not typical of other approaches utilized in the research or field, using the results from this formulation as an example. The summary essentially highlights the uniqueness of the smart crystal approach when it comes to dermal nanosuspension formulations. As demonstrated in the production of rutin nanocrystals, this technique blends high-pressure homogenization with low-energy pearl milling. When applied to human volunteers' skin, a nanosuspension containing 5% rutin in the form nanocrystals showing 500-fold more antioxidant activity than a 5% water-soluble rutin-glycoside derivative. A nanocrystal containing active rutin, as a nanocrystal, leads to higher gradient between the topical formulation and the skin [10].

Media Milling Nanocrystals

High-shear media mills or pearl ball mills are used to create nanosuspensions. A milling chamber, a milling shaft, and a recirculation chamber make up the mill. After that, the medication is sent through the mill, which has tiny pearls or grinding balls in an aqueous solution. As the temperature is properly regulated, these balls rotate at a very high shear rate as they pass through the grinding jar's interior, striking the sample on the wall on the other side. The combined actions of impact and friction result in a significant reduction of particle size[11]. Materials like zirconium oxide, aluminum oxide sintered in ceramic, or tightly cross-linked polystyrene resin with exceptional abrasion

resistance are used to make the milling medium or balls. A planetary ball mill is a equipment that may achieve a particle size below 0.1 μm (PM100 and PM200; Retsch GmbH and Co., KG, Haan, Germany). A Zn-Insulin nanosuspension with particle size of 150 nm was created by wet milling technique[12].

High-Pressure Homogenization Method

Using the high-pressure homogenization (HPH) approach, which relies on the use of significant shear pressures, a solution is ejected from cavities and used to scatter drug crystals in order to carry out the cavitation process. Two homogenization concepts that are commonly used in conjunction with certain homogenizers that follow these principles are microfluidization and piston-gap homogenization[13]. The HPH technology can be separated into two categories based on the homogenization concept and technique: microfluidization and piston-gap homogenizers. The jet principle, put forth by Haynes in 1992, serves as the foundation for the microfluidization technology. The Z-type to Y-type reform has been applied to the microfluidic reactor channels. The main factors limiting HPH in pharmaceutical industrial applications are the small reaction channel, the high number of homogenization cycles (some patents describe as many as 75), and the high percentage of micron particles in the final product, particularly for drugs with relatively low hardness of the homogenization target[14]. In the mid-1990s, a new method for preparing nanosuspensions—piston-gap homogenizers—was created based on the potential issues with media milling and the microfluidization principle. Based on the various dispersed phases, the piston-gap homogenizers can be classified as either non-water phase homogenization (Nanopure) or water phase homogenization (Dissocubes). The initial iteration of piston-gap homogenization technology is called Dissocubes. But since this uniformly distributed phase is a water phase, drying and water removal are the factors that need to be looked into in order to govern the production of the next product. Certain medications of low melting points require drying treatment using expensive freeze-drying technologies[15]. The aforementioned drawbacks are addressed by the piston-gap homogenization technology for the creation of nanosuspensions, and the non-aqueous phase homogenization may distribute the medication in both the oil phase and the aqueous solution of PEG or glycerol. Gelatin soft capsules might be immediately inserted into drug nanosuspensions made by homogenizing oil or PEG 400/ PEG 600 as a dispersive phase. The non-aqueous dispersion phase is advantageous for drug storage and enhances the stability of some drug nanosuspensions with significant chemical instability, such hydrolysis. These drugs may be tailored into a dosage form that is redilution-ready prior to clinical use. For intravenous administration, the drug nanosuspensions that use glycerol-water as a dispersed phase could be diluted into an isotonic suspension with water[16].

Nanoedge Method

The foundation of nanoedge technology is the combination of homogenization and precipitation. The drugs are first dissolved in an organic solvent and for precipitation, this solution is then mixed with a miscible anti-solvent. Because of its poor solubility in the water-solvent mixture, the medication precipitates. Quick precipitation and high-pressure homogenization are combined to create high-shear processing, which goes hand in hand with this precipitation method[17].

Microemulsion technique

The emulsion, which is created by employing a somewhat water-miscible solvent as the dispersed phase, can also be made into nanosuspensions by simply diluting it. Drugs that are partially water miscible or soluble in volatile organic solvents can be used using the emulsion technique. Nanosuspensions can also be created using microemulsion templates. Surfactants or cosurfactants stabilize microemulsions, which are dispersions of two immiscible liquids, like water and oil, thermodynamically. The medication can be saturated through close drug mixing and is either loaded into the internal or preformed phase of the microemulsion. Using the microemulsion process, glieofulvin nanosuspension is made with lecithin, butyl lactate, water, and the sodium taurodeoxycholate salt[18].

Precipitation Method

A common technique for creating submicron particles of medications that are poorly soluble is the precipitation method. This approach involves dissolving the drug in a solvent, then solution is mixed with another solvent that

renders the drug insoluble when surfactant is present. When a solution is quickly added to a solvent, usually water, the medication in the solution becomes supersaturated and forms an ultrafine amorphous or crystalline form. This process includes crystal growth and nuclei production, both of which are mostly temperature-dependent. The main prerequisites for creating a stable suspension with the smallest possible particle size are a high nucleation rate and a low crystal growth rate [19].

Nanosuspensions Herbal products

Curcumin nanosuspensions

Turmeric rhizome (*Curcuma longa*) is the primary source of curcumin (diferuloylmethane), a polyphenolic chemical that is utilized extensively as a nutraceutical ingredient in food and drink products. Anticancer, antiviral, antioxidant, anti-inflammatory, antibacterial, hypoglycaemic and antirheumatic qualities are among curcumin's health advantages. [20]However, its clinical use and therapeutic impact are limited by its poor oral bioavailability, stability, and water solubility[21]. Curcumin decrases the toxicity of heavy metals like lead and cadmium by binding with them. This characteristic of curcumin explains why it protects the brain. Curcuminoids are components of turmeric, primarily curcumin, demethoxycurcumin, and bisdemethoxycurcumin. These substances are all not very soluble in water. Curcumin's primary disadvantages, including instability, low solubility, poor bioavailability, and rapid metabolism, significantly restrict its therapeutic use. It has been tried to make curcumin fully dispersible in an aqueous medium by using nanosuspension-based delivery[22]. When compared to native curcumin, curcumin nano-formulations exhibit more anticancer efficacy. Curcumin nanoparticles of about 100–200 nm in size can be produced using polymer nanoparticles based on PLGA, which is a biodegradable and biocompatible polymer. While appropriate surface coating permits escape from macrophage absorption, this particle size range is sufficiently tiny to permit intracapillary transit. Numerous studies have been conducted on these curcumin nanoformulations using animal and cell culture models. Dendrimer-based nanoparticles can create a curcumin nanoformulation that has the capacity to selectively kill human neurotumor cells while maintaining biological activity[23]. The formulation has a zeta potential of -25 mV, a modest diameter (211 nm), and low polydispersity (0.06). On Caco-2 and HT-29-MTX cell lines, the prepared nanosuspensions demonstrated a lower cytotoxicity profile and better chemical stability at pH values that resemble the gastrointestinal tract as compared to the solubilised curcumin. This formulation of redispersible curcumin nanosuspensions might open up new possibilities for curcumin oral dosage. When curcumin/TPGS nanosuspensions or curcumin/Brij78 nanosuspensions were administered, the oral bioavailability of curcumin increased by several times respectively, in comparison to suspension. [24] Targeting curcumin to the hair follicles as a unique method of transdermal delivery may create a depot from which the active ingredient is released into the hair follicle and have a significant therapeutic impact on the management of skin conditions. Investigations were conducted on the ability of curcumin nanosuspensions to penetrate hair follicles and the impact of gels in which the nanosuspensions are suspended[25]. The gels were loaded with various characteristics, including viscosity, lipophilicity and polarity. Using comparable concentrations of the nanosuspensions and evaluated for their capacity to direct the nanosuspensions into the hair follicles. It was discovered that regardless of the kind and concentration of gelling agent used, all gels loaded with nanosuspensions demonstrated an effective uptake of the nanosuspensions into the hair follicles [26].

***Scoparia dulcis* nanosuspension**

This plant species is mostly used to treat inflammation, kidney stones, diabetes, hypertension, stomach ailments, analgesia, and fever. Alcoholic extracts of Scoparia dulcis are employed in this investigation. The best technique for creating a polyherbal nanosuspension is ionic gelation. Qualitative phytochemical screening was used to identify the phytochemicals contained in the extract. Carbohydrates, tannins, flavonoids, saponins, steroids, terpenes, and phenols are all present in the alcohol extract. A herbal nanosuspension was made by varying the chitosan content. Ionic gelation was the technique utilized to create the nano suspension. Sodium benzoate is used as a preservative and PEG as a stabilizer. In addition to stomach ailments, kidney stones, hypertension, diabetes, and inflammation, the findings offer scientific proof for the traditional use of *Scoparia dulcis* herbal nanosuspension for analgesic, antipyretic, and urinary problems. Following the scale-up investigations of the optimized formulation, clinical and human trials must be conducted[27].

***Phyllanthus amarus* nanosuspensions**

About 800 species of *Phyllanthus amarus*, a plant belonging to the Euphorbiaceae family, may be found worldwide in tropical and subtropical regions. It is frequently utilized in traditional Indian medicine and as dietary supplements to treat a variety of physiological conditions, including liver diseases. In both cultivated and waste areas, *Phyllanthus amarus*, a branching annual glabrous herb, is a common weed. Its absorption following oral administration may be restricted due to the poor water solubility of its main ingredients, such as flavanoids and lignans. Thus, *Phyllanthus amarus* nanosuspension was created to increase the drug's solubility. The ethanolic extract of Phyllanthus amarus extract (PAE) and its nanoparticles (PAN) were investigated for their hepatoprotective effects on acute liver damage caused by paracetamol in Sprague-Dawley rats. By lowering levels of aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, and bile salts, it was discovered that an oral dose of PANs that is five times lower than the oral dose of PAE had a comparable hepatoprotective effect. Rat liver biopsy analyses corroborated these biochemical evaluations[28].

***Coriander sativum* nanosuspensions**

Since coriander (*Coriandrum sativum* L.) is an annual herb, an extract was made from the entire plant. Although they have different flavours and purposes, the plant's seeds, leaves, and roots can all be eaten. Because the leaves of coriander are perishable, it can be utilized as a complete plant and processed to improve the flavour of ripe fruits before adding it to various meal preparations. [29] The coriander plant is a great source of nutritious components and minerals. Due to its many health advantages and ability to prolong food preservation, coriander is also well-known for its anti-oxidant, anti-diabetic, anti-mutagenic, anti-anxiety, and antibacterial activity, as well as its analgesic and hormone-balancing properties.[30] The primary cause of a drug's removal from the market is hepatotoxicity. The study examined the hepatotoxic effects of a nanosuspension containing extracts from *coriander sativum*. According to the results, the nanosuspension significantly reduces the hepatotoxic liver damage caused by carbon tetra chloride in male Wistar albino mice[31].

Baicalin nanosuspensions

Baicalin, a naturally occurring flavonoid that is isolated from the dried root of Scutellaria baicalensis Georgi, is one of the most widely used bioactives in Chinese medicine and has been utilized extensively in China from ancient times. It has numerous positive health impacts on people and can be used to treat a range of illnesses either by itself or in combination with other medications[32]. Baicalin is a possible therapeutic medication for the treatment of stroke and Alzheimer's disease because of its herbal, which might protect and improve cerebral ischemia reperfusion as well as protect nerve and endothelial cells[33]. Nevertheless, this substance is nearly insoluble in water and poorly soluble in ethanol. Its insolubility has long been a significant issue limiting its use in medicine.

***Cuscuta chinensis* Nanosuspension**

A popular traditional remedy for supporting the kidneys and liver is *Cuscuta chinensis*. This parasitic plant is a member of the Convoluceae family. It is frequently used as an aphrodisiac, pain reliever, anti-aging, and anti-inflammatory. Its absorption following oral administration may be restricted due to the poor water solubility of its main ingredients, such as lignans and flavonoids. Therefore, to increase the drug's solubility, the nanosuspension approach was employed[34]. Ethanolic extract (CE) of *Cuscuta chinensi* was studied for its hepatoprotective and antioxidant properties in relation to acetaminophen-induced hepatotoxicity in rats. The hepatoprotective activity of an oral dose of *Cuscuta chinensis* nanosuspension, which is five times lower than the oral dose of Cucuta chinensis extract, was found to be comparable. Furthermore, superoxide dismutase, catalase, glutathione peroxidase, and malondialdehyde were all markedly elevated by the antioxidant activities of CE and CN. Additionally, the results demonstrated that Cuscuta chinensis nanosuspension had more effective hepatoprotective and antioxidant effects than Cuscuta chinensis extract[35].

Quercetin nanosuspensions

Fruits, vegetables, and therapeutic herbs all naturally contain quercetin, a polyphenolic flavonoid. Quercetin is frequently used as a nutritional supplement and phytochemical medicine to prevent and treat a variety of illnesses

because of its most notable dietary antioxidants and pharmacological activities. Quercetin, one of the most well-known dietary antioxidants, is said to have positive health effects, such as preventing osteoporosis, some types of cancer, heart and lung conditions, and aging, without any indication of toxicity, carcinogenicity, or genotoxicity associated with ingestion[36]. The enhanced dissolve performance and significantly increased dissolution rate of freeze-dried quercetin nanosuspensions were due to the increase in surface area and solubility brought about by the reduction in particle size. The physical mixture, the freeze-dried quercetin nanosuspensions, and the coarse medication all dissolved at cumulative rates of 52.30%, 53.07%, and 70.90% within 120 minutes, respectively[37]. Nanosuspensions pharmaceutics technology has the potential to improve not only the dissolution and, thus, the oral bioavailability of the herbal product, but also the transdermal efficiency. By using the wet media milling technique quercetin nanosuspensions was stabilized by taking Tween 80 and Poloxamer 188 showed encouraging accumulation in the skin layers and biocompatibile in human keratinocytes and the capacity to mitigate the oxidative effect of hydrogen peroxide on cells[38].

***Berberine* nanosuspensions**

The active component of *Coptis berberine* is an alkaloid that is an isoquinoline derivative and has a variety of pharmacological actions. Berberine and its derivatives as well as when combined with other pharmaceutically active substances or in formulations, have applications in a number of therapeutic areas, including diabetes, depression, cancer, inflammation, hypertension, and numerous infectious diseases. But because of its low oral bioavailability, berberine's therapeutic use is restricted[39]. In diabetic mouse models, the antidiabetic effects of Berberine nanosuspension were assessed in comparison to the effectiveness of bulk Berberine. Furthermore, by changing comparing berberine and metformin, the berberine nanosuspension demonstrated greater hypoglycemic, total cholesterol, and reduction body weight. Thus, using a nanosuspension to administer berberine is a viable method of treating type 2 diabetes[40]. Malignant cancers like hepatocarcinoma pose a serious threat to human life. The anti-hepatocarcinoma properties of berberine nanosuspension were assessed in vitro and in vivo in comparison to the effectiveness of bulk berberine. While in vivo studies also demonstrated greater anticancer activity, berberine nanosuspension had notable inhibitory effects against human HepG2 and Huh7 cells. Therefore, a promising method of treating hepatocarcinoma is to give berberine as a nanosuspension[41].

Silybin nanosuspensions

In order to shield the liver from oxidative stress and chronic inflammatory processes, silybin, a hepatoprotective herbal medication, has been used extensively in clinics to treat hepatitis. Its benefits include a certain therapeutic impact and less negative side effects. Additionally, silybin has a variety of biological activities, including antioxidant and anticancer properties. Its low water solubility and, as a result, low oral bioavailability are characteristics of silybin[42]. Therefore, the development of silybin products is currently hampered by its poor water solubility and restricted gastrointestinal absorption[43]. In addition to the standard dose form, multifunctional PEGchitosan-stabilized silybin nanosuspensions could also be delivered via smart nanocaged carriers[44]. The b-poly(ε-caprolactone)-methoxypoly(ethylene glycol) (mPEG-b-PCL) For the manufacture of silybin nanosuspensions, methoxypolythylene glycol-grafted chitosan (PEG-CS), a multifunctional stabilizer and propargyl modified were used as nanocaged carriers. Using an anti-solvent precipitation technique, two silybin nanosuspensions formulations like PEG-CS and nanocaged nanosuspensions were prepared. The nanocaged nanosuspensions were found to have exceptional stability. Additionally when compared to the solution group, the AUC of the nanocaged and PEG-CS nanosuspensions increased by 5.54 and 2.58 fold, respectively. As a result, the technology of nanocaged nanosuspensions, which has superior stability, enhanced dissolving velocity, and relative bioavailability, may be suggested as a practical and effective method for the delivery of medications that are water insoluble. The viability of employing smart nanocaged carrier technology in herbal medicine has also been investigated[45].

Camptothecin nanosuspensions

Topoisomerase is a DNA enzyme that is inhibited by the cytotoxic quinoline alkaloid camptothecin. It was extracted from the stem and bark of the tree *Camptothecia acuminate,* which is used in traditional medicine to cure cancer. In early clinical trials, camptothecin shown impressive anticancer activity, but it also had poor solubility and a high rate

of adverse medication reactions[46]. The study examined the anticancer activity of camptothecin nanosuspension both in vitro and in vivo, as well as its dose-dependent toxicity in comparison to topotecan. The MTT assay was used to examine the cytotoxicity of camptothecin nanosuspension and topoecan against MCF-7, HCT-8, and PC-3 cells; the HCT-8 xenograft model was used to assess the antitumor activity in vivo; and changes in body weight and relative organ weight were used to examine the dose-dependent toxicity in vivo during the treatment. When applied to MCF-7 cell lines, camptothecin nanosuspension exhibits approximately six times the in vitro cytotoxicity activity of TPT, almost the same anticancer activity and less toxicity. Camptothecin nanosuspension is a unique prospective formulation with minimal toxicity and strong antitumor activity, according to the data[47].

Resveratrol nanosuspensions

After being identified in 1924, resveratrol, a non-flavonoid polyphenol natural product was extracted from *Veratrum grandiflorum* roots in 1940. Resveratrol, an antitoxin released by plants during times of stress or pathogen attack, was also discovered in grape leaves in 1976. It is known as phytoalexin because resveratrol synthesis rose dramatically in response to UV light, mechanical stress, and fungal infection[48]. D-α-tocopherol polyethylene glycol 1000 succinate (TPGS) and folate modified distearoylphosphatidyl ethanolamine-polyethylene glycol-folate (DSPEPEG-FA) were utilized as stabilizers and target delivery carriers to create two resveratrol nanosuspensions that are RSV-NA nanosuspension and RSV-NB nanosuspension, that were made using the antisolvent precipitation method[49].

CONCLUSION

An overview of advancements in the effectiveness of nanosuspensions technology for the delivery of natural products is given in this review. Topics covered include methods for preparing nanosuspensions, typical formulations of herbal product nanosuspensions, screening guidelines for stabilizers, the effects of stabilizers and particle size on the in vivo study of the nanosuspensions administered intravenously as well as how to accomplish intravenous target delivery. An essential strategy for enhancing the effectiveness of insoluble active ingredients in herbal products is the use of nanosuspensions. Although this drug delivery technology has advanced significantly in recent years, there are still numerous issues with stabilizer selection and use, the in vivo drug delivery process, targeted drug delivery and other areas and there is a lack of basic research.

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RESEARCH ARTICLE

Using the Gymnastics Functional Measurement Tool for Injury Prediction in Indian Male Gymnasts Aged 18 - 30 Years

Arshi Mehta[1]*, Jaimin Ranparia[2], Rohan Thakor[3], Manoj Patil[4], Kishan Rakholiya[5], Pratik Katariya[2]

[1]Assistant Professor, Shree Bharatimaiya College of Optometry and Physiotherapy, Vesu Road, (Affiliated to Veer Narmad South Gujarat University), Surat, Gujarat, India.
[2]Clinical Therapist, BIOFIT Physio and Fitness Clinic, Athwa Gates, Surat, Gujarat.
[3]Clinical Therapist, Surat, Gujarat, India.
[4]MPT Student, Shree Bharatimaiya College of Optometry and Physiotherapy, Vesu Road, (Affiliated to Veer Narmad South Gujarat University), Surat, Gujarat, India.
[5]Clinical Therapist, Mitra Physio Hub, Surat, Gujarat, India.

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***Address for Correspondence**
Arshi Mehta
Assistant Professor,
Shree Bharatimaiya College of Optometry and Physiotherapy, Vesu Road,
(Affiliated to Veer Narmad South Gujarat University),
Surat, Gujarat, India.
E.Mail: arshimehta1@gmail.com

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ABSTRACT

Gymnastics is a demanding sport requiring strength, power, agility, flexibility, balance, coordination, and cardiovascular endurance, but it also carries a high risk of injury, particularly with its growing popularity. This study aimed to predict injuries among Indian male gymnasts aged 18–30 using the Gymnastic Male Functional Measurement Tool (GFMT). A total of 45 participants were selected based on specific criteria, and data were collected through consented demographic details, injury-related questions, and performance of the 10 GFMT tests. Descriptive analysis using SPSS v21 revealed a mean age of 24.16 years and an injury prevalence of 18%. The shoulder (75%) was the most frequently impacted area, followed by the lower back and ankle (25%), with the neck, upper back, and elbow each accounting for 13% of cases. Athletes who sustained injuries showed lower GFMT scores than their uninjured counterparts, reflecting decreased performance efficiency. The study found that poor GFMT scores correlate with higher injury risk, suggesting it is a useful predictive tool. Inadequate training and lack of proper cool-down routines were also highlighted as contributing factors to injury.

Keywords: Gymnast, GFMT tool, Indian gymnast, Injury, Prediction.

INTRODUCTION

Sports contribute significantly to physical fitness, mental well-being, and overall quality of life. Regular participation improves cardiovascular endurance, muscular strength, flexibility, and coordination while also promoting personal growth and psychological development[1]. However, sports involving intense physical demands also come with a risk of injury. Injury patterns vary depending on the sport, training intensity, age, and experience [2, 3]. Gymnastics, one of the most demanding sports, requires a combination of strength, agility, flexibility, and balance [4]. The repetitive, high-impact nature of gymnastics increases the risk of both acute and overuse injuries [5]. As the sport gains popularity, injury prevention has become a growing concern, especially since many gymnasts start intensive training at a young age[6, 7]. Male gymnasts often face high training volumes, leading to common injuries in the shoulders, back, neck, and ankles due to repeated loading and extreme ranges. These injuries can interrupt training, limit performance, and lead to long-term consequences[8-10]. Early detection of risk factors is, therefore, essential for injury prevention, and field-based screening tools are useful in assessing physical readiness and limitations [11]. The Gymnastic Functional Measurement Tool (GFMT) is a sport-specific assessment designed to evaluate critical physical attributes in gymnasts[12, 13]. The male version includes ten practical tests that assess strength, flexibility, agility, balance, and endurance using accessible equipment. These tests reflect the physical demands of gymnastics and help identify potential risk factors[11, 14]. Lower scores in specific tests may signal performance limitations that could increase injury risk. Thus, the GFMT is not only valuable for performance evaluation but also for predicting injuries [11]. Understanding injury risk in gymnasts also requires consideration of regional differences in physical characteristics and training conditions. Indian gymnasts may differ from athletes in other regions, making localized data essential. This study focuses on injury prevalence among Indian male gymnasts and evaluates the GFMT's utility in predicting injury within this population, intending to inform safer training practices and personalized conditioning programs. The study's significance lies in its potential to provide relevant insights into injury patterns and to support the development of targeted interventions. By using the GFMT as a screening tool, coaches and healthcare professionals can better guide gymnasts toward improved safety, performance, and long-term athletic development. The primary aim of this study is to predict injuries among Indian male gymnasts aged 18 to 30 years using the Gymnastic Male Functional Measurement Tool (GFMT), helping to strengthen injury prevention strategies through functional assessment.

METHODOLOGY

This correlational study aimed to examine the relationship between physical performance and injury prediction in male gymnasts. Using purposive sampling, 45 gymnasts aged 18–30 years were recruited from coaching centers across Surat. Participants had a minimum of six months of active training and were excluded if currently injured, recovering from recent injuries (<3 months), or outside the age/experience range. The study was conducted over six months (October 2023 to April 2024) with prior ethical approval and informed consent. Data collection was divided into three parts. First, gathered demographic and injury-related information, including training experience, competition level, injury history, and protective measures. The another is Male Gymnastic Functional Measurement Tool (MGFMT), which mainly incudes different ten sport-specific tests targeting agility, flexibility, strength, balance and endurance. Tests included the rings hold, handstand push-ups, star excursion balance, over-grip pull-ups, vertical jump, hanging pike, shoulder flexibility, splits (left, right, and middle), agility, and handstand duration. Standard procedures and basic equipment (e.g., tape measure, stadiometer, stopwatch) were used for all tests. (Fig: 1 to Fig: 8) Each test targeted specific physical capacities: the rings hold and push-ups assessed upper body and core strength; the star excursion balance test evaluated dynamic balance; pull-ups tested muscular endurance; the vertical jump and hanging pike assessed leg power and core flexibility, respectively. Related to range of joints and flexibility of lower body, the quantification is being carried out by shoulder flexibility and split test. Agility and handstand endurance were measured through directional sprints and time held on parallel bars. (Table-1) Raw scores were interpreted per GFMT scoring criteria and analyzed statistically. The results aim to determine GFMT's predictive validity in identifying injury risk and guiding prevention strategies among Indian male gymnasts.

RESULT

A total of 45 Indian male gymnasts aged 18–30 years participated in this study. The mean age was 24.16 ± 3.02 years, with average height and weight of 1.70 ± 0.08 m and 65.18 ± 8.17 kg, respectively. Based on BMI, 37% of participants were underweight, 60% were within the normal range, and 4% were classified as obese. Most participants (62%) had less than one year of experience in gymnastics, and the majority (75%) competed at the district level. Regarding injury prevalence, 18% of the participants reported gymnastics-related injuries, with the shoulder (75%) being the most commonly affected site, followed by the ankle (25%), lower back (25%), neck (13%), and upper back (13%). Knee, wrist and head injuries were less reported. Training frequency analysis showed that both injured and non-injured athletes commonly trained 3–5 days per week. All participants performed warm-ups, while cool-downs were slightly more common in the non-injured group (95%) compared to the injured group (87%). Protective equipment use was reported by 50% of injured and 49% of non-injured athletes. When comparing performance on the Male Gymnastic Functional Measurement Tool (GFMT), non-injured athletes had consistently higher mean scores across most of the 10 test components except the agility, as shown in table 2.

DISCUSSION

The present study aimed to predict injuries among Indian male gymnasts aged 18–30 years using the Gymnastic Male Functional Measurement Tool (GFMT), providing valuable insights into how performance-based field testing can help identify injury risk. With an injury prevalence of 18%, the results are slightly lower than those reported in earlier studies, such as the 24% prevalence among male gymnasts documented in 2015[15], which may be attributed to differences in training intensity, competition level, or preventive practices. The most frequently injured site was the shoulder (75%), followed by the ankle and lower back (25% each), with less frequent injuries reported in the neck and upper back. These findings align partially with those of prior studies, such as the 2021 systematic review[6], which noted high injury rates across upper limbs, the trunk, and lower limbs in gymnasts. The findings of the present study affirm the GFMT's effectiveness in forecasting injury risk at specific sites based on performance outcomes. Athletes who sustained injuries consistently exhibited lower average scores on the majority of GFMT components, particularly those assessing shoulder function (such as ring hold, shoulder flexibility, over-grip pull-ups, and handstand push-ups, back and trunk (hanging pike and pull-ups), and ankle (vertical jump, splits, and star excursion balance).This indicates reduced strength, endurance, balance, and flexibility in injured gymnasts, reinforcing that those physical inefficiencies directly correlate with higher susceptibility to injury. In line with Bostjan Jakse's research[16], which emphasized the role of body composition and tailored training in athlete performance, this study also revealed that 37% of participants were underweight and 4% were obese, which may reflect inadequate nutritional support or inconsistent training loads—factors that could influence injury risk. Furthermore, most participants had less than one year of experience and primarily competed at the district level, which may suggest a developmental stage where physical conditioning and technical adaptation are still maturing. Bostjan also noted that a structured training schedule is crucial for safe progression[16]; in this study, most injured athletes trained only 3–4 days a week, whereas a higher proportion of non-injured gymnasts trained up to 6 days weekly, suggesting better physical adaptation and workload management. The importance of recovery routines was also evident: although all athletes performed warm-ups, cool-downs were more common among non-injured athletes (95%) than injured ones (87%), consistent with the understanding that proper recovery reduces post-exercise stress and injury risk[17].

The use of protective equipment was similar in both groups, indicating that while protective gear is standard practice, it alone may not be sufficient without proper conditioning and recovery strategies. Notably, the agility test was the only GFMT component where injured athletes performed marginally better, possibly due to its emphasis on cardiovascular and speed elements rather than muscular strength or flexibility. This further supports the need to emphasize strength and balance training in injury prevention programs. As the GFMT has demonstrated good reliability and construct validity[11, 14], its continued application in gymnastic screening—especially among populations like Indian male gymnasts, for whom limited data currently exists—can greatly support injury

prevention. By pinpointing individual physical weaknesses and adjusting training to correct them, coaches and sports medicine practitioners can boost athlete safety, minimize injury-related downtime, and improve overall performance. These findings validate the role of sport-specific functional assessment tools like GFMT in structured athlete management and highlight the importance of integrating them into regular practice, especially in high-risk sports like gymnastics. This study had a few limitations. It included only male Indian gymnasts, so the findings may not apply to female athletes. The sample size was small and limited to gymnasts from Surat, which may affect the generalizability of the results. Additionally, the study did not differentiate between types of gymnastics, which may have influenced the injury patterns observed. Future studies are recommended with larger sample sizes to improve data reliability and avoid saturation. Research should also include Indian female gymnasts to understand gender-specific injury patterns. Additionally, comparing different assessment tools could help identify the most effective method for injury prediction. Studies focusing on specific gymnastics disciplines and their associated injury risks would also provide more targeted insights.

CONCLUSION

There is 18% prevalence of injury among Indian male gymnast, where, using male GFMT tool the injury can be predicted for the Indian male gymnast, as lesser the score for the items, less the efficiency to perform the task leading to lack of specific strength and ability leading them to lend up with the injury. Also, lack of specific training and not carrying out proper cool down can be a factor for getting injured while performing gymnastics.

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Table 1. GFMT items and targeted area

Sr. No.	Item	Targeted Area
1	Ring hold test	Shoulder adductor strength and endurance
2	Vertical jump test	Lower extremity power
3	Hanging pike test	Abdominal strength, hip flexor strength and flexibility as well as grip strength
4	Shoulder flexibility test	Shoulder complex flexion flexibility
5	Agility test	Speed, endurance, and agility
6	Over grip pull up test	Upper extremity strength and muscular endurance
7	Split test	Pelvis and lower extremity flexibility
8	Handstand push-up test	Shoulder and upper extremity strength and endurance
9	Star excursion balance test	Balance and muscular control
10	Handstand hold test	Strength, endurance and balance

Table 2. Data analysis of Male GFMT tool components

Male GFMT Items	Non-injured athlete (N=37)			Injured athlete (N=8)		
	Min.	Max.	Mean	Min.	Max.	Mean
Hanging pike test	2	6	3.63	1	6	2.57
Ring Hold test	2	5	4.00	2	5	3.03
Vertical Jump test	3	7	5.13	2	7	4.19
Shoulder flexibility test	3	6	4.13	1	5	2.76
Handstand Pushup	2	5	3.50	1	7	3.43
Agility test	2	5	2.88	2	5	3.11
Star excursion test	10	10	10.00	9	10	9.89
Over-grip pull-up test	4	9	6.50	1	10	5.24
Splits test	0	2	1.63	0	2	1.38
Handstand hold test	1	4	2.25	1	4	1.95



Figure 1. Rings hold test

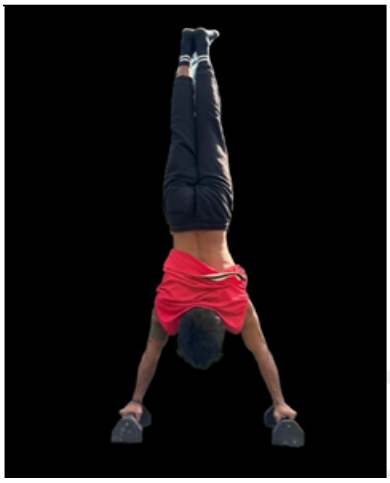


Figure 2. Handstand push-up test

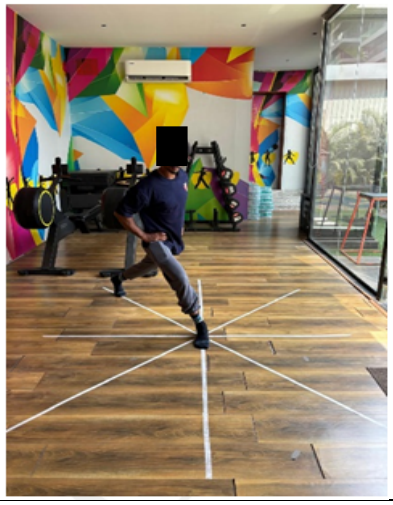


Figure 3. Star excursion balance test



Figure 4. Over-grip pull up test

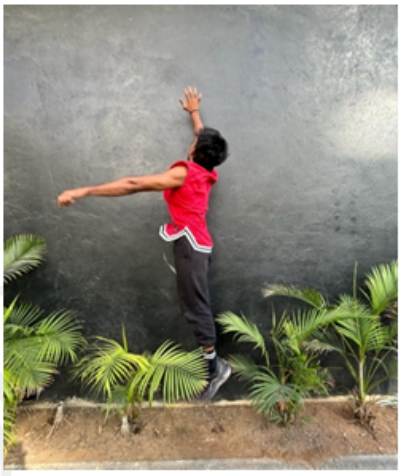


Figure 5. Vertical jump test

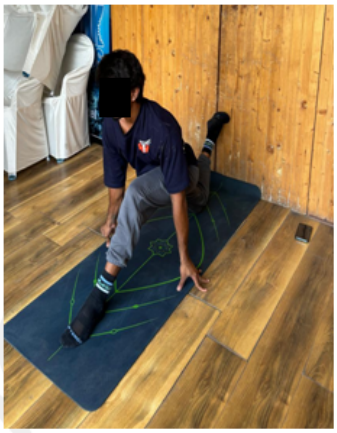


Figure 6. Right split test



Figure 7. Shoulder flexibility test



Figure 6. middle split test

The most commonly affected areas were the shoulder (75%), lower back, and ankle (25%), followed by the neck, upper back, and elbow (13% each). Injured athletes had lower GFMT scores compared to non-injured athletes, indicating reduced performance efficiency. The study found that poor GFMT scores correlate with higher injury risk, suggesting it is a useful predictive tool.

REVIEW ARTICLE

Coogan-Reese Syndrome : A Detailed Review

Khushi Kansal* and Ravindra Kumar Manik

Assistant Professor, Subharti College of Allied and Healthcare, Faculty of Allied Health Sciences, Swami Vivekanand Subharti University, Meerut, Uttar Pradesh, India.

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***Address for Correspondence**
Khushi Kansal
Assistant Professor,
Subharti College of Allied and Healthcare,
Faculty of Allied Health Sciences,
Swami Vivekanand Subharti University, Meerut,
Uttar Pradesh, India.
E.Mail: khushikansal02@gmail.com

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ABSTRACT

Coogan-Reese syndrome, also referred to as Cogan-Reese syndrome or Iris Nevus Syndrome, is a rare and distinct disorder within the Iridocorneal Endothelial [ICE] syndromes. These conditions are typically unilateral, nonhereditary, and are characterized by the abnormal proliferation and migration of corneal endothelial cells, which form a membrane-like structure that disrupts the anterior segment of the eye. This disruption can lead to secondary glaucoma by obstructing the drainage of aqueous humour. Coogan-Reese syndrome is distinguished by its characteristic features, including pigmented nodules or diffuse pigmentation of the iris, progressive iris atrophy, corectopia, and peripheral anterior synechiae. These abnormalities contribute to elevated intraocular pressure and visual impairment. The exact aetiology of Coogan-Reese syndrome remains unclear, though both genetic susceptibility and environmental factors, such as viral infections, have been suggested as possible contributors. Recent diagnostic advancements, including anterior segment optical coherence tomography [AS-OCT] and ultrasound biomicroscopy [UBM], have significantly improved the ability to identify the characteristic features of this syndrome. Differential diagnoses include conditions with overlapping clinical features, such as Fuchs' Heterochromic Iridocyclitis, neovascular glaucoma, and neurofibromatosis type 1. Management of Coogan-Reese syndrome primarily focuses on controlling secondary glaucoma and corneal oedema through both medical and surgical interventions. Initial treatment typically involves topical intraocular pressure-lowering medications; however, in refractory cases, procedures like trabeculectomy, glaucoma drainage devices, or cyclophoto coagulation may be necessary. Corneal transplantation techniques, such as Descemet's Membrane Endothelial Keratoplasty [DMEK] or Penetrating Keratoplasty [PK], are considered for severe corneal decompensation. Despite these treatments, the progressive nature of the disease necessitates ongoing monitoring and multidisciplinary care. The prognosis of Coogan-Reese syndrome varies depending on the severity of glaucoma and

corneal involvement. Early diagnosis and proactive treatment can preserve vision; however, the aggressive and often treatment-resistant nature of the condition underscores the need for further research to develop improved therapeutic strategies. This review article was prepared through a comprehensive process to gather, evaluate, and synthesize relevant information from a wide range of scientific sources. A systematic search was conducted across multiple databases, including PubMed, Google Scholar, and Scopus, using key terms such as "Coogen-Reese Syndrome," "clinical features of Coogen-Reese," "genetic basis of Coogen-Reese," and "treatment of Coogen-Reese" to ensure broad coverage of the existing literature. To maintain the relevance and quality of the sources included, specific inclusion and exclusion criteria were applied. Peer-reviewed articles, case reports, and clinical studies that addressed the pathophysiology, diagnosis, treatment, and outcomes of Coogen-Reese Syndrome, with a focus on both adult and paediatric populations, were included. Studies providing original data, systematic reviews, or meta-analyses were prioritized. Exclusion criteria consisted of studies on unrelated disorders, articles in languages other than English, and opinion pieces or unpublished data lacking clinical evidence or peer review. This review provides a comprehensive overview of the pathophysiology, clinical presentation, diagnostic strategies, differential diagnosis, treatment modalities, and prognosis of Coogan-Reese syndrome, thereby enhancing the understanding and management of this complex ocular disorder.

Keywords: Coogen Reese Syndrome, Iridocorneal, Syndrome, atrophy, genetic, nodules.

INTRODUCTION

Coogan-Reese syndrome, also referred to as Cogan-Reese syndrome or Iris Nevus Syndrome, is a rare and distinct disorder within the broader category of Iridocorneal Endothelial [ICE] syndromes. ICE syndromes are a group of unilateral, nonhereditary conditions characterized by abnormal proliferation and migration of corneal endothelial cells [1]. These aberrant endothelial cells possess the unique ability to form a membrane-like structure that extends across the anterior chamber, affecting critical ocular structures, including the iris and trabecular meshwork [2]. This pathological extension of endothelial cells disrupts the normal anatomy and function of the anterior segment of the eye, often leading to secondary glaucoma, as the aqueous humor drainage pathway is compromised [3]. Coogan-Reese syndrome stands out among ICE syndromes due to its distinct iris-related manifestations. The defining feature of this syndrome is the presence of pigmented nodules or a diffuse, patchy pigmentation across the anterior surface of the iris. This pigmentation creates an irregular, speckled pattern that is often readily visible during clinical examination, and it may vary in intensity over time [4]. As the condition progresses, patients typically experience worsening iris atrophy, corectopia [a condition where the pupil is displaced from its normal central position], and an irregularly shaped pupil. These changes can lead to significant cosmetic concerns and visual disturbances, further complicating the clinical management of the condition [5]. In addition to these iris abnormalities, peripheral anterior synechiae [PAS] may develop, where pathological adhesions form between the iris and cornea. This can exacerbate the elevation of intraocular pressure, contributing to the progression of glaucoma [6]. The precise aetiology of Coogan-Reese syndrome remains elusive, although it is generally believed to arise from a combination of genetic susceptibility and environmental factors [7]. Viral infections, particularly those caused by herpes simplex virus or Epstein-Barr virus, are thought to play a role in triggering the condition in individuals with a predisposition to ICE syndromes [8]. Advances in diagnostic imaging techniques, such as anterior segment optical coherence tomography [AS-OCT] and ultrasound biomicroscopy [UBM], have revolutionized our ability to diagnose Coogan-Reese syndrome with greater precision. These tools allow clinicians to visualize the subtle changes in the anterior segment and differentiate this syndrome from other similar ocular conditions [9]. This review provides an extensive examination of the pathophysiology, clinical manifestations, diagnostic approaches, differential diagnosis, treatment strategies, and prognosis of Coogan-Reese syndrome. By delving into these aspects, we aim to enhance the understanding and management of this complex ocular disorder.

Historical Background

Coogan-Reese syndrome, a rare ocular disorder, was first described in the mid-20th century and is recognized as a distinct subtype within the broader spectrum of Iridocorneal Endothelial [ICE] syndromes [10]. This condition owes its name to ophthalmologists James D. Coogan and Albert Reese, who were pivotal in identifying and characterizing the unique iris abnormalities and associated ocular complications that set it apart from other forms of ICE syndrome[11]. Unlike related conditions such as Chandler syndrome, known for corneal oedema and endothelial abnormalities, and Essential Iris Atrophy, which involves progressive thinning and distortion of the iris, Coogan-Reese syndrome is distinguished by the presence of nodular or diffuse pigmented lesions on the iris surface[12]. These distinctive features, along with its potential to cause glaucoma and other vision-threatening complications, underscore its clinical importance [13]. The detailed observations and pioneering work of Coogan and Reese contributed significantly to the understanding and classification of ICE syndromes, paving the way for further research into their pathophysiology and management.

Pathophysiology

The hallmark of ICE syndrome, including Coogan-Reese syndrome, is an abnormal proliferation of the corneal endothelium, a thin layer of cells on the inner surface of the cornea [14]. Normally, these cells help maintain the cornea's clarity by keeping it dehydrated. However, in Coogan-Reese syndrome, these cells multiply and start to act like epithelial cells, a transformation known as epithelial-like metaplasia [15].This leads to a series of problems in the eye, including corneal swelling, iris abnormalities, and glaucoma [16].This abnormal proliferation spreads from the corneal endothelium to the iris and the trabecular meshwork, resulting in various clinical manifestations

Corneal Endothelial Dysfunction: The proliferating endothelial cells lead to corneal edema as they lose their ability to maintain proper fluid balance in the cornea [17].

Trabecular Meshwork Involvement: The abnormal endothelial cells can spread into the trabecular meshwork, obstructing aqueous humour outflow and leading to secondary glaucoma [18].

Iris Changes: In Coogan-Reese syndrome, the abnormal endothelium migrates over the iris, leading to the formation of pigmented nodules or diffuse pigmentation, along with progressive iris atrophy and pupil distortion [corectopia] [19]. The exact cause of Coogan-Reese syndrome is still unknown, but some studies suggest that a viral infection, especially the herpes simplex virus [HSV], might trigger the disease [20]. HSV could potentially change the behavior of the corneal endothelial cells, leading to their abnormal growth and spread. However, this theory remains controversial, and not all cases of ICE syndrome show clear viral involvement [21].

Clinical Features

Coogan-Reese syndrome is a rare condition with distinct clinical features primarily affecting the iris and corneal endothelium. It typically manifests in middle-aged adults, with a higher prevalence observed among females [22]. The disease is almost exclusively unilateral, impacting only one eye, and belongs to the broader spectrum of Iridocorneal Endothelial [ICE] syndromes [23].

Pigmented Iris Nodules [Iris Nevi]

The defining feature of Coogan-Reese syndrome is the presence of pigmented nodules on the surface of the iris, commonly referred to as iris nevi. These nodules vary widely in size, shape, and number, imparting a distinct spotted or bumpy texture to the iris [24]. Occasionally, instead of discrete nodules, there may be diffuse pigmentation of the iris, creating a uniformly darker appearance. Patients might notice these changes cosmetically, with alterations in iris color or texture being particularly striking. The pigmented nodules are not only a diagnostic hallmark but also contribute to the unique visual presentation of the condition [25].

Iris Atrophy and Corectopia

As the disease advances, the iris undergoes progressive atrophy, characterized by thinning and the development of holes in the iris stroma. This deterioration frequently leads to corectopia, a displacement or distortion of the pupil. Corectopia is a hallmark sign of ICE syndromes and may cause visual disturbances or contribute to patient concern over aesthetic changes [26]. Additionally, the iris can form abnormal adhesions to the cornea, termed peripheral anterior synechiae, which can further distort the normal anatomy of the eye and exacerbate functional impairments [27].

Corneal Edema

Dysfunction of the endothelial cells in Coogan-Reese syndrome impairs their ability to maintain corneal deturgescence, resulting in corneal oedema [28].This oedema manifests clinically as blurred vision, glare, and halos around lights, particularly noticeable in dim settings. Corneal clarity may deteriorate over time, and patients often describe a sensation of visual "haze." The degree of corneal oedema can fluctuate, worsening in the presence of elevated intraocular pressure [IOP] or other stressors to the eye [29].

Secondary Glaucoma

Secondary glaucoma is a frequent and potentially sight-threatening complication of Coogan-Reese syndrome. Abnormal endothelial cells proliferate and invade the trabecular meshwork, leading to obstruction of aqueous humour outflow and a consequent rise in IOP [30]. This type of glaucoma is typically aggressive and refractory to conventional medical management, often necessitating surgical intervention to control IOP and preserve vision [31]. The coexistence of glaucoma and corneal oedema amplifies the risk of vision loss, underscoring the importance of early diagnosis and vigilant monitoring [32].

Diagnosis

The diagnosis of Coogan-Reese syndrome is primarily clinical, based on the characteristic ocular findings. A thorough eye examination is essential to identify the key features of the syndrome. Diagnostic tools and tests include:

Slit-Lamp Examination: This reveals the characteristic pigmented nodules or diffuse iris pigmentation. The slit-lamp examination also helps identify corneal oedema and assess the degree of iris atrophy and corectopia. The corneal endothelium may exhibit a "hammered silver" or "beaten metal" appearance, indicative of endothelial abnormalities [33].

Gonioscopy: Gonioscopy is used to evaluate the anterior chamber angle of the eye, which is the area responsible for draining aqueous humour from the eye [34]. In Coogan-Reese syndrome, the trabecular meshwork is often covered by abnormal endothelial cells, which can be detected during gonioscopy. This blockage is what leads to the development of glaucoma. This test is essential in evaluating the extent of angle closure caused by the abnormal cells and anterior synechiae formation. [35]

Specular Microscopy: Specular microscopy provides an in-depth assessment of the corneal endothelium.In patients with Coogan-Reese syndrome, the endothelial cells appear irregular and lose their typical hexagonal shape, which is key to maintaining the cornea's transparency [36]. This abnormality is often described as having a "hammered silver" or "beaten metal" appearance [37].

Intraocular Pressure [IOP] Measurement: Regular monitoring of IOP is critical, as glaucoma is a common and potentially sight-threatening complication of the syndrome [38].

Anterior Segment Optical Coherence Tomography [AS-OCT]: This non-invasive imaging technique can provide detailed cross-sectional images of the cornea, iris, and anterior chamber angle. This tool helps in assessing the structural changes in the eye, including the presence of synechiae [iris sticking to the cornea] and corneal edema [39].

Differential Diagnosis

Coogan-Reese syndrome must be carefully differentiated from other ocular conditions that share overlapping features, to ensure accurate diagnosis and effective management. The following conditions are particularly relevant:

Fuchs' Heterochromic Iridocyclitis [FHI]: This condition is characterized by chronic, mild uveitis, which frequently presents with heterochromia and cataract formation. Unlike Coogan-Reese syndrome, FHI does not exhibit the nodules on the iris or the severe glaucoma that are hallmarks of Coogan-Reese syndrome [40]. Additionally, the inflammation in FHI is typically low-grade and non-granulomatous, with the absence of peripheral anterior synechiae or endothelial changes [41].

Neovascular Glaucoma [NVG]: NVG is a type of secondary glaucoma caused by the growth of abnormal new blood vessels on the iris and trabecular meshwork [42]. While NVG may mimic the synechiae observed in iridocorneal endothelial [ICE] syndromes, including Coogan-Reese syndrome, its underlying cause is often distinct, typically linked to retinal ischemia or systemic conditions such as diabetes mellitus or central retinal vein occlusion [43]. Unlike Coogan-Reese syndrome, NVG is primarily associated with severe underlying vascular pathology rather than primary endothelial dysfunction [44].

Neurofibromatosis Type 1 [NF1]: NF1 is a genetic disorder that may present with Lisch nodules, which are pigmented, benign hamartomas found on the iris. While Lisch nodules superficially resemble the iris nodules seen in Coogan-Reese syndrome, they do not result in the corneal changes, peripheral anterior synechiae, or secondary glaucoma that are characteristic of Coogan-Reese syndrome [45]. NF1 is typically accompanied by other systemic manifestations, such as café-au-lait spots and neurofibromas, which further aid in differentiation [46].

Management

The management of Coogan-Reese syndrome primarily targets the control of secondary glaucoma and the reduction of corneal oedema, with a combination of medical and surgical treatments. The condition is complex, and its progression requires careful monitoring and individualized care. Below is an overview of the treatment strategies and their respective roles in managing this rare disorder.

Medical Therapy for Glaucoma

The primary goal of medical therapy is to lower intraocular pressure [IOP] to prevent optic nerve damage and vision loss [47]. Initial treatment usually consists of topical medications aimed at reducing IOP. Common classes of drugs include

Prostaglandin Analogs: These medications, such as latanoprost, are effective at lowering IOP by enhancing the outflow of aqueous humour through the uveoscleral pathway.

Beta-blockers: Agents like timolol reduce aqueous humour production, thereby lowering IOP. However, they may not be as effective in advanced stages of ICE syndrome due to the progressive nature of the trabecular meshwork obstruction.

Carbonic Anhydrase Inhibitors [CAIs]: Drugs such as dorzolamide reduce aqueous humor production, helping control IOP.

Despite the initial benefits, these medications may become less effective over time due to the progressive and obstructive nature of the trabecular meshwork in ICE syndrome, requiring additional interventions [48].

In addition, hypertonic saline eye drops are used to reduce corneal oedema by drawing excess fluid from the corneal stroma. Although this provides temporary relief, it does not address the underlying causes of corneal swelling and is typically a short-term measure [49].

Surgical Intervention for Glaucoma

When medical management fails to adequately control IOP, surgical interventions may be necessary. These procedures aim to either improve aqueous humour drainage or decrease its production [50]. The following are commonly considered surgical options:

Trabeculectomy: This filtration surgery creates a new drainage pathway for aqueous humor to reduce IOP. However, its effectiveness is limited in ICE syndrome due to endothelial cell proliferation and fibrosis at the surgical site, which can obstruct the drainage mechanism [51].

Glaucoma Drainage Devices [GDDs]: When trabeculectomy is not effective, the implantation of drainage devices, such as the Ahmed valve or Baerveldt implant, may offer a better alternative. These devices create a controlled outlet for aqueous humour, thereby helping to maintain adequate IOP levels. GDDs are often more successful in patients with ICE syndrome due to the reduced risk of trabecular meshwork obstruction and endothelial proliferation [52].

Cyclophotocoagulation: This laser procedure involves the selective destruction of the ciliary body to reduce aqueous humor production. It is typically reserved for refractory cases of glaucoma that do not respond to medical or conventional surgical treatments. Cyclophotocoagulation can be an effective tool in controlling IOP in severe, treatment-resistant cases [53].

Management of Corneal Oedema

Corneal oedema, a hallmark of Coogan-Reese syndrome, can lead to significant visual impairment if not properly managed. The primary aim is to reduce swelling and preserve corneal clarity:

Hypertonic Saline Eye Drops/Ointments: These agents help temporarily reduce corneal oedema by drawing excess fluid from the corneal stroma, providing short-term relief [54].

Corneal Transplantation: In cases where corneal oedema becomes severe and leads to significant vision loss, a corneal transplant may be required. There are two primary types of corneal transplants considered:

Descemet's Membrane Endothelial Keratoplasty [DMEK]: This selective transplant involves replacing only the damaged endothelial cells of the cornea. DMEK has shown promising results in patients with ICE syndrome, as it focuses on the specific layer affected by the condition [55].

Penetrating Keratoplasty [PK]: For advanced cases where the damage to the cornea is more widespread, a full-thickness corneal transplant may be necessary [56]. PK is considered when DMEK is not an option or in cases with more significant corneal scarring [57].

Monitoring and Follow-Up

Given the progressive nature of Coogan-Reese syndrome, continuous monitoring is essential to ensure optimal management and timely intervention. Patients should undergo regular assessments of the following:

Intraocular Pressure [IOP]: Frequent monitoring of IOP is necessary, as glaucoma progression can lead to optic nerve damage and vision loss [58].

Corneal Health: Regular evaluation of corneal thickness and swelling helps guide treatment decisions, particularly with respect to managing corneal oedema [59].

Visual Acuity: Regular tests of visual function are crucial to monitor any changes in vision and determine the need for surgical interventions [60]. Long-term follow-up is critical, as the disease can progress despite treatment. Ongoing

management requires a multidisciplinary approach, involving both glaucoma and corneal specialists, to address the complex interplay of intraocular pressure and corneal health in Coogan-Reese syndrome [61].

Prognosis

The prognosis for Coogan-Reese syndrome [also known as Iridocorneal Endothelial [ICE] syndrome] can vary significantly depending on the severity and progression of associated complications, particularly those involving glaucoma and corneal changes [62]. Early detection of elevated intraocular pressure [IOP] and prompt, effective management are crucial for preserving vision. However, glaucoma related to ICE syndrome can be particularly challenging to manage due to its tendency to be resistant to conventional treatments [63]. This necessitates the use of advanced, often multifaceted therapeutic strategies, including medical therapy, laser procedures, and sometimes surgical intervention [64]. In cases where corneal clarity is markedly compromised, corneal transplantation may become an essential treatment to restore vision. While corneal grafting can effectively improve visual outcomes, it carries substantial risks, including graft rejection and the recurrence of endothelial dysfunction, which can result in further corneal decompensation. Additionally, the progression of ICE syndrome itself may lead to complications that complicate the success of such procedures [65]. The potential for significant vision loss is particularly high in the advanced stages of the disease, making early diagnosis and proactive, individualized treatment plans critical[66]. The implementation of a personalized approach to managing Coogan-Reese syndrome is vital to optimizing visual outcomes and enhancing the long-term prognosis. Given the complexity of the condition, patients should undergo regular, thorough follow-up appointments to monitor disease progression and adjust treatment regimens as necessary. Even with aggressive interventions, the condition may continue to evolve, requiring careful attention [67]. A multidisciplinary approach that includes collaboration between ophthalmologists, corneal specialists, and glaucoma experts is essential to manage the complex and often unpredictable course of the syndrome. This collaborative effort helps ensure that patients receive the most appropriate and effective care, improving their chances of maintaining functional vision and reducing the risk of further complications [68].

Future Directions and Research Perspectives

Advances in Imaging Techniques

The evolution of ocular imaging has significantly enhanced the diagnosis and monitoring of Coogan-Reese Syndrome [CRS]. Traditional slit-lamp biomicroscopy remains a cornerstone of clinical assessment, but newer modalities such as anterior segment optical coherence tomography [AS-OCT] and ultrasound biomicroscopy [UBM] have improved our ability to visualize the intricate changes in the anterior segment.

Anterior Segment Optical Coherence Tomography [AS-OCT]: AS-OCT is a non-invasive imaging technique that offers high-resolution cross-sectional images of the anterior segment [69]. It is particularly useful in assessing the abnormal corneal endothelial proliferation characteristic of ICE syndromes, including Coogan-Reese syndrome [70]. Future advancements in AS-OCT may provide automated detection and quantification of endothelial membrane progression, allowing for early intervention before significant complications arise [71].

Ultrasound Biomicroscopy [UBM]: UBM is a high-frequency ultrasound technique that allows detailed visualization of the anterior chamber angle, including peripheral anterior synechiae [PAS] formation and trabecular meshwork abnormalities [72]. This imaging method plays a crucial role in differentiating Coogan-Reese syndrome from other ICE variants. Further refinements in UBM technology could enhance real-time imaging capabilities, improving clinical decision-making [73].

Specular and Confocal Microscopy: Specular microscopy enables the assessment of endothelial cell morphology, revealing characteristic changes such as cell polymorphism, pleomorphism, and abnormal migration [74]. Confocal microscopy further aids in visualizing endothelial proliferation, distinguishing Coogen-Reese Syndrome from other endothelial pathologies [75].

Artificial Intelligence in Imaging Analysis: Machine learning and AI-based algorithms are being explored for the automated analysis of anterior segment images. These technologies have the potential to improve diagnostic accuracy, predict disease progression, and assist in individualized treatment planning [76].

Genetic and Molecular Studies

Despite extensive research, the exact genetic basis of Coogan-Reese syndrome and other ICE syndromes remains unclear. Recent research suggest that a combination of genetic predisposition and environmental triggers [such as viral infections] contributes to disease development. Future genetic and molecular research could offer critical insights into the pathogenesis of this disorder.

Potential Genetic Markers: Some studies have suggested an association between polymorphisms in endothelial cell-related genes and the development of Coogan-Reese syndrome. The role of mutations in genes regulating endothelial cell adhesion, proliferation, and apoptosis is currently under investigation [77].

Role of Viral Infections: The association between herpes simplex virus [HSV] and Epstein-Barr virus [EBV] with ICE syndromes suggests a possible viral-mediated mechanism in endothelial cell dysregulation. Further studies using viral DNA/RNA sequencing and immunohistochemical analysis of corneal tissues could clarify the role of viral infection in triggering disease onset [78].

Molecular Pathways in Endothelial Cell Transformation: Research into the molecular mechanisms underlying corneal endothelial cell migration and proliferation is crucial for developing targeted therapies. Potential areas of interest include exploring the involvement of TGF-β signalling, endothelial-mesenchymal transition [EMT], and matrix metalloproteinases [MMPs] in disease progression [79,80].

Emerging Therapeutic Approaches

Current treatments for Coogan-Reese syndrome primarily focus on managing intraocular pressure [IOP] and preventing complications like secondary glaucoma. However, novel therapeutic strategies targeting the underlying disease process are being explored.

Targeted Pharmacological Therapy

Rho-Kinase [ROCK] Inhibitors: These medications, such as ripasudil and netarsudil, have shown promise in improving aqueous outflow and reducing intraocular pressure. Future research may evaluate their role in modifying endothelial cell behaviour to prevent disease progression [81].

Anti-Fibrotic Agents: Since ICE syndromes involve abnormal endothelial fibrosis, drugs targeting fibrotic pathways [such as pirfenidone or anti-TGF-β therapies] could be investigated for their potential to slow disease progression [82].

Anti-Viral Therapies: Given the suspected viral etiology, long-term antiviral therapy [e.g., valganciclovir or acyclovir] may be considered for patients with a history of viral infections to prevent further endothelial damage [83].

Stem Cell Therapy and Regenerative Medicine

- Research into corneal endothelial cell regeneration using induced pluripotent stem cells [iPSCs] or ex vivo cultured endothelial cells holds promise for future treatment options [84].
- Tissue-engineered endothelial cell grafts or gene-edited endothelial transplants could potentially restore normal corneal function in advanced cases [85].

Gene Therapy and RNA-Based Interventions

- Advances in gene-editing technologies like CRISPR-Cas9 may offer potential solutions for correcting genetic mutations associated with ICE syndromes [86].

- RNA-based therapies, such as small interfering RNA [siRNA] or antisense oligonucleotides, could be designed to target specific molecular pathways involved in endothelial cell proliferation [87].

Minimally Invasive Glaucoma Surgery [MIGS]

- MIGS procedures, including microstents and canaloplasty, are being increasingly explored as safer and less invasive alternatives to traditional trabeculectomy for glaucoma associated with Coogan-Reese syndrome [88].
- Future advancements in implantable microdevices with sustained drug delivery mechanisms could offer long-term IOP control with minimal surgical risks [89].

CONCLUSION

Coogan-Reese syndrome is an uncommon yet severe subtype of Iridocorneal Endothelial [ICE] syndrome, distinguished by the presence of iris nodules, corneal endothelial dysfunction, and secondary glaucoma [90]. This rare condition presents a considerable diagnostic and therapeutic challenge due to its progressive nature and the complex interplay of ocular structures involved. The progressive damage to the corneal endothelium and the development of glaucoma can lead to significant visual impairment if not promptly and effectively managed [91]. Early diagnosis and proactive management are critical to preserving vision and minimizing complications. Diagnosis often relies on clinical examination and imaging modalities, such as anterior segment optical coherence tomography [AS-OCT] or specular microscopy, which aid in identifying characteristic changes in the corneal endothelium and iris architecture. Therapeutic strategies primarily focus on controlling intraocular pressure [IOP] to mitigate glaucoma-related damage, typically through medical therapy, laser procedures, or surgical interventions like trabeculectomy or glaucoma drainage devices [92]. Corneal edema resulting from endothelial dysfunction often necessitates corneal transplantation, such as Descemet's stripping endothelial keratoplasty [DSEK] or penetrating keratoplasty [PKP], particularly in advanced cases. Despite these interventions, long-term outcomes may remain guarded due to the progressive and recurrent nature of the disease [93]. While current management is largely palliative, aimed at stabilizing IOP and addressing corneal complications, there is growing interest in advancing treatments through a better understanding of the underlying pathophysiology [94]. Research into endothelial cell therapy, regenerative medicine, and the molecular mechanisms driving ICE syndromes offers hope for novel, more effective therapies in the future. Emerging technologies, such as tissue-engineered corneal grafts and targeted gene therapy, may revolutionize the care of patients with Coogan-Reese syndrome, potentially improving both visual outcomes and quality of life [95].

Methods for Locating, Selecting, Extracting, and Synthesizing Data

In the preparation of this review article on Coogen-Reese Syndrome, a structured and comprehensive approach was utilized to gather, evaluate, and synthesize relevant information from a diverse range of scientific sources. A systematic search strategy was implemented across multiple established scientific databases, including PubMed, Google Scholar, and Scopus, to ensure the inclusion of high-quality, peer-reviewed articles. The search terms used encompassed a broad spectrum of relevant phrases, such as “Coogen-Reese Syndrome,” “clinical features of Coogen-Reese,” “genetic basis of Coogen-Reese,” and “treatment of Coogen-Reese,” which were combined in various ways to maximize the retrieval of pertinent literature. To ensure that only the most relevant and reliable sources were included in the review, specific inclusion and exclusion criteria were applied during the selection process. The inclusion criteria prioritized peer-reviewed articles, case reports, and clinical studies that provided in-depth insights into the pathophysiology, diagnosis, treatment, and outcomes of Coogen-Reese Syndrome. Special attention was given to studies that focused on both adult and pediatric populations diagnosed with the disorder. Articles that presented original data, systematic reviews, or meta-analyses were considered most relevant and were given preference during the selection process. Conversely, exclusion criteria were set to eliminate sources that were not directly related to Coogen-Reese Syndrome. Studies focusing on unrelated ocular or systemic disorders were excluded, as well as articles published in languages other than English due to the language limitations of the review process. Additionally, opinion pieces and unpublished data lacking peer review or clinical evidence were excluded to

maintain the rigor and quality of the sources considered. This comprehensive approach ensured that the literature included in the review was not only relevant and scientifically sound but also provided a broad and balanced view of the current understanding of Coogen-Reese Syndrome. By systematically gathering and synthesizing data from a range of sources, this review offers a thorough and up-to-date summary of the clinical, genetic, and therapeutic aspects of this rare ocular disorder.

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RESEARCH ARTICLE

Formulation and Evaluation of Antioxidant Cream, Moisturizing Lotion and Gel Face Wash from *Ananas comosus*

Sipra Sarkar Banerjee[1]*, Dhrubo Jyoti Sen[2], Sudip Kumar Mandal[3] and Sayan Pal[4]

[1]Associate Professor, Department of Pharmacy, Brainware University, Barasat, West Bengal, India.
[2]Professor, Department of Pharmacy, Techno India University, Salt Lake, Kolkata, West Bengal, India.
[3]Associate Professor, Department of Pharmaceutical Chemistry, Dr. B.C Roy College of Pharmacy and Allied Health Sciences, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata,West Bengal, India.
[4]Student, Department of Pharmacy, BCDA College of Pharmacy and Technology, (Affiliated to Maulana Abul Kalam Azad University of Technology) Kolkata, West Bengal, India.

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***Address for Correspondence**
Sipra Sarkar Banerjee
Associate Professor,
Department of Pharmacy,
Brainware University, Barasat,
West Bengal, India.
E.Mail: sipra.2000@gmail.com

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ABSTRACT

This project investigates the formulation and evaluation of antioxidant-based topical products: a cream, lotion, and gel face wash. The aim is to develop formulations with enhanced antioxidant properties to combat oxidative stress in the skin, contributing to improved skin health and appearance. The different pharmaceutical formulations are prepared with synthetic chemicals but there are side effects. Herbal formulation is safer and having customer acceptability. That's why we are formulating the antioxidant cream, gel. and facewash there may be other herbal formulations but formulation with pineapple is less.
The aim is to develop formulations with enhanced antioxidant properties to combat oxidative stress in the skin, contributing to improved skin health and appearance. The formulations were prepared using Bromelain, isolated from pineapple with an antioxidant property. Each formulation was designed to suit all skin types and applications. The cream is for intense hydration and long-lasting effects, the lotion is for light and non-greasy application, and the gel face wash is for an effective cleansing effect. The products were subjected to physicochemical tests to assess stability, pH balance, viscosity, and antioxidant capacity. In vitro assays, such as DPPH, are used to measure antioxidant activity. Additionally, preliminary skin irritation tests and sensory evaluations were performed to confirm the safety and acceptability of the products. All three formulations demonstrated good stability and acceptable pH levels. The antioxidant activity varied among the products. The cream was rich and

moisturizing, suitable for dry skin. The lotion provided moderate hydration with a lighter feel, making it suitable for normal skin. The gel face washes effectively cleansed while providing antioxidant benefits, suitable for all skin types. The antioxidant cream, lotion, and gel face wash successfully incorporated antioxidants and showed potential results. Further studies could focus on optimizing the formulations and testing their effectiveness through long-term use.

Keywords: *Ananas comosus*, antioxidant property, moisturizing cream, lotion, gel face wash.

INTRODUCTION

Pineapple is a tropical fruit, a member of the Bromeliaceae family. It is mostly consumed raw but can also be processed into juice, drinks, jams, and jellies (1). The *Ananas comosus* is a herbaceous perennial that may reach heights of 1.0 to 1.5 meters (3.3 to 4.9 feet), while it can occasionally reach much higher heights. The plant itself is visually striking, with a short, stocky stem and stiff, waxy leaves (2). When it blossoms, the separate fruits of the flowers come together to form a pineapple. Commercial growers "Following the first fruit, the main stem's leaf axils produced side shoots, which are known as suckers by Commercial growers(3). Pineapple is one of the most beneficial fruits for producing value-added compounds such as antioxidants, organic333 aci33ds, bromelain, Sesquiterpene, and phenolic compounds, according to its physicochemical makeup and nutritional value (4). Pineapple stems are the source of the potent chemo-responsive proteolytic enzyme bromelain. It is isolated and purified using various techniques and contains several thiol endopeptidases (5). Though scientists have also shown that it has antibacterial and anticancer properties, it is most typically utilized as an anti-inflammatory medication. (6)

MATERIALS USED

DPPH Solution, Bee wax, Methyl Paraben, Glycerin, Petroleum Jelly, Tween 80, SLS, Carpool 934, Borax, Liquid Paraffin, Rose Oil, Castor Oil, Cetyl Alcohol, Sodium Benzoate, Triethanolamine, Ethanol were taken from different manufacturer like Sigma Aldrich, Nice Chemicals, CDH Ltd, Loba Chemicals, Himedia, Changshu Song sheng, Fine chemical. Different Instruments are used like Lyophilizer Labard Instruchem, Centrifuge Machine, Vinayak Enterprise, Magnetic Stirrer, Remi, Electronic Balance, Mettler Toledo MF204, pH meter, IR Spectroscopy, Bruker Alpha II, BOD incubator, Vinayak Enterprise, Brookfield Viscometer, AMETEK Brookfield, Hot air Oven, Vinayak Enterprise.

METHODS

Preparation of extract

Fresh fruit of pineapple (*Ananas comosus*) was purchased from the markets in and around Barasat, West Bengal, India. Slices of the pineapple peels were removed. Next, chop the core from the pineapple into small pieces. These small pieces were put into the mixer grinder for smashing. After complete smashing the extract was filtered through a gauge. The filtrate of pure juices of pineapple is collected in a container. The extracted juice is subjected to Lyophilization for 3 days and we get a shiny and sticky substance (7).

Antioxidant Study of Pineapple Extract

Ethanol was added into the filtered juice at 1:4 ratio and stored for 8h at 8°C then centrifuged at 15000RPM for 25 min. Obtained Sediment was dried at 30°C. 40 µg/mL DPPH solution was prepared and absorbance was measured at 200-400 nm. Five different concentrations of Vitamin C solution and pineapple extract were added into 40 µg/mL DPPH solution. Mix it properly. Then the mixture was placed in a dark cabinet for 30 minutes. The absorbance was

checked at 517 nm by using UV- VIS spectrophotometer. The blank solution was Ethanol. Percentage of inhibition activity was performed by using formula (8).

% of inhibition = (ADPPH - Asample / ADPPH) × 100% Preparation of cream

All water-soluble ingredients (Table 1) (Borax, and pineapple extract) dissolved in water, serving as an Aqueous phase. All ingredients (Bees wax and Liquid paraffin) which were oil soluble mixed at 75°C±5°C in separate beakers. The oil phase was then poured into the aqueous phase slowly with vigorous stirring. Finally, other ingredients like Rose oil and preservatives were added with continuous mixing (9). The cream that was prepared is shown in Fig 1.

Determination of antioxidant capacity of cream

Cream was dissolved in ethanol and three different concentrations of Cream and Vitamin- C solution. 40 µg/mL DPPH solution was made and absorbance was checked at 200-400 nm. After that, three different concentrations of Cream solution and Vitamin- C solution were added into the DPPH solution and incubated for 30 min at 30˚C. The absorbance of the solution was measured at 517 nm using a UV-visible spectrophotometer. Ethanol was used as a blank. The free radical scavenging activity was calculated using the following formula (10).Figure 1(a) represents the pineapple cream.

% of inhibition = (ADPPH - Asample / ADPPH) × 100%

Preparation of Lotion

The ingredients used in the preparation of cream are listed in Table 2. Oil in water(O/W) emulsion of 1% and 2% of drugs were prepared (11). The emulsifier (glyceryl monostearate) and other components which are oil soluble components i.e sunflower oil, (mineral oil, cetyl alcohol petroleum jelly) were dissolved in the oil phase (part A) and heated up to 80℃. Extract and water-soluble components glycerine, propylparaben. methylparaben, were solubilized in (part B) and heated up to 80˚C. Once heated, Until the emulsifier cooled, the aqueous phase was gradually added to the oil phase while being continuously stirred. (12). When the temperature dropped to 45˚C, the perfume was added. Figure 1(b) represents the pineapple cream.

Determination of antioxidant capacity of lotion

Lotion was dissolved in ethanol and prepare three different concentrations of Lotion and Vitamin- C solution. 40 µg/mL DPPH solution was prepared and absorbance was measured at 200-400 nm. After that, three different concentration of Lotion solution and Vitamin- C solution was added into the DPPH solution and incubate for 30 min in 30˚C. The absorbance of the solution was measured at 517 nm using a UV-visible spectrophotometer. The blank was ethanol. Using the formula below, the percentage of scavenging activity was determined. (10,13)

% of inhibition = (ADPPH - Asample / ADPPH) × 100%

Preparation of Gel face wash

The ingredients used in the preparation of cream are listed in Table 3. Carbopol 934 was dissolved in distilled water and left overnight for shock. After that extract, Sodium Lauryl Sulphate (SLS) / Tween 80 and preservative was added with continuous stirring. Then Triethanolamine was added as a pH adjuster(14).The figure 1(c) represents the gel face wash.

Determination of antioxidant capacity of gel face wash

40 µg/mL DPPH solution was prepared and absorbance was measured at 200-400 nm. Three different concentrations of Vitamin C solution and gel face wash solution were added into 40 µg/mL DPPH solution. Mix it properly. After that, the mixture was incubated for half an hour. At 517 nm, the absorbance was determined using a UV-VIS spectrophotometer. The blank solution was Ethanol. Percentage of scavenging activity was calculated by using formula (10, 13)

% of inhibition = (ADPPH – A sample / ADPPH) × 100%

Evaluation of Cream

Sensory assessment of cream

The formulated cream was evaluated at time of preparation by its organoleptic properties lick color, odor texture etc (15). Results are listed below Table. 4.

Determination of pH cream

Digital pH meter was calibrated using standard buffer solution.0.5 g cream was dissolved in 50 ml distilled water and determined the pH (16).

Determination of Viscosity of cream

The viscosity of the cream formulation was determined by Brookfield viscometer by using LV- 4 spindle. The adaptor was filled with the cream formulation and viscosity was measured.

Spread ability studies of cream

The spread ability refers to a cream's ability to cover the skin evenly. This quality is determined by the amount of time, in seconds, it takes for two slides to glide off the cream, held between them, under a specified weight. The faster the two slides separate, the better the Spread ability. Two pairs of standard-sized glass slides were collected. After that, a slide of appropriate size was chosen and the cream mixture was applied to the slide (17). Next, another slide was positioned on top of the mixture. After that, a weight or specific load was added to the top slide to evenly press the cream between the two slides into a thin. Next, the weight was taken off and any extra formulation sticking to the slides was scraped away. The weight attached to it allowed the upper slide to slip off freely due to gravity. The duration for the upper slide to slide off was recorded. The data was presented in table 4.(18)

Spreadability = (m*l)/t
m = weight tied to the upper slide (2g)
l =length of glass slide (7.5cm)
t =time taken in seconds.

Stability Studies of Cream

Stability testing of drug products begins as a part of drug discovery and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done. The ICH guidelines were followed for conducting the stability investigations. The cream is placed in a bottle and stored for two months at 40 ± 2 °C and 75 ± 5% relative humidity. Samples were examined for viscosity and physical characteristics at the conclusion of the investigations (19). The Spreadability of the cream is tabulated in table 5.

Homogeneity of cream

Through touch and visual inspection, the homogeneity of the formulations was evaluated.

Removal of cream

The cream's ease of removal was assessed by using tap water to wash the region that received the cream application.

After feel effect of cream

After applying a certain quantity of cream, the degree of emolliency, slipperiness, and residue were assessed.

Evaluation of Lotion

Organoleptic evaluation lotion

The formulated lotion was evaluated by its organoleptic properties lick color, odor texture etc.

Determination of pH

Digital pH meter was calibrated using standard buffer solution. 0.5 g lotion was dissolved in 50 ml distilled water and determined the pH.

Determination of Viscosity

The viscosity of the lotion formulation was determined by Brookfield viscometer by using LV- 4 spindle. Lotion formulation was poured into the adaptor and viscosity was measured

Spreadability refers to a lotion's ability to cover the skin evenly

This quality is determined by the amount of time, in seconds, it takes for two slides to glide off the lotion, held between them, under a specified weight. The faster the two slides separate, the better the Spreadability. Two pairs of standard-sized glass slides were collected. After that, a slide of appropriate size was chosen and the lotion mixture was applied to the slide. Next, another slide was positioned on top of the mixture. After that, a weight or specific load was added to the top slide to evenly press the lotion between the two slides into a thin layer. The data are depicted in Table 7. Next, the weight was taken off and any extra formulation sticking to the slides was scraped away. The weight attached to it allowed the upper slide to slip off. The duration for the upper slide to slide off was recorded (20).and ends with the demise of the compound or commercial product. To assess the drug and formulation stability, stability studies were done. The stability studies were carried out as per ICH guidelines. The lotion is filled in a bottle and kept in a humidity chamber maintained at 40 ± 2 °C / 75 ± 5 % RH for two months. At the end of studies, samples were analyzed for the physical properties and viscosity. The Spreadability of the lotion is tabulated in table 5.

Homogeneity

Through touch and visual inspection, the homogeneity of the formulations was evaluated.

Removal

By using tap water to wash the area where the lotion was applied, the lotion's ease of removal was assessed.

After feel

After applying a certain quantity of lotion, the degree of emolliency, slipperiness, and residue were assessed.

Greasiness

In this instance, the lotion was applied to the skin's surface as a smear, and its oiliness or grease- like consistency was assessed.

Evaluation of Gel face Wash

Organoleptic evaluation

The formulated gel face wash was evaluated by its organoleptic properties lick colour, odour texture etc.

Determination of pH

Digital pH meter was calibrated using standard buffer solution. 0.5 g gel face wash was dissolved in 50 ml distilled water and determined the pH.

Determination of Viscosity

The viscosity of the gel formulation was determined by Brookfield viscometer by using LV-4 spindle. gel formulation was poured into the adaptor and viscosity was measured.

Stability Studies of Gel Face wash

Drug product stability testing starts during the drug discovery process and concludes when the chemical or commercial product degrades. Stability studies were conducted to evaluate the stability of the medication and

formulation. The ICH guidelines were followed for conducting the stability investigations. The cream is placed in a bottle and stored for two months at 40 ± 2 °C and 75 ± 5% relative humidity. Samples were examined for viscosity and physical characteristics at the conclusion of the investigations. The Spreadability of the lotion is tabulated in table 5.

Homogeneity
Through touch and visual inspection, the homogeneity of the formulations was evaluated.

Removal
By using tap water to wash the area where the gel face was applied, the gel has ease of removal was assessed.

After feel
After applying a certain quantity of gel face wash, the degree of emolliency, slipperiness, and residue were assessed.

RESULT

According to the literature review, the DPPH solution's maximum wavelength was 517 nm. The DPPH solution was changed to reveal antioxidant activity (21). The DPPH solution turns from purple to yellow, and the color's intensity increases and is directly correlated with the moles of the stabilized molecule present. Stability testing of drug products starts with drug discovery and ends with the demise of the compound or commercial product. Antioxidants derived from plants, such as bromelain, shield the body against illnesses caused by free radicals with minimal to no adverse effects. By stopping the oxidative chain reaction, which prevents oxidative damage, antioxidants reduce oxidative stress (22). The antioxidant activity of vitamin C and crude pineapple extract is presented in Table 6. The DPPH solution's color changes from purple to yellow, and the amount of the stabilized molecule present exactly correlates with how intense the color is. Antioxidants derived from plants, such as bromelain, shield the body against illnesses caused by free radicals with minimal to no adverse effects. By stopping the oxidative chain reaction, which prevents oxidative damage, antioxidants reduce oxidative stress (23). Table 6: Determination of antioxidant activity of vitamin C and Crude Pineapple extract

Determination of antioxidant capacity of lotion
The color of the DPPH solution transitions from purple to yellow, with the intensity of the color being directly correlated with the moles of the stabilized molecule present. Antioxidants derived from plants, such as bromelain, shield the body against illnesses caused by free radicals with minimal to no adverse effects. By stopping the oxidative chain reaction, which prevents oxidative damage, antioxidants reduce oxidative stress (24, 25). The data are depicted in table no 09. Antioxidants derived from plants, such as bromelain, shield the body against illnesses caused by free radicals with minimal to no adverse effects. Antioxidants reduce oxidative stress by stopping the oxidative chain reaction, which prevents oxidative damage (26).

Determination of pH of the cream
The pH of the cream was shown to be between 6.4 and 7.9, which is a suitable range for the pH of skin. All of the lotion formulations had pH values that were closer to skin pH. The results are listed below in Table 8.

Determination of viscosity of cream
The cream's viscosity, which ranged between 40000 and 33000 cps, suggests that it may be readily distributed with little shear. The viscosities of the all three formulations are given in Table 8.

Homogeneity of cream
The extracts are distributed uniformly throughout the cream in all formulations, which is determined by touch and visual appearance.

Removal of cream

All three cream formulation was applied on the skin and were easily removed by washing with tap water.

After feel effect of cream. The change of different organoleptic properties of different formulation are shown in Table 9. The amount of residue left behind, the cream's emolliency, and its slipperiness were all deemed satisfactory after application.

Determination of pH of the lotion

The lotion's pH was found to be between 6.4 to 7.6, which is a suitable range for the pH of skin. All of the lotion formulations had pH values that were closer skin pH. The result is listed below.

Determination of viscosity of lotion

The lotion's viscosity, which ranged between 150000 and 380000 cps, suggests that it may be readily distributed with little shear. The viscosity of the all three formulations is given in Table.

Homogeneity lotion

The extracts are distributed uniformly throughout the lotion in all formulations which is determined by touch and visual appearance.

Removal of lotion

All three lotions applied on skin were easily removed by washing with tap water.

After feel effect of lotion

After applying the lotion, it was determined that the emolliency, slipperiness, and amount of residue left were all satisfactory.

Determination of viscosity of gel face wash

The viscosity of the gel face wash, which ranged between 5209 and 5630 cps, suggests that it may be readily distributed with little shear.

Homogeneity of gel face wash

The formulations are distributed uniformly throughout the gel face wash in all formulations which is determined by touch and visual appearance.

Removal of gel face wash

The all three gel applied on skin was easily removed by washing with tap water.

After feel of gel face wash

Washing with tap water made it simple to remove all three gels that had been applied to the skin.

Following gel face wash feel: Following gel face wash application, it was found that the amount of residue left, emolliency, and slipperiness were all acceptable.

FTIR Study

From the FTIR Spectrum of Crude Bromelain the peak was observed at 3328.81 cm^{-1} which represents N-H bond and represents amino group and having enzyme or protein in nature (27). The 2124.10 cm^{-1} peak shows presence of C≡C triple bond which represents alkyne group contain organic compound like monounsaturated hydrocarbon and 1635.89 cm^{-1} peak shows presence of C=N or cyano or nitrile group containing compound like amine, imine and nitrile compound (28).

The FTITR study of Cream

The pineapple cream F1 shows peak at 2920.27 cm^{-1} and 2851.66 cm^{-1} which represents presence of Strong OH bond containing compound like aldehyde, carboxylic acid or ketone and incorporation of that group with amino group (29). The next peak shows at 1461.67 cm^{-1} and 1376.87 cm^{-1} which shows presence of aromatic benzene ring which is the mother molecule of every compound. The 720.75 cm^{-1} peak shows presence of C-H (methyl group) containing compound (30). The pineapple cream F2 shows peak at 2918.98 cm^{-1} and 2850.72 cm^{-1} which reflect the presence of C-H stretching which suggests that the presence of Aliphatic hydrocarbons means the presence of compounds such alkenes(31).At peak 1736.89 cm^{-1} represent C=O stretching which indicates the presence of esters, aldehydes, ketones, or carboxylic acids. The next peak shows at 1462.09 cm^{-1} and 1376.89 cm^{-1} which shows presence of aromatic benzene ring which is the mother molecule of every compound. The C-O stretches at 1279, 1167, and 1048 cm^{-1} further indicate functional groups such as esters or ethers.

The 720.33 cm^{-1} peak shows presence of C-H methyl group containing compound (32)

The IR spectrum suggests the presence of Free hydroxyl groups (3778 cm^{-1}) and amine groups (3339 cm^{-1}), indicating isolated alcohols or phenols and primary or secondary amines or amides, respectively. Aliphatic hydrocarbon chains (2923 and 2854 cm^{-1}), suggesting the presence of methylene and methyl groups. The presence of Carbonyl group (1715 cm^{-1}), characteristic of esters, ketones, or aldehydes. C-O stretching at 1106 and 1035 cm^{-1} pointing to alcohols, ethers, or esters (33). The IR spectrum of Pineapple lotion F2 suggests the presence O-H stretching at 3774.82 cm^{-1} is associated with alcohols and phenolic substances (34). At 3353.21 cm^{-1} presence of N-H stretching indicate that the presence of primary amines. C=O stretching at 1641.06 cm^{-1} indicate the presence of amides. The IR spectrum suggests that the sample likely contains hydroxyl groups (indicating alcohols or phenols), aliphatic hydrocarbons (alkanes), carbonyl groups (ketones, aldehydes, carboxylic acids, or esters), alkenes or aromatic rings, and ethers or esters. The broad and strong O-H peak, along with the distinct carbonyl and C-H stretching vibrations.

FTIR Spectrum of Pineapple Gel Face Wash (F1)

FTIR spectrum of Pineapple Gel Face Wash (F1) shows the broad peak around 3300 cm^{-1} suggests the presence of O-H stretching vibrations, typical of hydroxyl groups found in alcohols or phenols. Aliphatic hydrocarbons are indicated by the peak close to 2920 cm^{-1}, which is typical of C-H stretching vibrations. The strong and sharp peak around 1633 cm^{-1} corresponds to C=O stretching vibrations, which is typical of carbonyl groups present in compounds such as ketones, aldehydes, carboxylic acids, or esters. The IR spectrum displays the broad absorption around 3347 cm^-1 suggests the presence of hydroxyl (O-H) groups, typical of alcohols or phenols. Aliphatic hydrocarbons are shown by the peak near 2926 cm^-1, which corresponds to C-H stretching vibrations. C=O stretching vibrations are characterized by a strong peak at about 1633 cm^-1, which indicates the presence of carbonyl groups such as those found in ketones, aldehydes, carboxylic acids, or esters. Additional peaks around 1031 cm^-1 suggest C-O stretching vibrations, indicating the presence of ethers or esters(35).

FTIR Spectrum of Pineapple Gel Face Wash (F3)

The FTIR spectrum shows key absorption peaks at 3346.86 cm^{-1}, 2360.41 cm^{-1}, 1632.39 cm^{-1}, 1214.47 cm^{-1}, and 692.23 cm^{-1}. The prominent broad peak around 3346.86 cm^{-1} typically indicates O-H or N-H stretching, suggesting the presence of alcohols, phenols, or amines. The peak near 2360.41 cm^{-1} could be attributed to C≡N stretching or CO_2. The peak at 1632.39 cm^{-1} is likely due to C=O stretching vibrations from carbonyl groups or amide bands. The peak at 1214.47 cm^{-1} suggests C-O stretching, possibly from esters, ethers, or carboxylic acids(36).

DISCUSSION

This study successfully formulated and evaluated three antioxidant skincare products: a cream, lotion, and gel face wash, utilizing pineapple extract for its antioxidant properties. The aim was to assess and compare the antioxidant capacity, pH, viscosity, and stability of each formulation. The antioxidant capacity of the formulations was determined using IC_{50} values, which reflect the concentration required to inhibit 50% of free radicals. The IC_{50} value

for the pineapple extract was shown to be 41.67 μg/mL. With an IC_{50} value of 44.10 μg/mL, the lotion had the highest antioxidant capacity among the developed formulations, closely approximating the antioxidant strength of the pineapple extract itself. The gel face wash followed with an IC_{50} value of 47.19 μg/mL, demonstrating substantial antioxidant activity. The cream, while effective, showed a slightly lower antioxidant capacity with an IC_{50} value of 50.84 μg/mL. These results suggest that the lotion is the most effective in retaining antioxidant properties, making it potentially beneficial for enhancing skin protection against oxidative stress. The pH and viscosity of the formulations were also evaluated to ensure compatibility with the skin and ease of application. The cream exhibited a pH range between 6.4 to 7.9 and a viscosity range of 33,000 to 40,000 cps, indicating that it is well-suited for skin application and can be easily distributed with moderate shear. The lotion, with a pH range from 6.4 to 7.6 and a higher viscosity range of 150,000 to 380,000 cps, offers a thicker consistency, which is ideal for more intensive miniaturization. The gel face wash had a pH range of 6.4 to 7.5 and a lower viscosity range of 5,209 to 5,630 cps, suggesting it is easily spreadable and suitable for cleansing while maintaining compatibility with the skin's natural pH balance (37). FTIR (Fourier Transform Infrared) spectroscopy provided further insights into the chemical composition of the formulations, confirming the presence of functional groups associated with antioxidant activity. The cream showed peaks indicating the presence of aldehyde, carboxylic acid, ketone, aromatic benzene ring, and methyl groups. The lotion's FTIR spectrum revealed hydroxyl, amine, carbonyl, and aliphatic hydrocarbon chains. The gel face wash demonstrated the presence of hydroxyl, aliphatic hydrocarbons, and carbonyl groups, supporting the incorporation of antioxidant compounds.

CONCLUSION

In conclusion, this project demonstrated that the formulated cream, lotion, and gel face wash successfully utilized the antioxidant properties of pineapple extract, each offering distinct benefits for skin care. The lotion showed the highest antioxidant capacity among the products, suggesting it can effectively protect the skin from oxidative damage. The cream, with moderate antioxidant activity and excellent moisturizing properties, is particularly suitable for dry skin. The gel face wash provides effective cleansing while offering antioxidant protection, making it suitable for daily use on various skin types. These formulations offer a range of options for different skincare needs and preferences, contributing valuable antioxidant benefits. Overall, this project highlights the potential of pineapple extract in developing antioxidant-rich skincare products. Future studies could focus on evaluating the long-term effectiveness and user satisfaction of these formulations to further improve and adapt them for wider use.

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Table 1: Formulation table of Cream

Ingredients	F1	F2	F3
Pineapple Extract(API)	2 ml	3 ml	4 ml
Beeswax(Base)	3 gm	3.2gm	3.5gm
Borax(In-situemulsifier)	0.2gm	0.4gm	0.3gm
Liquid Paraffin(Soothing agent)	10 ml	15 ml	12 ml

Methyl Paraben(Preservative)	0.02gm	0.04gm	0.03gm
Water(Vehicle)	Q.S.	Q.S.	Q.S.
Rose oil(Fragrance)	Q.S.	Q.S.	Q.S.

Table 2: Formulation table of Lotion

Ingredients	F1	F2	F3
Pineapple extract(API)	1 ml	3 ml	2 ml
Glycerine (Humectant)	1 ml	1.5ml	2 ml
Castoroil (Emollient)	2.8ml	3.3ml	2.5ml;
Petroleum Jelly (Base)	0.8gm	1.3gm	1 gm
Cetyl Alcohol (Emulsifier)	0.5gm	0.6gm	1 gm
Tween 80(Surfactant)	1 ml	2 ml	1.5ml
Sodium Benzoate(Preservative)	0.08gm	0.11gm	0.05gm
Water(Vehicle)	Q.S.	Q.S.	Q.S.

Table 3: Table of Gel Formulation face wash

Ingredients	F1	F2	F3
Pineapple Extract (API)	3 ml	4 ml	3 ml
Sodium Lauryl Sulphate (Surfactant)	0.4gm	-	0.3gm
Tween 80(Surfactant)	-	2.5ml	-
Sodium Benzoate (Preservative)	0.06gm	0.04gm	0.03gm
Triethanolamine (pH adjuster)	0.3ml	0.4ml	0.1ml
Carbopol 934(Gelling agent)	0.4gm	0.2gm	0.1gm
Distilled Water(vehicle)	36 ml	50 ml	40 ml
Orange Oil (Flavouring agent)	Q.S.	Q.S.	Q.S.

Table 4: Organoleptic evaluation of the formulation at the time of preparation

Specification	Cream	Lotion	Gel face wash
State	Semi-solid	Suspension	Colloidal
Colour	White	Transparent	Transparent
Odour	Characteristic	Characteristics	Characteristics
Texture	Smooth	Smooth	Smooth

Table 5: Spreadability test of formulation

		Spreadability (gcm/sec)		
Formulation	Time(sec)	Cream	Lotion	Gel face wash
F1	5	3	2.5	2.3
F2	7	2.14	3.1	3.4
F3	4	3.75	3.5	3.5

Table 6: Determination of antioxidant activity of vitamin C and Crude Pineapple extract

	%DPPHinhibition	
Concentration(µg/ml)	**Vitamin C**	**Crude Pineapple extract**
20	**18.60**	**12.29**
40	**28.90**	**25.24**
60	**37.54**	**58.14**
80	**50.83**	**48.83**
100	**57.14**	**52.15**
IC_{50} (µg/ml)	**78.71**	**41.67**

Table 7: Antioxidant study of pineapple formulation

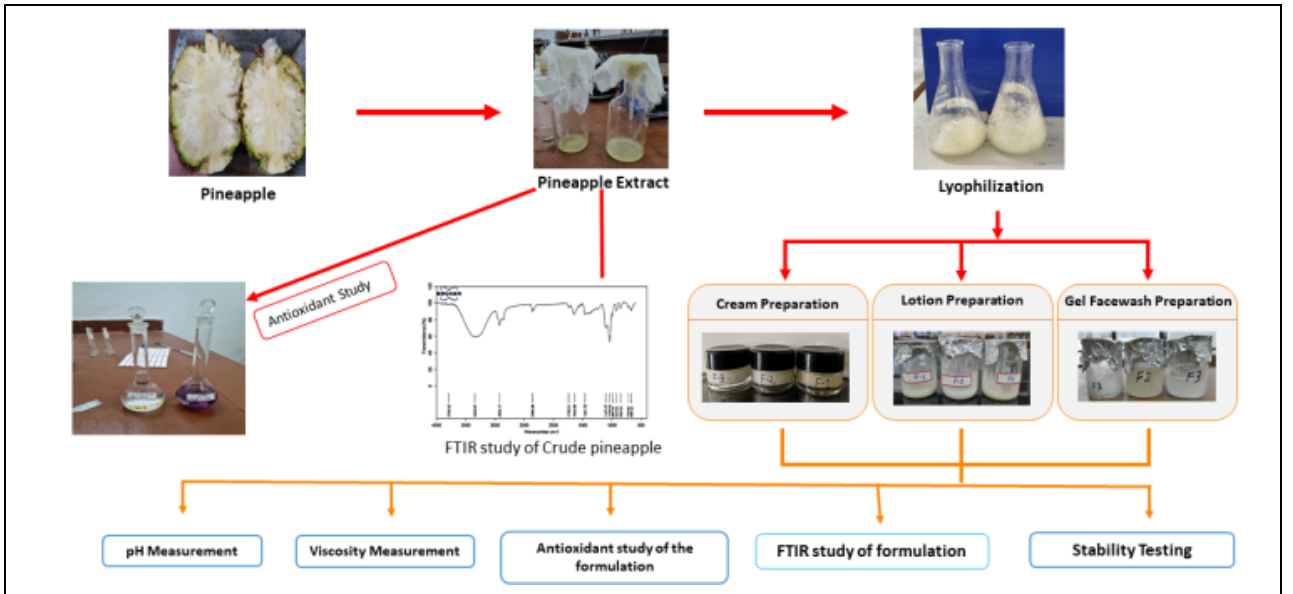
Concentration(µg/ml)	**%DPPHinhibition**		
	Cream	Lotion	Gel face wash
20	37.26	19.35	15.23
40	57.48	52.98	43.04
60	44.25	35.95	51.32
IC_{50}(µg/ml) Value	50.84	44.10	47.19

Table 8: Stability testing of different formulations for pH and viscosity after keeping in three different conditions.

	Observation after 15 days which are kept in Normal temp. (25°C)			**Observation after 15 days which are kept in Refrigerator**			**Observation after 15 days, which are kept under humidity chamber (40°C and 75% RH)**		
	Cream-F1	**Lotion-F1**	**Gel facewash-F1**	**Cream-F2**	**Lotion-F2**	**Gel facewash-F2**	**Cream-F3**	**Lotion-F3**	**Gel facewash-F3**
pH	7.7	7.4	7.1	6.5	6.7	7.3	8.5	7.9	8.4
Viscosity (CPS) At 0.6 RPM	39000	290000	5401	41000	170000	5420	47000	310000	7580

Table 9: Change of organoleptic properties of different formulations after keeping in three different condition.

Specification	**Observation after 15 days which kept in Normal temp. (25°C)**			**Observation after 15 days which kept in Refrigerator**			**Observation after 15 days which are kept under humidity chamber (40°C and 75% RH)**		
	Cream-F1	Lotion-F1	Gel facewash-F1	Cream-F2	Lotion-F2	Gel facewash-F2	Cream-F3	Lotion-F3	Gel facewash-F3
State	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid	Semi-solid
Colour	White	White	White	White	White	White	Yellowish	Yellowish brown	White
Odor	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic	Characteristic
Texture	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth	Tacky	Smooth	Tacky



Graphical Abstract



Fig.1(a)Pineapple cream (b) Pineapple lotion (c) Pineapple gel face wash

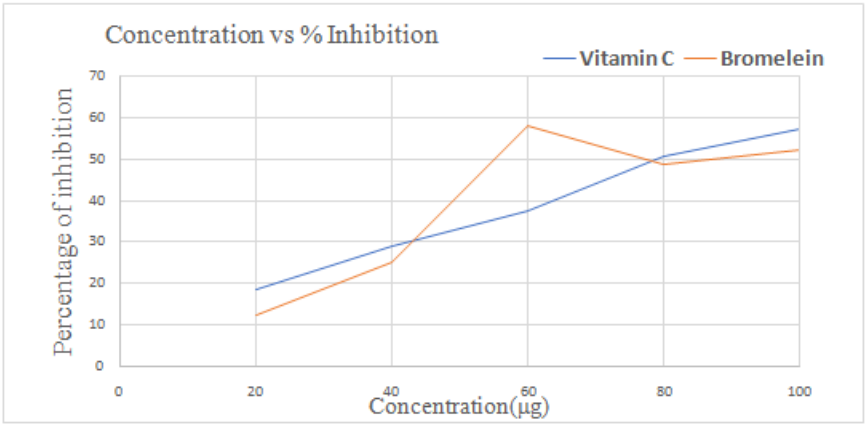


Fig 2: Antioxidant study of (a) Pineapple Crude, (b)Vitamin C

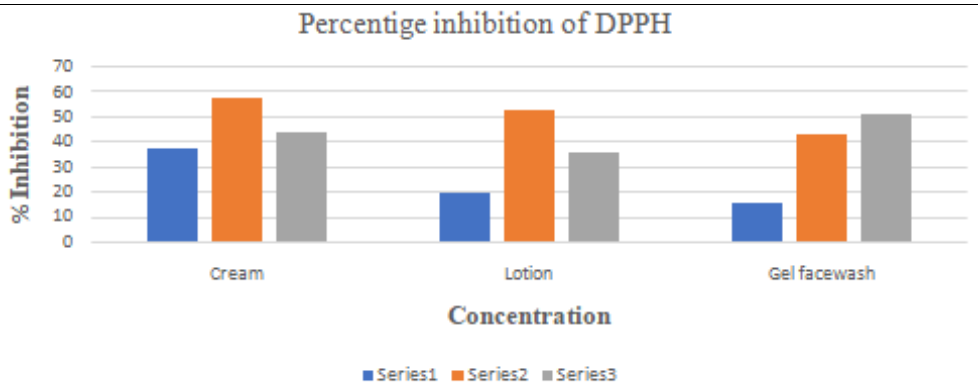


Fig 3: Antioxidant study of Cream, Lotion and Gel Facewash

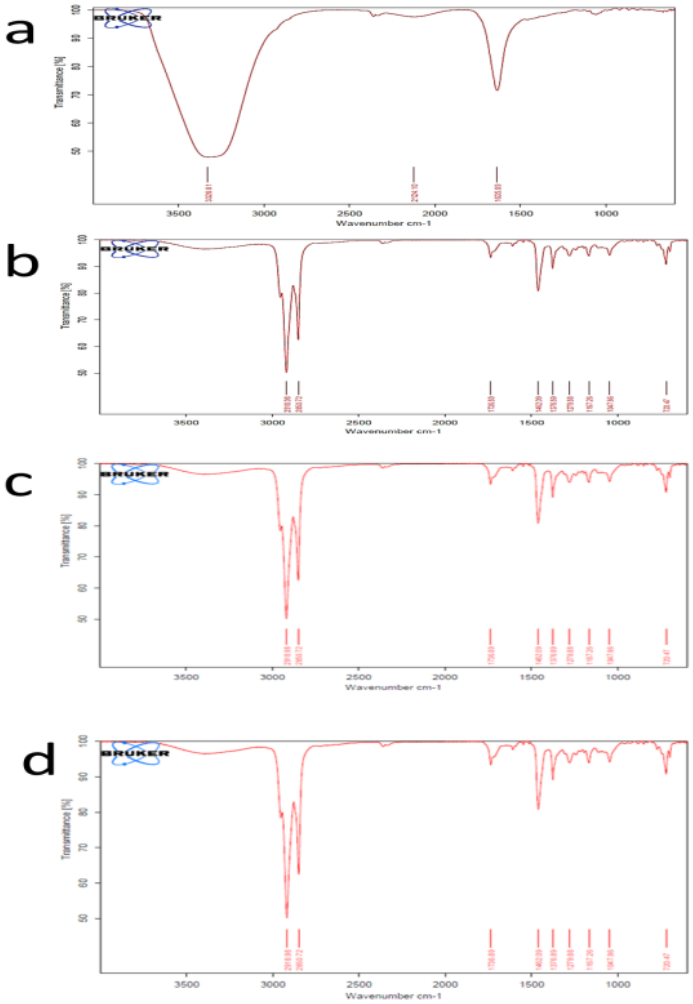


Fig 4:FTIR data for (a)Crude pineapple (b)Pineapple cream (c) Pineapple lotion(d)Gel face wash

RESEARCH ARTICLE

Evaluation of Post Operative Pain After Root Canal Treatment Following Activation with Different Irrigation Techniques in Maxillary and Mandibular Molars with Symptomatic Irreversible Pulpitis : An *In vivo* Study

Aparna Palekar[1], Deepali Gupta[2]*, Basawaraj Biradar[3] and Mukund V Singh[3]

[1]HOD and Professor, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Ahmednagar, (Affiliated to Pravara Institute of Medical Sciences), Maharashtra, India.
[2]Postgraduate Student, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Ahmednagar, (Affiliated to Pravara Institute of Medical Sciences), Maharashtra, India.
[3]Professor, Department of Conservative Dentistry and Endodontics, Rural Dental College, Loni, Ahmednagar, (Affiliated to Pravara Institute of Medical Sciences), Maharashtra, India.

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***Address for Correspondence**
Deepali Gupta
Postgraduate Student,
Department of Conservative Dentistry and Endodontics,
Rural Dental College, Loni, Ahmednagar,
(Affiliated to Pravara Institute of Medical Sciences),
Maharashtra, India.
E.Mail: deepaligupta1802@gmail.com

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ABSTRACT

AIM: To estimate and compare the incidence of post operative pain after irrigation activation using Conventional needle irrigation , Passive ultrasonic irrigation (PUI) and Sonic irrigation methods during root canal therapy using Visual Analogue Scale. Thirty patients with symptomatic irreversible pulpitis in maxillary or mandibular molars were selected for the study. The teeth were randomly assigned to three groups depending on the final irrigation technique applied. In Group 1 (conventional needle irrigation), a 27-gauge needle was used to perform the irrigation. In Group 2, Sonic agitation was done using endoactivator (Dentsply). It is device operating at 10,000 rpm and 3-5 mm vertical strokes. In Group 3, Passive ultrasonic irrigation was done using an ultrasonic device (Satelec). The tips in all groups were fitted 2mm short of working length. Volumes of 5ml 2.5% of NaOCl, 2ml 17% of EDTA, were used for 1 minute. Teeth were then obturated with gutta-percha and a resin containing sealer using the lateral compaction technique. Postoperative pain was evaluated at 6, 12, 24, and 48 hours, as well as after 1 week, using the Visual Analogue Scale (VAS). At 24 h, the severity of pain experienced by patients in the NI group was found significantly higher compared to the patients in the Sonic and Ultrasonic group ($P < 0.05$). Significant difference was found between groups at 24 h ($P < 0.05$). At 48 h pain continued to

decrease in all groups, the sonic irrigation group demonstrated lower values in VAS scale than the needle irrigation group. The highest PP scores were noted at 6 h in all groups and observed to diminish over time. At 6 h, 12h, 24 h and 48 hours, no significant difference was found among the groups in relation to pain intensity ($P > 0.05$). Within the limitations of the present study, the highest recording of Post op pain at the end of the 24th h was detected in the NI group and the lowest in the Sonic and Ultrasonic group. In all the irrigation methods, the pain showed a decrease after 24th h and the difference between the PP levels related to the irrigation methods disappeared. This study provides valuable insights into the effectiveness of different irrigation activation techniques in minimizing postoperative pain following root canal treatment. By identifying the most effective method, clinicians can improve patient comfort and treatment outcomes, contributing to enhanced patient satisfaction and adherence to endodontic procedures.

Keywords: Conventional needle irrigation, EDTA, Irrigation activation, Passive ultrasonic irrigation, Postoperative pain, Root canal treatment, Sodium hypochlorite, Sonic irrigation, Symptomatic irreversible pulpitis, Visual Analogue Scale,

INTRODUCTION

Successful endodontic treatment means effectively cleaning out the infected or dead pulp and removing any harmful microorganisms from the root canal system[1]. Clinically, it is defined by the lack of symptoms, sinus tract, swelling, and radiographically by normal periodontal ligament space or a progressive reduction in the size of the periapical radiolucency over follow-up periods[1]. The discomfort experienced following endodontic therapy is known as post-endodontic pain[1,2]. Both patients and dentists find it to be an uncomfortable scenario[1,2]. Post-treatment endodontic pain has been reported to occur in approximately 16% to 25% of cases. It usually appears in the first 24 to 48 hours following obturation and goes within few hours, however it can sometimes last for many days[2]. A number of factors can affect the likelihood of pain following root canal therapy: inadequate instrumentation; irrigant extrusion; intracanal dressing extrusion; hyperocclusion; missed canals; preoperative pain; periapical pathosis; apical debris extrusion; and apical patency at the time of root canal preparation[3]. Most authors used different endodontic materials and techniques, as well as varied criteria for defining and assessing post-endodontic pain. Pain perception is mainly subjective and variable. Because of its ease of use, the visual analogue scale (VAS) is frequently employed to describe the degree of pain. A 100-mm line with two endpoints representing no pain and the worst pain served as the VAS intensity rating[3]. Sodium hypochlorite (NaOCl) is frequently employed as an irrigant owing to its excellent antimicrobial activity and its effectiveness in breaking down organic matter. The activation of such irrigant using different techniques makes the areas that are difficult to access such as curved canals, dilacerated canals, calcified canals easily accessible. Conventional manual irrigation using a syringe and needle has been shown to be ineffective at delivering irrigant to places with limited access, like the apical and isthmus regions[3,4]. Passive ultrasonic irrigation (PUI) involves moving an ultrasonic tip that has been placed inside the canal, 2 mm short of the WL, passively up and down so as not to bind with the root canal walls[3,4]. The Endo Activator tips increase the activity of irrigation solutions without causing any dentin cutting The tips enables the safe activation of intracanal solutions and has the ability to activate intracanal fluid[3,4]. The purpose of this study is to evaluate and compare the incidence of postoperative pain with the application of conventional needle irrigation, passive ultrasonic irrigation, endoactivator during root canal therapy.

MATERIALS AND METHODS

This study was conducted in the Department of Conservative Dentistry and Endodontics, RDC,Loni after IEC approval.

Inclusion Criteria

- Teeth with symptomatic irreversible pulpitis (pulp vitality test will be done using EPT)
- Fully mature multirooted teeth. (will be assessed radiographically and clinically)
- Age: 18 to 65 years
- Systemically healthy patients
- Teeth with enough crown structure for adequate isolation
- Restorable teeth undergoing endodontic treatment

Exclusion Criteria

- Patients with systemic diseases, including diabetes, auto-immune disease, and cancer.
- Immunodeficient Patients
- Teeth with calcified canals, root resorption, immature/open apex, or previous root canal treatment.
- Teeth with severe damage.
- Teeth presenting with a sinus tract or associated periapical abscess
- Patients who have previously taken any medication.

A preoperative radiograph was taken to examine the periodontal and periapical tissues. The procedure began with the administration of local anesthesia, followed by rubber dam isolation of the teeth. Carious lesions were eliminated, and conventional access to the root canal system was achieved with diamond burs under continuous water irrigation. An apex locator was used to determine the working length (WL), which was subsequently verified by digital radiography using a 10k initial apical file. Initial filing was performed up to 25k hand files, followed by rotary filing of each canal using the Hero Gold Rotary file system. The instrumentation sequence followed the manufacturer's guidelines and involved using 4% taper files in sizes 20, 25, and 30, followed by 6% taper files in sizes 20, 25, and 30, in a crown-down technique. Copious irrigation was performed between the use of each file with 2.5% sodium hypochlorite and normal saline. A final rinse with 17% EDTA was used to irrigate all root canals for one minute, and they were then dried with sterile paper points. Thirty patients with maxillary or mandibular molars diagnosed as symptomatic irreversible pulpitis were selected. "The allocation of teeth into three groups was done randomly, based on the final irrigation procedure applied."

Group1: Conventional needle activation (control group).

Group2: Passive ultrasonic activation.

Group3: Sonic activation (Endoactivator)

In Group 1 Conventional needle irrigation was used, employing a 27-gauge needle.

In Group 2 Sonic agitation was done using endoactivator (Dentsply). It is device operating at 10,000 rpm and 3-5 mm vertical strokes.

In Group 3 Passive ultrasonic irrigation was done Using an ultrasonic device (Satelec)

The tips in all groups were inserted 2mm short of working length

Volumes of 5ml 2.5% of NaOCl, 2ml 17% of EDTA, were used for 1 minute

Teeth were then obturated with gutta-percha and a resin-based sealer using the lateral compaction technique

Postoperative pain was evaluated at intervals of 6, 12, 24, and 48 hours, as well as after one week, using the Visual Analogue Scale (VAS).

Patients to be asked to describe their pain experience using one of five well defined categories as

0-No PAIN

1-3- Mild pain

3-5- Moderate pain

5-9- Severe pain

10- Worst possible pain

RESULTS

- At 24 h, the severity of pain experienced by patients in the NI group was found significantly higher compared to the patients in the Sonic and Ultrasonic group ($P < 0.05$). Significant difference was found between groups at 24 h ($P < 0.05$)
- At the 48-hour mark, the sonic irrigation group demonstrated a significantly lower VAS score compared to the needle irrigation group.
- The highest PP scores were noted at 6 h in all groups and observed to diminish over time.
- At 6 h, 12h, 24 h and 48 hours, no significant difference was found among the groups in relation to pain intensity ($P > 0.05$).
- **6 hours**: All groups experienced the highest pain, with no statistically significant difference.
- **12 hours**: Pain began to decrease in all groups, with no significant difference among them.
- **24 hours**: Needle Irrigation (NI) group had significantly higher pain than Sonic and Ultrasonic groups ($P < 0.05$).
- **48 hours**: Pain continued to decrease in all groups, showed a significantly lower VAS score in the sonic irrigation group than in the needle irrigation group.

DISCUSSION

Apical extrusion of debris or irrigants varies depending on the root canal irrigation method used. Any irrigation delivery system that minimizes the risk of extrusion into periapical tissues benefits both the patient and the clinician by reducing postoperative discomfort[7]. However, the available data regarding debris and irrigant extrusion using different irrigation methods are primarily limited to in vitro studies. Furthermore, to the best of our knowledge, no study has evaluated the effect of different irrigation agitation techniques on the incidence and severity of postoperative pain (PP) after root canal treatment (RCT) in symptomatic molar teeth. This study aimed to compare the effects of various irrigation agitation methods on PP severity following RCT. Postoperative pain (PP) is largely subjective and can be affected by a variety of factors. Measuring subjective variables presents a considerable challenge, leading to the use of various scales and methods to assess PP [8]. The Visual Analog Scale (VAS) (100 mm) ranging from 0 to 10 was used to measure PP intensity. The VAS is simple, valid, reliable, and widely used in previous studies evaluating PP after RCT [9,10]. To minimize errors, the scale was described in detail to patients, who were also assisted in recording preoperative pain conditions to ensure familiarity with the scale. Limitations in evaluating PP arise due to differences in study designs, including variations in preoperative tooth conditions and treatment protocols [11]. To address these concerns, only teeth diagnosed with symptomatic irreversible pulpitis treated in a single visit were included. The potential effects of infected necrotic pulps and the use of intracanal medicaments were excluded. Standardization was maintained across all groups, including the use of a consistent irrigation agitation technique. A previous study by Rodríguez-Figueroa *et al*. [12] compared irrigant extrusion using passive ultrasonic irrigation (PUI), the EndoActivator system, and needle irrigation (NI), concluding that PUI and EndoActivator tips positioned within 1 mm of the working length (WL) appeared to be relatively safe. Based on these findings, PUI and EndoActivator tips within 1 mm of the WL were used in this study. It is well established that radiologic and electronic root canal measurements may not always correspond [15]. In this study, WL determination was achieved using an electronic root canal length measurement device and confirmed radiologically. This approach helps clinicians confine preparation within the root canal system, reducing the risk of over-instrumentation, which is considered a primary factor in postoperative discomfort. Several studies suggest that variables such as age, sex, and preoperative pain significantly impact PP [16,17]. However, no significant differences among demographic variables and preoperative pain scores between the groups were found, allowing these factors to be excluded from consideration. PP scores following RCT were significantly higher in certain groups at the 6- and 24-hour time intervals, leading to the rejection of the null hypothesis. Previous studies have evaluated the extent of apical extrusion associated with different irrigation approaches.. Karataş *et al*. [18] compared the effects of NI, sonic activation (SA), and PUI on apical debris extrusion and found no significant differences among these methods. Yost *et al*. [19] assessed the extrusion of NaOCl using both the EndoActivator and a conventional needle, finding that both

methods resulted in comparable levels of extrusion. Azim *et al*. [20] compared the volumes of irrigants extruded apically using different irrigation methods in an artificial model simulating clinical conditions and found that NI and EndoActivator methods resulted in similar volumes of extrusion. Boutsioukis *et al*. [23] investigated the effects of PUI and SA on irrigant extrusion using a newly developed method. Pain scores were highest in all groups within the first 6 hours, followed by a significant reduction at subsequent observation intervals of 24, 48, and 72 hours, and at 7 days. This pattern aligns with previous studies examining the duration of PP after RCT [21, 22]. Further studies are necessary to evaluate the influence of additional variables on PP.

CONCLUSION

Within the limitations of the present study, the highest recording of Post op pain at the end of the 24th h was detected in the NI group and the lowest in the Sonic and Ultrasonic group. Across all irrigation techniques, pain levels decreased after 24 hours, and the differences in postoperative pain associated with each method were no longer evident.

List of abbreviations
PP – Postoperative Pain
PUI – Passive Ultrasonic Irrigation
VAS – Visual Analogue Scale
WL – Working Length
NI – Needle Irrigation
NaOCl – Sodium Hypochlorite
EDTA – Ethylene diaminetetra acetic Acid
RCT – Root Canal Treatment
EPT – Electric Pulp Testing
RCT – Root Canal Therapy
RPM – Rotations Per Minute
G – Gauge (Needle Size)
mm – Millimeter
h – Hours

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Table 1: Comparison of Post-operative VAS score between three groups

Interval	Needle Irrigation		Sonic Irrigation		Ultrasonic Irrigation		p-value
	Mean	SD	Mean	SD	Mean	SD	
6 hrs	3.40	2.22	2.80	1.62	2.80	1.55	0.556
12 hrs	2.50	2.07	2.00	1.49	2.10	1.52	0.646
24 hrs	1.80	1.69	0.60	0.52	1.00	0.82	0.211
48 hrs	0.60^{a}	0.84	0.00^{b}	0.00	$0.10^{a,b}$	0.32	0.044*

Kruskal Wallis test; Post hoc Bonferroni test; * indicates a significant difference at p≤0.05

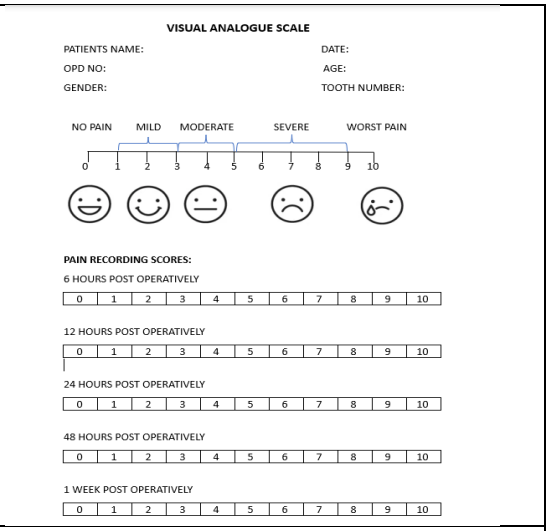


Fig. 1: Visual Analogue Scale

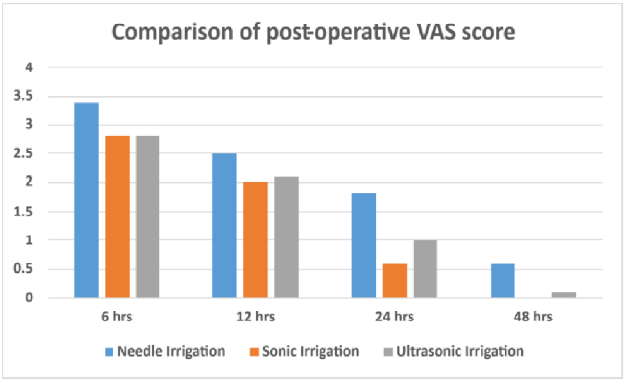


Fig.2: Comparison of Post-Operative VAS Score



Fig. 3: Cc syringe with 27 gauge needle

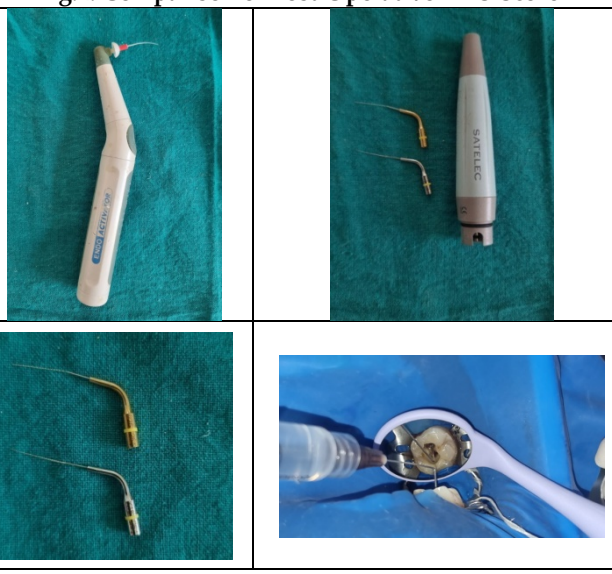


Fig.4: Irrigation Using Needle Irrigation

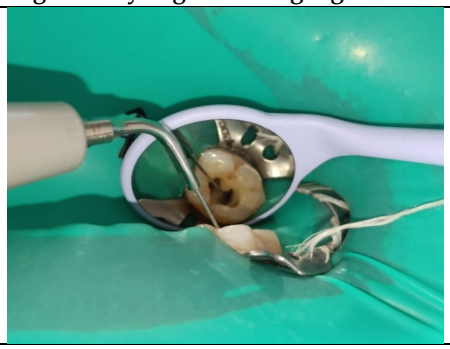


Fig.5: Irrigation using ultrasonic

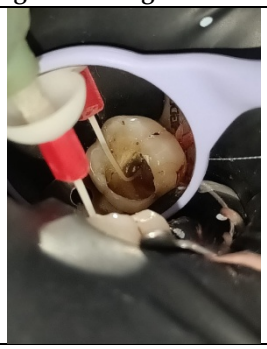


Fig.6: Irrigation Using Endoactivator

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RESEARCH ARTICLE

The Performance Model to Enhance the Lung Cancer Risk Analysis with Machine Learning Techniques

M Usha Devi[1]* and P. Karuppusamy[2]

[1]Assistant Professor, Department of Computer Science, Rathinam College of Arts and Science, Eachanari, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Information Technology, PPG College of Arts and Science, Saravanampatti, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
M Usha Devi
Assistant Professor,
Department of Computer Science,
Rathinam College of Arts and Science, Eachanari,
(Affiliated to Bharathiar University), Coimbatore,
Tamil Nadu, India.
E.Mail: usha.devi145@gmail.com.

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ABSTRACT

This study evaluates the performance of three machine learning models Logistic Regression, Random Forest, and Support Vector Machine in predicting lung cancer risk based on demographic and lifestyle factors. Using a dataset of 60 individuals with features including age, smoking habits, alcohol consumption, and residential area quality, compare model performance through various metrics and provide comprehensive data visualizations. The Random Forest classifier demonstrated superior performance with 88.3% accuracy, SVM is 85%and Logistic Regression is 81.7%. Our findings predict that ensemble methods may be particularly effective for medical risk prediction tasks with moderate-sized datasets.

Keywords: Lung Dataset, Data Pre-processing, Logistic Regression, SVM, Random Forest.

INTRODUCTION

Lung cancer continues to be one of the most prevalent and deadly malignancies worldwide, accounting for a significant portion of cancer-related morbidity and mortality. While tobacco smoking remains the predominant risk factor, accounting for approximately 85% of cases, emerging research highlights the growing significance of environmental exposures, genetic predispositions, and lifestyle factors in lung carcinogenesis. Recent epidemiological studies demonstrate notable shifts in disease patterns, including increasing incidence rates among never-smokers and changing histological profiles. Concurrently, advances in screening modalities and the

implementation of tobacco control policies have shown promising results in early detection and prevention. This paper synthesizes current evidence on the multifactorial etiology of lung cancer, examines contemporary trends in disease epidemiology, and evaluates the effectiveness of various prevention and early detection strategies. The analysis aims to provide a comprehensive understanding of this complex disease and inform future public health initiatives and clinical approaches to reduce the global burden of lung cancer.

LITERATURE REVIEW

Siegel *et al*. (2023)provides an updated statistical overview of cancer incidence, mortality and survival rates in the United States. The authors highlight key trends in lung cancer, emphasizing the impact of smoking and early detection. The study serves as a comprehensive resource for understanding current cancer epidemiology and is widely cited in public health research. Islami *et al*. (2021) This study quantifies the proportion of cancer cases and deaths linked to modifiable risk factors, such as smoking, alcohol use, and obesity. The findings underscore the significant role of tobacco in lung cancer mortality and advocate for stronger preventive measures. The research supports policy interventions aimed at reducing exposure to carcinogens. Alberg *et al*. (2022)this review explores the epidemiology, diagnosis, and management of lung cancer. It discusses risk factors, including smoking and environmental exposures, and provides clinical guidelines for early detection. The study is a key reference for pulmonologists and oncologists. O'Keeffe *et al*. (2021) This meta-analysis in *BMJ Open* examines gender differences in lung cancer risk among smokers. The authors conclude that smoking poses a similar relative risk for both men and women, contradicting earlier beliefs about gender-based susceptibility. The study reinforces the universality of smoking-related lung cancer risks. Malhotra *et al*. (2021)this global study identifies smoking as the leading risk factor for lung cancer across diverse populations. It also highlights regional variations in lung cancer incidence linked to tobacco use patterns, air pollution, and occupational hazards. Jha and Peto (2022)This evaluates the global impact of smoking, cessation, and tobacco taxation on lung cancer rates. The authors argue that higher taxes and anti-smoking policies significantly reduce mortality, providing evidence for public health strategies. Tindle *et al*. (2021)Using data from the Framingham Heart Study, this research demonstrates that cumulative smoking exposure strongly predicts lung cancer risk, even among former smokers. The study emphasizes the long-term benefits of quitting smoking early. Bade and Dela Cruz (2020)This review in Clinics in Chest Medicine summarizes the latest findings on lung cancer etiology, with a focus on smoking and emerging risk factors like radon and genetic predispositions. It serves as a primer for clinicians. De Groot *et al*. (2021)This article discusses epidemiological shifts in lung cancer, including rising adenocarcinoma rates and the impact of screening programs. It highlights the need for targeted prevention strategies. Smith and Winstanley (2022)this study links stricter tobacco policies (e.g., advertising bans, smoke-free laws) to declines in lung cancer mortality. It provides empirical support for legislative action. Wakelee *et al*. (2021)This research focuses on lung cancer in never-smokers, identifying second hand smoke, radon, and genetic factors as key contributors. It challenges the stereotype that lung cancer only affects smokers. Pesch *et al*. (2022)A pooled analysis of case-control studies, this paper quantifies the risk of lung cancer subtypes (e.g., squamous cell, adenocarcinoma) associated with smoking intensity and duration. Thun *et al*. (2023)This study tracks 50-year trends in U.S. smoking-related mortality, showing declines due to reduced smoking rates but warning of persistent disparities among low-income populations. Travis *et al*. (2021)A landmark classification system for lung adenocarcinoma, endorsed by major respiratory and oncology societies. It standardizes diagnostic criteria for researchers and pathologists. Ezzati and Lopez (2022)This study estimates global deaths attributable to smoking in 2000, highlighting tobacco's role in preventable mortality and advocating for international tobacco control. Lortet-Tieulent *et al*. (2021)This study analyzes lung cancer trends by histological subtype, noting a rise in adenocarcinoma among women. It calls for gender-specific research on smoking and lung cancer.

RESEARCH METHODOLOGY

Data Collection

The dataset taken from kaggle, which is used to predict the lung disease detection. The dataset consists of Name, Surname, Age, Smoke, AreaQ, Alkhol and Result.

Data-Pre-processing

In figure 1, The dataset shown contains information on 59 individuals with 7 attributes for each. These attributes include personal identifiers such as Name and Surname, along with health-related and environmental factors like Age, Smokes, AreaQ , and Alkhol. Result is the target variable, indicates that lung disease affected means 1 and not affected means 0

Model Evaluation

In the model evaluation, the three models are compared for the prediction of the lung disease such as Logistic Regression, Random Forest, and Support Vector Machine (SVM). In this work, Random Forest shows the improved performance of among all the models. Its provide the better results in the performance of the evaluation of the model.

RESULTS AND DISCUSSIONS

Correlation Map

The Result (lung disease presence) has a strong positive correlation with Age (0.64), Alkhol (0.81), and Smokes (0.37), suggesting these features are significant contributors to predicting lung disease. In contrast, AreaQ has a strong negative correlation with Result (-0.75), implying that poorer air quality (lower AreaQ) is associated with a higher likelihood of disease. In figure 2, displays a feature correlation matrix visualized as a heatmap, which illustrates the pairwise correlation coeff. Features like Name and Surname show negligible correlation with all other variables and are irrelevant for prediction. Overall, this matrix is useful for identifying the most influential features for building an effective predictive model.

Results of Cancer Status

Each subplot shows the distribution or correlation between two variables, with data points color-coded by cancer lung disease status pink for Result = 1 and teal for Result = 0. In figure 3, apair plot in scatter plot matrix that visualizes relationships between various features in the dataset based on lung disease status that is Result.

Comparison of Models

In figure 4, a comparison of model accuracies for lung disease prediction using three different machine learning algorithms that is Logistic Regression, Random Forest, and Support Vector Machine. Among the models evaluated, the Random Forest model achieves the highest accuracy, close to 0.88, indicating its superior performance in correctly predicting disease outcomes. Logistic Regression follows with an accuracy of approximately 0.85, while the SVM model performs the least effectively with an accuracy around 0.83.

Overall Performance Metrics

In Table 1, summarizes the performance of three machine learning models Logistic Regression, Random Forest, and Support Vector Machine used for lung disease prediction. In this, the Random Forest model shows the highest performance, achieving the highest accuracy of 88.3%, precision of 87.5%, recall of 88.9%, F1-score with 88.2%, and ROC-AUC with 0.89. The SVM model performs moderately well, with an accuracy of 85% and balanced precision, recall, and F1-score is 83.3%, along with a ROC-AUC of 0.86. Logistic Regression, while still fairly effective, lags slightly behind with an accuracy of 81.7%, precision of 80.0%, recall of 77.8%, F1-score of 78.8%, and a ROC-AUC of 0.82. In this results, Random Forest is the most reliable and accurate model for detecting lung disease among the three.

CONCLUSION

The analyses of the lung disease prediction task using machine learning techniques using dataset, consisting of health and lifestyle attributes such as age, smoking frequency, alcohol consumption, and environmental factors, which shows strong correlations between lung disease and features like age, alcohol use and area quality. The correlation matrix and pair plot confirm that these features are significant predictors, while identifiers like names and surnames hold no predictive value. Model comparison through accuracy and performance metrics reveals that the Random Forest classifier consistently outperforms both Logistic Regression and SVM. It achieves the highest scores across all key metrics accuracy (88.3%), precision, recall, F1-score, and ROC-AUC indicating its robustness in correctly identifying both positive and negative cases of lung disease. SVM shows moderate performance, and Logistic Regression, while still effective, is the least accurate among the three. Overall, the findings predict that Random Forest is the most suitable model for this classification task.

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Table 1. Model Performance Comparison

Model	Accuracy	Precision	Recall	F1-score	ROC-AUC
Logistic Regression	81.7%	80.0%	77.8%	78.8%	0.82
Random Forest	88.3%	87.5%	88.9%	88.2%	0.89
SVM	85.0%	83.3%	83.3%	83.3%	0.86

```
RangeIndex: 59 entries, 0 to 58
Data columns (total 7 columns):
 #   Column    Non-Null Count  Dtype
---  ------    --------------  -----
 0   Name      59 non-null     object
 1   Surname   59 non-null     object
 2   Age       59 non-null     int64
 3   Smokes    59 non-null     int64
 4   AreaQ     59 non-null     int64
 5   Alkhol    59 non-null     int64
 6   Result    59 non-null     int64
dtypes: int64(5), object(2)
memory usage: 3.4+ KB
None
      Name        Surname  Age  Smokes  AreaQ  Alkhol  Result
0     John           Wick   35       3      5       4       1
1     John  Constantine     27      20      2       5       1
2   Camela      Anderson     30       0      5       2       0
3     Alex         Telles    28       0      8       1       0
4    Diego      Maradona     68       4      5       6       1
```

Figure 1: Attributes values

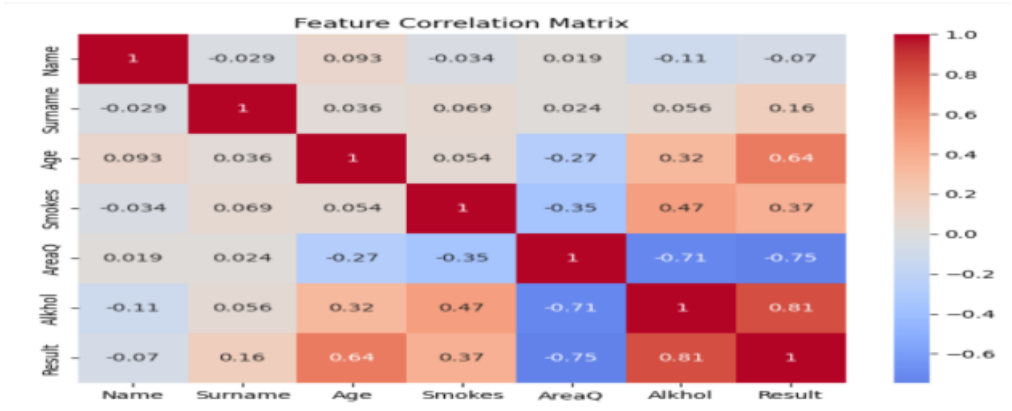


Figure 2: Feature Correlation Matrix

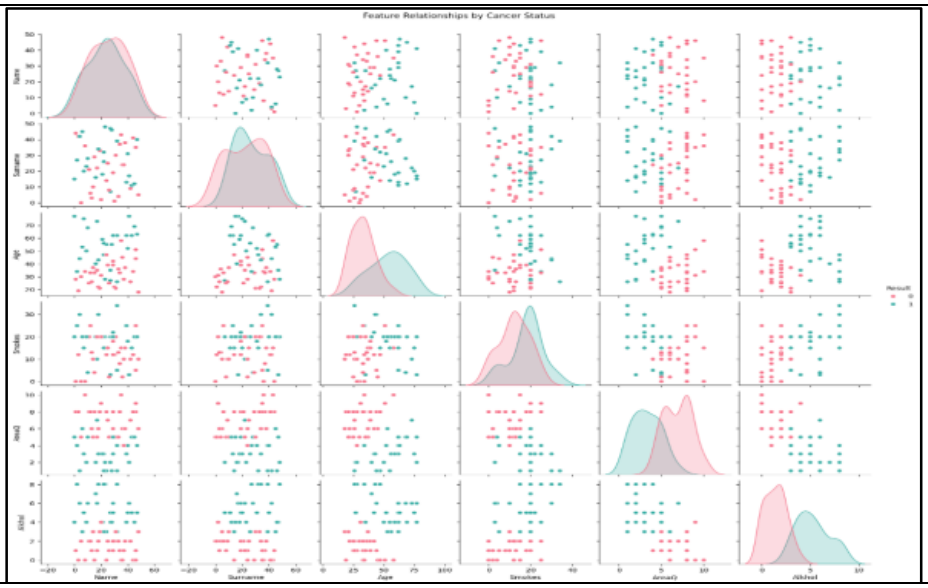


Figure 3: Cancer Status

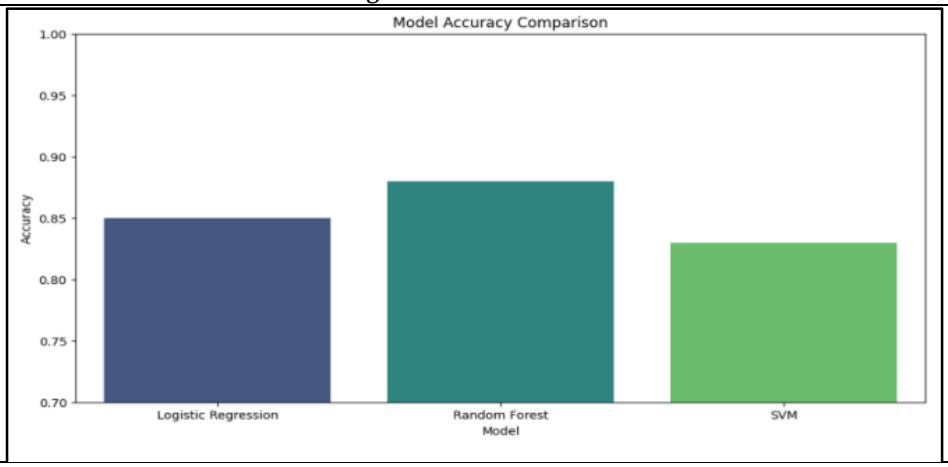


Figure 4: Performance evaluation of Model

RESEARCH ARTICLE

Towards Holistic and Inclusive Menstrual Health : Bridging Research, Policy and Practice for Indian Adolescents

Anjali Pushkar Tiwari[1]*

Associate Professor, Department of Obstetrics and Gynecological Nursing, Manikaka Topawala Institute of Nursing, (Affiliated to Charotar University of Science and Technology), Anand, Gujarat, India.

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***Address for Correspondence**
Anjali Pushkar Tiwari
Associate Professor,
Department of Obstetrics and Gynecological Nursing,
Manikaka Topawala Institute of Nursing,
(Affiliated to Charotar University of Science and Technology),
Anand, Gujarat, India.
E.Mail: anjalitiwari.nur@charusat.ac.in

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ABSTRACT

A good menstrual health plays a vital role in determining the future reproductive life of a female. Although being so important, it is often an under-looked aspect, when it comes to adolescent wellbeing, particularly in low and middle income countries like India. In India, there have been significant progress towards creating public health awareness but still menstrual problems among adolescents are deeply rooted in cultural stigma, poor health literacy and skimpy healthcare access. This review attempts to extract vital excerpts from the existing literature on Indian adolescents' menstrual health, identifying key gaps in research, exploring strengths and limitations of the existing policy initiatives and also evaluating the practices related to menstrual health. This was achieved by comprehensively reviewing peer reviewed articles, data from Government policy documents as well as the grey literature from various non-governmental organizations. The focal point of this review was adolescent menstrual irregularities, their psychosocial and lifestyle factors with their health seeking behavior in terms of the existing policy frameworks. This review findings highlight that in spite of growing awareness about menstrual health and management, there still persists critical gaps in both evidence and action.

Keywords: Menstrual health, Indian Adolescents, Menstrual irregularities, Psychosocial impact, Lifestyle factors, Health seeking behavior, Public health policy.

INTRODUCTION

Adolescence in humans is a crucial period of growth and development involving pivotal changes and adaptations in one's physical, physiological, psychological and social environments. It is a period which marks the transition from pre-reproductive to reproductive maturity. Menarche, the female's first menstrual period, often occurring during adolescence, serves as a significant milestone of reproductive wellbeing. Menstruation is a major biological event, which not only influence a girl's reproductive health, but have additional significant implications for her educational participation, mental wellbeing and social interactions[1,2]. India, housing over 253 million adolescents, where nearly 47% of them are females, menstrual health continues to be a profoundly overlooked matter[3]. To make things worse, menstrual health in India has always been a bounded issue, often encircled by sociocultural taboos and myths[2]. Empirical evidence highlights that a substantial proportion of Indian adolescent girls experience several menstrual irregularities such as dysmenorrhea, menorrhagia, irregular menstrual cycles, premenstrual syndrome and others, which often result in psychosocial distress among them[4]. Additionally, studies have also shown that majority of the adolescent girls with menstrual health related complaints, do not actually seek formal medical care, but instead rely on self-medication or home remedies. These treatment seeking behaviors were attributed to hesitation, shyness, lack of correct information and a shortage of adolescent friendly care options[5,6]. Menstruation and its related irregularities have a considerable impact on the academic performance of adolescent girls and highly account for their school absenteeism[7,8,9]. The worldwide understanding surrounding menstrual health has progressively broadened to encompass a multidimensional framework that includes hygiene, sanitation, menstrual irregularities, mental wellbeing and access to adolescent friendly healthcare services. However, in the Indian context, the adoption of this holistic perspective still remains in an emergent stage. The government of India have definitely made a positive attempt to work upon these aspects by introducing certain policies/programs. However these policies/programs largely focus on menstrual hygiene, with very skimpy focus on broader aspects such as mental support, nutritional need and various social determinants of menstrual health[9]. The researcher presents this review which forms a part of a larger mixed method research project currently underway in Gujarat, India, which aims to explore psychosocial and lifestyle correlates of menstrual irregularities and treatment seeking behavior of adolescent girls. This review attempts to synthesize existing evidence on menstrual health among Indian adolescents, critically evaluate any gaps in public health policies and programs and advocate for a more holistic and integrated approach to menstrual health from adolescent centered perspective.

Current Research Landscape

Prevalence and Distribution of Menstrual Irregularities

Studies across the country consistently suggest that there exists a heavy burden of menstrual irregularities among adolescents, with about 50-75% of them having suffered from at least one type of irregularity[5,10,11,12]. A meta-analysis done among Indian adolescent girls identified the pooled prevalence irregularities like dysmenorrhea, premenstrual syndrome, oligomenorrhoea, menorrhagia and polymenorrhoea to be among the highest[13]. Although menstrual irregularities are rampantly prevalent, yet they are failed to be adequately reported, often due to the socio-cultural beliefs surrounding it, which stigmatize and inhibit the open discourse around menstruation.

Psychosocial Impact

Menstruation in Indian context, is continually surrounded by several centuries old cultural restrictions and taboos. Menstruating females are considered as impure[2]. Discussing about it openly is often stigmatized. This has led to a feeling of shame, hesitance and misinformation among the female demography, specially the adolescents. Researches have shown strong association between menstrual disorders and various psychosocial factors like anxiety, stress, social isolation and body image issues[14,15]. Skipping school is a commonly cited collateral reaction to menstrual discomfort[7,8,15]. Additionally, lack of proper sanitary facilities in schools, worsened the school attendance during menstruation. A study done in the capital of India found that girls remained absent from schools citing reasons like anxiousness, embarrassment, shame along with lack of private place to manage periods in school, lack of adequate water supply and waste disposal system and lack of a separate bathroom for the girls[16]. A systematic review and

meta-analysis on management of menstrual hygiene among Indian adolescent girls reported that a quarter of them missed school during menstruation due to the lack of proper washrooms[17].

Lifestyle and Behavioral Determinants

Empirical evidences strongly suggest that lack of physical activities, a bad sleep routine and unhealthy eating habits have a strong association with menstrual irregularities[4,13]. Research has also indicated that menstrual problems were more prevalent among adolescent girls with high BMI[12,13,18].

Health-Seeking Behavior and Barriers to Care

In spite of the widespread prevalence of menstrual irregularities among Indian adolescents, there is a strikingly low utilization of healthcare services. Instead of taking professional help, girls were found more comfortable to confide their menstrual related problems with their mothers and friends[14,15,18]. Barriers like hesitance, prevailing stigma, lack of knowledge and lack of access to health care facilities, influenced the treatment seeking behavior of girls.

Methodological Gaps in Research

Most of the studies done in this area are predominantly cross sectional and focusses on school going adolescents. Large gap exists in findings related to adolescents not going to school as well as girls with disabilities. Additionally, studies involving the qualitative exploration of the experiences of such adolescents is minimal. This is a pivotal aspect when it comes to the in-depth understanding of the dynamic interrelationship that exists between various determinants of menstrual health.

Policy Landscape

National Initiatives and Government Programs

In order to address the disparities noted in the equitable access to menstrual healthcare services, the Government of India has introduced several national initiatives which primarily focusses on improving adolescent menstrual health. Two such prominent schemes are the Menstrual Hygiene Scheme (MHS), also known as the Scheme for Promotion of Menstrual Hygiene and the Rashtriya Kishor Swasthya Karyakram (RKSK). The Ministry of Health and Family Welfare launched MHS in the year 2011, with the main goal of benefitting adolescent girls by increasing their awareness as well as by increasing their access to high quality sanitary napkins and ensuring their safe disposal in an environmentally friendly manner[19]. The Accredited Social Health Activists (ASHAs) were also trained under this initiative, to play a pivotal role in distributing sanitary napkins along with creating awareness regarding menstrual hygiene management[20]. This scheme was a commendable step towards empowering adolescent girls. However, its impact was constrained by irregular supply of the sanitary napkins and minimal subsidies[21]. In 2014, RKSK was also introduced, whose one of the core strategy focused on improving adolescent menstrual health which was integrated within the sexual and reproductive health related component. Despite its holistic approach, RKSK faced its own challenges like lack of trained counsellors, procurement issues and inadequate participation of adolescents[22,23]. In the education sector, the Ayushman Bharat-School Health Programme has also included the component of menstrual health. However the programme too have faced considerable implementation gaps, with many of its intended interventions failing to materialize at ground-level[24].

Policy Gaps and Systemic Challenges

Despite the notable policy advances made by India in addressing menstrual health among adolescents, programmatic challenges such as being narrowly focused, having limited adolescent engagement, being underfunded with inconsistent percolation till grass-root level, have all collectively undermined the effectiveness of these initiatives.

Public Health Practice Landscape

Public Health Interventions: Successes

Over the past decade, India has proactively worked upon bettering the menstrual health of adolescents. The government has shown immense perseverance by adopting innovative models and methods in order to facilitate

better implementation of its various initiatives. By collaborating with Non-Governmental organizations, the country has successfully attempted to expand its services like distribution of sanitary pads, raising awareness and fighting against stigmatization, to the actual needful demography[24].

Public Health Practice: Programmatic Gaps

In spite of significant strides in promoting menstrual health, the actual impact of various initiatives have been restricted by several pressing shortcomings. These include being excessively fixated on distribution of sanitary products with limited attention to the physical, emotional and social aspects of menstrual health; insufficient supportive infrastructure in schools; limited focus on adolescents who are outside the formal education system; insufficient training and coordination among stakeholders involved in implementation[22,23,24].

Challenges in Health Workforce

Under the various initiatives aimed to address adolescent menstrual health, several challenges persists as highlighted by various studies. It is reported that the frontline workers - primarily ASHAs and Peer educators - are often not able to sustain their efforts effectively due to factors such as inadequate supply of sanitary pads for distribution, workforce shortages compounded with overburdened existing taskforce and insufficient refresher training opportunities, both in frequency and duration[21,22,23,24].

Call for Holistic and Equity Centered Approaches

To have a lasting effect, menstrual health must be approached with dignity, inclusion and must be recognized as a fundamental public health right. To achieve this would require additional efforts like robust implementation frameworks, active community engagement, a well-resourced and empowered taskforce and holistic care that caters both physical and psychosocial wellbeing.

Key Recommendations

At the moment, efforts towards menstrual health among Indian adolescents reflect a fragmented understanding based approach, with weak integrated systemic actions. The recommendations outlined in table 1, aims to support the creation of a transformative framework that embeds menstrual health within the bigger agenda of dignified and inclusive public health.

CONCLUSION

The dimension of adolescent menstrual health has recently picked up its momentum in the Indian subcontinent. Although the last decade has witnessed increased attention through various policies and programs, the discourse around menstrual health remains predominantly focused on hygiene and access to sanitary napkins. The critical concern of understanding the complex multifaceted interplay of biological, psychological and socio-cultural factors that usually affect adolescent girls, is often over-looked. Additionally, research in this domain remains scattered, with limited focus on psychosocial dimension and treatment seeking behavior. This review has attempted to synthesize the existing evidence on Indian adolescents' menstrual health, advocating for a more holistic approach that repositions adolescent menstrual health from a mere hygiene issue to an integral human right and public health priority.

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Table1: Key Recommendations for advancing adolescent menstrual health

Recommendation	Strategy
Redefine Menstrual Health	Expand from a hygiene issue to a holistic health and human right matter. Should also address menstrual irregularities, associated mental health, reproductive health awareness and psychosocial support.
Prioritize Adolescent focused research	Conduct and invest in multitude of scientific inquiries involving adolescents, particularly those from the over-looked demography like out of school adolescents.
Scale up school based Menstrual Health efforts	Ensure trained taskforce, adequate supplies, supportive infrastructure, inclusive education; Strengthen existing Adolescent Friendly Health Clinics; Support for frontline workers.
Targeted and Inclusive support	Enhance more inclusive efforts targeting adolescents who are outside the formal education system, have disabilities or belong to marginalized section.
Involvement of male counterpart	Engage male stakeholders like teachers, fathers and community leaders in the challenging journey of de-stigmatizing menstrual health and fostering social acceptance, through menstrual health initiatives.
Strengthen monitoring system	Promote independent monitoring and evaluation of both Government and Non-Government led actions. Establish built in feedback mechanism for continuous improvement.
Partnerships for United action	Foster collaborations between sectors like health, education, digital media and youth empowerment to develop and deliver sustainable menstrual health solutions.

REVIEW ARTICLE

Navigating Product Licensing in the Eu : An Approximate Overview

K. Chaithanya Lakshmi Ramadevi[1]* and Y. Koushik[2]

[1]Student (M. Pharmacy), Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, Lam, (Affiliated to Acharya Nagarjuna University, Nagarjuna Nagar), Guntur, Andhra Pradesh, India.
[2]Associate Professor, Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, Lam, (Affiliated to Acharya Nagarjuna University, Nagarjuna Nagar), Guntur, Andhra Pradesh, India.

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***Address for Correspondence**
K. Chaithanya Lakshmi Ramadevi
Student (M. Pharmacy),
Department of Regulatory Affairs,
Chalapathi Institute of Pharmaceutical Sciences, Lam,
(Affiliated to Acharya Nagarjuna University, Nagarjuna Nagar),
Guntur, Andhra Pradesh, India.
E.Mail: kuthanichaithanya@gmail.com,

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ABSTRACT

This literature overview," Navigating Product Licensing in the EU: AN approximate Overview," It delves into the Central, Decentralized, and multiple Recognition proceedings, breaking down their respective edges, minuses, and felicitous for multi-hued groups of pharmaceutical substances and drugs. The document emphasizes the intention of EMA to preside over the Central approval route; attributes and resemble as sanction time, disbursement, executive expenditure, and post-marketing guidance are juxtaposed across these strategies. It similarly recognizes novel tendencies and the obstacles in regularizing administrative necessities across EU constituent nations. It suggests fields for coming breakthroughs in optimizing these operations, leveling to upgrade demand obtain ability, mitigating interval-to-demand, and keeping up public security within the EU.

Keywords: Product Licensing consent, EMA, Central approval route Proceeding, Decentralized nonsupervisory proceeding, multiple Recognition route, European Union official cadre, Safety regulation, Post-market guidance.

INTRODUCTION

Product licensing consent proceedings serve as fundamental perspectives for pharma health care regulating system, governing how medications are approved and launched for different regions. In the EU, the purpose of these proceedings is not only to ensure the integrity, efficiency, and safeguarding of the medications but also to accelerate

the accessibility of innovative treatments to patients across the member states. Considering the society well-being impacts, these procedures are complex and dynamic and both scientifically supported and legally organized.
The EU establishes four unique routes for acquiring marketing authorization:

1. Central approval route Proceeding,
2. Decentralized non-supervisory proceeding,
3. Multiple Recognition route, and
4. Nationalized proceedings.

Designed for the Pharma formulations and directive goals, For instance, Central approval route Proceeding is compulsory for Novel treatments, advanced therapies, and drugs for rare or neglected diseases (orphan drugs), while decentralized and multiple recognition proceedings are typically adopted for generic products and widely accepted products targeting market access in multiple regions. This assortment allows organizations Agility in developing product introduction strategies, yet, it contributes to a more complex administrative landscape. The EMA and NCA significantly contribute to the structure of the administrative landscape by Administer assessments and sector frameworks by the chosen regulatory strategy.

Overview of the EU administrative framework

Approaches are essential in the pharmaceutical firm, as they confirm that only safe, effective, and premium-quality drugs are introduced to the market. It entails a detailed exhaustive examination of a medicine's quality, safety, and efficacy before public sale, forming a key pillar of public health safeguarding. A Simplified, Fast-tracked authorization method can substantially cut down time-to-market for Cutting-edge therapies, serving the needs of industry stakeholders and patients, In need of fast access to new therapeutic solutions. To address the varying characteristics of pharmaceutical products, the EU implements multiple authorization pathways, namely the Central approval route Proceeding, Decentralized non-supervisory Proceeding (DCP), and Multiple Recognition Proceedings (MRP). Each of these routes provides different advantages in terms of regulatory efficiency, adaptability, and scope of market entry—factors that are especially important considering the wide range of medicinal products, such as biologic, generics, and orphan medicines.

The Role of the European Medicines Agency (EMA)

Central to Eu's administrative frame, it was EMA, which oversees the assessment and Supervisory control of therapeutic agents. These pathways are especially important for novel treatment modalities addressing unmet medical needs those addressing critical social well-being. The EMA's role goes beyond initial examination, encompassing for the continuous Medicinal product oversight post-authorization, conducting data-driven reviews, and offering regulatory guidance to pharma regulatory and EU states. By maintaining strict analysis and Therapeutic risk assessment procedures, administration upholds the safe use, superiority, and effectiveness criteria necessary for medications to enter and remain in the EU market. This not only safeguards community well-being but also supports modernization by accessibility of emerging medical interventions. Eu's legislative structure—led by the EMA—provides a well-defined, transparent, and scientifically robust environment for the pharmaceutical sector. This system strikes a crucial balance between assuring patient well-being and facilitating timely access to indispensable medications.

SCOPE, PURPOSE AND CONTEXT OF REVIEW

Aim: To deliver a concise comparative assessment for Eu's primary marketing authorization procedures, by highlighting their procedural steps, timelines, costs, and regulatory obligations. It offers stakeholders practical insights for selecting the most suitable pathway based on product type and strategic goals.

Source Selection: Relevant peer-reviewed literature, EMA and European Commission guidelines, and regulatory reports from 2015–2024 were included. The focus was on materials discussing regulatory timelines, benefits, requirements, and challenges of each MA route.

Inclusion and Exclusion Criteria: Included sources provided substantial insight into EU MA procedures for pharmaceuticals. Excluded were outdated documents or those unrelated to human medicinal products (e.g., cosmetics, food, or veterinary fields).

Data Analysis: A qualitative, comparative approach was applied. Key themes were extracted to evaluate timelines, regulatory complexity, cost, flexibility, and post-marketing obligations for each pathway.
Outcome: The synthesized findings equip with an understandable, strategic overview of EU marketing authorization processes, guiding companies in selecting appropriate regulatory routes for efficient and compliant market access.

Overview of Product Licensing Procedures in the EU
Central Approval Route Proceeding
It was a streamlined process overseen by the EMA. It is specifically designed to simplify the Endorsement of pioneering and high-impact therapeutic formulations over the EU and the EEA.

Scope and Applicability: Mandatory for Biotech-derived medicines, ATMPs, orphan drugs, medicines for specific conditions, Encompassing malignancies, neuro-degenerative conditions, endocrine disorders, immunological diseases, and viral infections like HIV/AIDS. Permissible to other forward-thinking treatment that represents the major strides in clinical, regulatory, or technological domains.

Process Overview: An application was submitted directly to EMA, the CHMP conducts the legislative assessment. If the CHMP issues a favorable standpoint, EC grants a single and unified license throughout EU.

Key Benefits: Once granted, the authorization applies uniformly to all EU member states and promotes regulatory uniformity, particularly for high-tech or novel products through centralized Pharmacovigilance and post-marketing surveillance.

Decentralized Non-supervisory Proceeding
It was intended for medications that are still waiting for the consent in any EU state; this route enables pharma industries to simultaneously obtain MA in multiple countries, offering both flexibility and efficiency.

Scope and Applicability: Used for products which are under review in any EU state, often selected for reliability looking into extend the multiple segments.

Process Overview: The contender submits a single consent form to multiple EU states, one country is designated as the RMS, which conducts the systematic examination and oversees the procedure and the other involved countries are known as CMS. If RMS provides an assessment report, which the CMS must either accept or raise objections to within a defined time-frame.

Key Benefits: Cost-efficient than applying for separate national authorizations across all nations, expands market reach while maintaining flexibility and offers a lower regulatory burden.

Multiple Recognition Route Proceeding
It allows a medication, once approved in one constituent state of EU, to extend its provision to other EU states, which offers streamlined approach to gain endorsement across multiple nations based on initial local acceptance.

Scope and Applicability: Used when a medicinal product has already received consent in a single EU state, whereas other states that wish to sell the medication to recognize the initial consent which are typical to register well-established medicament's and generics with Established inclusion in one country.

Process Overview: The product primary license in a RMS and contender claims the appreciation of this endorsement by the CMS. These countries required embracing the RMS / raise exceptions undoubtedly handed within specified duration. It facilitates evaluation compared to initial endorsements.

Key Benefits: Delivers an productive way to develop distribution of outcomes that already endorsed in one constituent of EU, constricts organizational duties, as the supplies has previously subjected to consideration in RMS, accelerates enrollment into further segments within the EU/EEA.

Nationalized Proceedings

It was the foundational route within the EU, used when a product is purposed to justified in just one constituent state of EU. Unlike other EU-wide proceedings, the NP was strictly confined to the country where the application is filed and does not permit access to additional member states.

Scope and Applicability

- A country-specific legislative pathway, valid for member jurisdiction where the request is lodged.
- Commonly chosen for generic medications or niche materials not aiming for immediate broader product access.
- Suitability of materials in a market with restricted coverage or those does not fulfill prerequisites for other centralized or decentralized procedures.

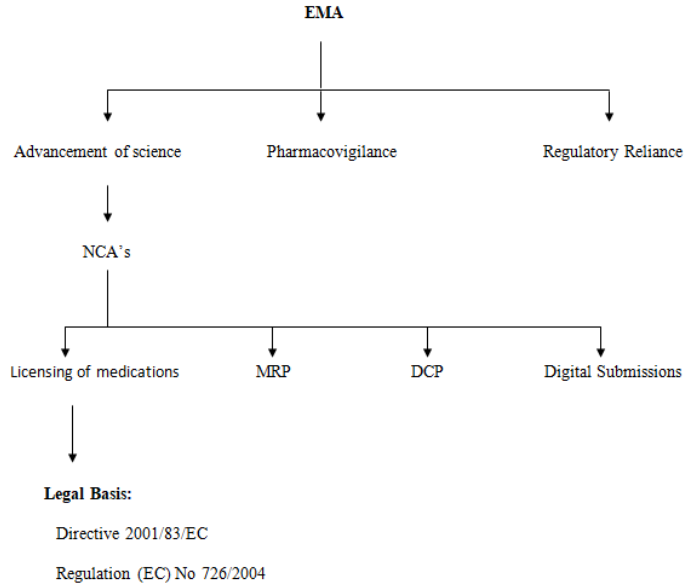
Process Overview: Entrant sends the Proposal to the NCA concern state; the NCA evaluates the product's security, therapeutic potency, and reliability by national compliance standards. Upon approval, medications merely market and sold in that defined boundaries.

Key Benefits

- A simpler and enhanced cost-effective route compared to wider EU approaches.
- Potentially faster endorsement timelines for commodities that do not require multinational access.
- Ideal for initial entry into a local market where a single-country authorization meets the commercial objectives.

Regulatory Authorities and Legal Framework In Pharmaceutical Regulation

EU Administrative



i.e.,

EMA

It plays a central role in coordinating the advancements for medicinal compounds even it also supervises Pharmacovigilance, ensuring that the risks of medicines are continuously assessed even after they are marketed.

NCA's

National administrative bodies in each EU state that accountable for proceedings related to MA. They play a significant role in the Multiple Recognition Proceedings and Decentralized Proceedings, helping facilitate the approval of medicines across multiple countries in the EU.

Legal Framework

EMA Regulatory activities and NCAs are operated under the Directive 2001/83/EC and EC - No 726/2004.

Directive 2001/83/EC sets out the regulatory structure for manufacture and distribution of therapeutic agents and materials within EU.

Regulation (EC) No 726/2004 outlines the centralized proceedings for legislative medications, which is particularly important for high-priority or complex medicines, such as biologics.

Recent Updates

Legislative Reliance: This concept allows administrative bodies to rely on the evaluations of other trusted regulators when making decisions about medicine, making the process better optimized and fostering international cooperation.

Digital Submissions: There has been an important turn to the digital submissions for regulatory approvals, which allows faster, more efficient processing of registration, reducing the burden on both administrative bodies and pharmaceutical companies. Comparative study of EU Authorization proceedings (Table 1)

Comparative Analysis with Global Regulatory Authorities

In the global regulatory landscape, the European Union (EU), United States (US FDA), and Japan (PMDA), India (CDSCO) represents four most influential regulatory systems. Each authority follows a distinct framework with its own benefits, challenges, and strategic approaches for Pharmaceutical approval.

U. S

FDA: US follows a single centralized marketing authorization system for all states.

Pathways

Standard Review

Expedited Pathways, including

Hasten: For critical medical conditions with unmet needs.

BTD: For interventions with preliminary evidence of Considerable advancement.

Accelerated Approval: Permitted on the basis of alternate endpoints.

Priority Review: Review in a span of 6 months instead of the standard 10 months.

Example: 'Keytruda' **(**pembrolizumab**)**: Received Breakthrough Therapy status for multiple indications.

Japan

PMDA centralized regulatory authority under the Ministry of Health, Labour, and Welfare, where Pre-submission consultations are mandatory.

Expedited Programs

i. Sakigake specification: For innovative products with initial stages in Japan.
ii. Priority Review and Conditional Approval are available based on urgency and medical need.

Example: Opdivo (nivolumab)**:** First approved in Japan for melanoma under priority review.

European Union

EMA with **NCAs**, the hybrid system combining a centralized agency with the decentralized national routes via NCAs**.**

Pathways

- Centralized Procedure(Mandatory for high-tech drugs),
- Decentralized and Mutiple Recognition(flexible national expansion),
- National Procedure (for single-country marketing).

Facing challenges like multiple legal frameworks (e.g., Directive 2001/83/EC, Regulation 726/2004), Linguistic diversity**:** Requires translations and national labeling adaptations, more complex coordination**:** RMS/CMS discussions in DCP/MRP.

Example: Zolgensma (onasemnogene abeparvovec)**:** Approved via centralized procedure.

India

CDSCO under Ministry of Health and Family Welfare works closely with state-level drug controllers and the Drug Consultative Committee. Offers a single national endorsement framework, where the Drug Controller general of India (DCGI) is capable for the authorization of drugs and biologics, Application, human studies, requires endorsement from the DCGI.

Expedited programs

Hasten: For drugs that fulfill neglected restorative needs or for life-threatening conditions.

Priority Endorsement: Required for quicker to get basic medicines.

Import Permitting: For imported drugs, the CDSCO has particular authorizing rules that guarantee item quality.

Examples: Covaxin: Endorsed beneath crisis utilize authorization by CDSCO with fast-track strategies amid the pandemic; Remdesivir: Affirmed beneath the same assisted pathways for COVID-19 treatment.

Global Regulatory Authorities Comparison (Table 2)

DISCUSSION

- The selection of a product licensing route is guided by the components characteristics, target markets, and prior regulatory experiences.
- The CP excludes the access for member states of EU through a single application, but involves greater complexity, time, and administrative effort.
- The DCP and MRP are more economical options, ideal for generics and well-established medications seeking selective market entry.
- Common challenges include inconsistent interpretations among member states, approval delays from CMS, and adaptation to refining digital expectations.
- New digital requirements, like eCTD format and use of PLM Portal, require pharmaceuticals to upgrade their administrative submission systems.
- Regulatory trends are shifting toward convergence, with increased use of reliance models and efforts to achieve digital harmonization across EU jurisdictions.
- These changes aim to simplify processes, enhance coordination, and support more efficient, faster outcomes approvals over Europe.

CONCLUSION

Comprehending the perspectives for EU product licensing applications were important for pharma industries. Focused on streamline enhancement and make their products available. Alternative options, like Central approval, Decentralized, and Multiple Recognition Proceedings, are each tailored to various kinds of pharma medications and endorsement strategies. A clear comprehension frame helps regulatory bodies and administrators make well-informed decisions, leading to faster approvals and even most productive entry into demand. Adopting digital technologies alongside adaptive licensing is modernizing the administrative frame, and facilitating accelerated and simplified legislative proceedings. Co-exist to align with EU benchmarks, these advancements were extremely streamlined processes, accelerate time-to-market, and rely on broader acceptance to advanced therapies for public. Carefully aligning administrative strategy with the medication and its demand expectations, it is the key to gain consent and attaining commercial viability. With regulatory enhancements and digital adoption on the rise, the EU is paving to approach agile and efficient pharma licensing procedure which supports immediate approaches to therapies for both the plant and public. Hence the transformation in regulation was scheduled for drive-in pharma progress and growth, making it possible to health care recipients for benefiting from reliable and effective therapies without undue waiting.

LIST OF ABBREVIATIONS

EU: European Union
EMA: European Medical Agency
CP: Central approval proceedings / centralizes procedure
DCP: Decentralized non-supervisory proceedings
MRP: Multiple recognition proceedings / mutual recognition procedure
NP: Nationalized proceeding / national procedure
NCA: National competent authorities
MA: Marketing authorization
EEA: European economic area
ATMPs: Advanced therapy medicinal products
CHMP: Committee for medicinal products for human use
RMS: Reference member states
CMS: Concerned member states
EC: European commission
CMDh: Coordination group for mutual recognition and decentralized procedures for human
CTD: Common technical document
IRIS: Integrated European industrial risk reduction system
eAF: Electronic application form
OTC: Over the counter
CDSCO: Central drugs standard control organization
FDA: Food and drug administration
US: United states
PMDA: Pharmaceuticals and medical devices agency
BTD: Breakthrough therapy designation
PLM: Product life cycle management.

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Table 1: Comparative study of EU Authorization proceedings:

Feature	Central approval Proceeding	Decentralized non-supervisory Proceeding	Multiple Recognition proceeding	Nationalized Procedure
Scope	Mandatory for biotech, orphan drugs, ATMPs, cancer, etc.	For products unapproved and not under the centralized scope	Used when product already authorized in one country of EU.	Used when product is intended for only one country
Purpose	To provide a single EU-wide license for innovative medicines	To apply for approval in multiple states at the same time	To extend existing authorization to further countries	To authorize products locally within a single country
Used For	High-innovation and EU-wide drugs	New generics, branded drugs across select countries	Nationally approved products needing wider access	Local/niche drugs, herbal/OTC products
Authority	EMA	RMS + CMS	RMS + CMS	NCA
Validity	All EU/EEA states	Selected countries	Selected countries	One country only
Application Type	One EU-wide	Simultaneous in several	Based on existing approval	National requirements
Timeline	~210 days	~210 days	~90 days	~210 days
Cost	High	Medium	Medium	Low to Medium
Examples	Comirnaty, Spinraza	Montelukast generic	Ibuprofen generic	Paracetamol syrup
Recent Amendments	Rolling review, IRIS	eAF mandate, digital RMS	CMDh updates, CTD portals	e-submissions, national portals

Table 2: Global Regulatory Authorities Comparison

Features	EU EMA / NCAs	India CDSCO	US FDA	Japan PMDA
Organization	Central and decentralized proceedings	Centralized with regional offices	Fully centralized	Centralized with early support

Expedited programs	PRIME, Accelerated Assessment	Fast Track, Priority assessment	Hasten, Breakthrough, etc.	Sakigake, Priority Review
Clinical Trial Support	Some support varies by procedure	Clinical trial approval, meetings	IND meetings, close interaction	Mandatory trial consultations
Timelines	~210 days Long - DCP/MRP	12-18 months	6–10 months	6–12 months
Linguistic Diversity	High (requires translation)	Minimal (English, Hindi, others)	Not applicable	Minimal
Approval Type	EU-wide	National approval, some mutual recognition	One national license	One national license
Legal Complexity	Multi-layered EU regulations	Single national framework	Single national framework	National framework

RESEARCH ARTICLE

Toxicity Potential of the Pesticide, Phoskill against the Freshwater Fish, *Oreochromis mossambicus* vis-a-vis Biochemical, Haematological, Histological and Molecular Characteristics

J.Jayashree[1], J.Roopavathy[1]*, K.Nanthini Devi[2], R.Mathupriya[3], G.Sathiyaraj[4], S.Sinduja[2], Anup Mandal[4], S.Kandan[4] , P. Santhanam[2] and P.Perumal[2]

[1]PG and Research Department of Zoology, Nirmala College for Women (Autonomous), Red Fields, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Department of Marine Science, School of Marine Sciences, Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.
[3]Department of Biotechnology, J.J. College of Arts and Science (Autonomous), Pudukkottai, (Affiliated to Bharathidasan University, Tiruchirappalli), Tamil Nadu, India.
[4]MPEDA-Rajiv Gandhi Centre for Aquaculture, Ministry of Commerce and Industries, Government of India, Sirkali, Tamil Nadu, India.

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***Address for Correspondence**
J.Roopavathy
Assistant Professor,
PG and Research Department of Zoology,
Nirmala College for Women (Autonomous), Red Fields,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: jroopavathy27@gmail.com

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ABSTRACT

Presently an evaluation was made on the effects of pesticide phoskillin specific body tissues of the common freshwater fish, *Oreochromis mossambicus*. The experimental fishes were 6.1±0.1cm in length and 5.78±0.8 mg in weight on average. Fishes were subjected to four distinct concentrations (0.1 - 0.4 ml/L) at various time intervals *viz*., 6 hrs, 12 hrs, 24 hrs, 36 hrs, 48 hrs, 60 hrs, 72 hrs, 84 hrs, and 96 hrs. After 96 hrs exposure, fish under a 0.2 ml/L concentration exhibited 50% mortality and the eyes and skin were completely damaged. The protein, carbohydrate and lipid-contents of phoskill-ingested fish showed a significant difference ($p>0.05$) against control-without phoskill ingested fish at different time intervals. The RBC and WBC counts were found to be decreased in phoskill treated fish blood (when compared to control group) for96hrs exposure. The blood parameters were reduced in phoskill ingested fishes *viz*; Total protein- 13.64±0.37%; Glucose - 115.4±1.62% and Granulocytes - 1.8±0.06%. Histology revealed the significant deformations found in gill and liver of tilapia fish. Gene expression results showed that myostatin gene was inhibited in phoskill treated fish compared to control fish. Phoskill ingested fish showed a reduced enzyme activity (TAA, Ache, and CAT) being significantly different ($p>0.05$), while total peroxide (TPX), TBARS and SOD were significantly increased ($P>0.05$) in fish tissues compared to control fish. In view of recent findings, further-more

experimental works are required so as to safely use these pesticides and to estimate the impacts of agricultural pollution on freshwater invertebrates.

Keywords: Toxicity, *Oreochromis mossambicus*, Biochemical composition, Haematology, Histology, Myostatin, Antioxidants.

INTRODUCTION

The vast majority of research on pesticide effects on fish has been limited to their testing for acute toxicity with fish mortality as the endpoint. The effects of pesticides on fish could include everything from altered physiology, biochemistry, behaviour, growth and reproduction to death. Recent population growth of human has put pressure on the world's agricultural system, and now, the main goal of most nations is to increase the food production to meet the demands of a growing human population which is predicted to increase to be about 10 billion by 2050[1, 2]. Meanwhile, a variety of harmful compounds, most notably the use of pesticides and fertilizers by agricultural sector, severely contaminate both the freshwater and terrestrial ecosystems [3]and which has become a major issue on a global scale. Pesticides typically runoff into the reservoirs or drainage systems from agricultural areas during rainfall or irrigation processes [4]. The three main ways that aquatic organisms are being exposed to pesticides are;(i) through the skin, (ii) through breathing, (iii) orally; aquatic organisms typically become exposed to pesticides during their feeding on pesticide-contaminated prey or by drinking contaminated water.

The aquatic environments inhabit a variety of animal and plant species, including fishes, amphibians, plants, and invertebrates. The pesticides can have both directly and indirectly an impact on these organisms; the direct effect being the physiological changes within an organism [5]. For instance, the exposure of pesticides to water fleas causes their demise, which can be regarded as a direct effect of pesticides, and it may produce a dramatic rise in the biomass of algae due to the relaxation from grazing pressure, which is regarded as a secondary effect. Globally, herbicides mostly 'glyphosate' are being used to manage both the terrestrial and aquatic weeds, and in recent years, its usage has been found to be increased. And thus, it has been well recognized that this herbicide has a harmful impact on non-target animals in aquatic environs [6]. Of late, pesticides are a necessary component of modern life because they are utilized to get rid of dangerous pests. In theory, they should only be dangerous to the intended organisms, but emerging research provides evidence that industrial chemicals, pesticides, heavy metals, and other toxic compounds interfere with the normal functioning of many species, including humans and aquatic organisms. Pesticides that are found in drinking water at higher concentrations are required to be banned towards public safety. It's important to keep industrial and agricultural wastes from being dumped into the aquatic environs and the regular monitoring of trace elements in water and other consumables are essential [7].

There have been reports of fish deaths in India as a result of water pollution and the pesticides are considered to be a quick aquatic-contaminant. Depending on the pesticide's solubility, once it enters the aquatic environment, it may stay there for several days to weeks, that could cause mass mortality as well as morphological, physical, biochemical, and behavioural changes in aquatic organisms. Further, the pesticide overdose can cause the organisms reduction in growth, and reproductive capacity and would find it more difficult to adjust to environmental changes [8]. In addition to the ichthyofauna, the aquatic invertebrates such as zooplankton are also being impacted by pesticide contamination. Since the fish and other aquatic animals are commercially valuable species, maintaining the biological balance of the aquatic ecosystems and maximizing the fish-yield solely depends on the careful management and protection of their natural habitats [9]. The most significant cause of aquatic pollution is thought to be pesticides, their metabolites, and consequences of their decomposition. Numerous research examined the insecticides' toxicological concerns like; alteration in oxygen consumption, damages in biomolecules and enzymes, histopathology and health-status of animals. Researchers focus on pesticide effects on ecosystems, particularly on their biota has increased in recent years as the sciences of ecology and ecotoxicology have made rapid progress/advancements in relation to fishes. Numerous recent studies have examined the effects

of various types of pesticide on fish biology [10, 11]. Despite the availability of abundance of scientific data on the toxicity of malathion to *Oreochromis* in India, nothing is known about how this pesticide affects the biochemical, antioxidant and enzymatic-characteristics of *Oreochromis mossambicus*. The aim of the current study was to assess the effects of the pesticide, phoskill at sub-lethal doses on certain antioxidant activities, hematological, biochemicals and histological characteristics in specific body tissues of the common freshwater fish, *Oreochromis mossambicus*.

MATERIALS AND METHODS

Species selection

The *Oreochromis mossambicus*, often known as the *Tilapia mossambica,* is a member of cichlidae family of freshwater fishes. The fish adapts well to lab conditions and is an economically important one. *Mosambique tilapia* is good for aquaculture since it is not very difficult to produce and gather. It has a substantial body with a long dorsal and is packed along the sides. The front portion of the animal bears spines to balance the body. Males have a larger mouth than females due to sexual dimorphism, and its scales are cycloid type. The adults can grow to a length of around 35 cm. Dorsal blade with 10 to 13 fragile beams and 15 to 18 spines. Due to ecological and reproductive weights, hostage and naturalized populations may vary in size and color. It is an omnivorous fish that live both saline water and fresh water as well as in new waters.

Experimental Design

The samples of tilapia fish, *Oreochromis mossambicus* (n=300; Length - 10+0.5cm; Weight - 20+1gm) were obtained from the Azhiyar fish farm, Pudukkottai District (India) and they were stocked at density of 15 numbers in each plastic bucket (10L). To supply constant aeration for all buckets, the air compressors were individually connected with each bucket to avoid hyperactivity – physical injuries and stress. The photoperiod was maintained at 12:12 hours L:D cycle. Fishes were fed with commercial pellet-feed at3% of body weight, thrice a day (9 am, 1 pm and 5 pm).Varied concentrations from 0.2 to 2.0 ppm were prepared and used for toxicity studies in fishes, *O. mossambicus* according to OECD Guidelines with slight modifications (OECD, 1992).

Toxicity Study

The 96-hour half-lethal concentration (LC_{50}) was determined by following the method (Islam et al., 2021).Five plastic containers with a 10L capacity each and tap water were kept with the fish (n=15).The dechlorinated tap water was used and maintained at a constant temperature, aeration, and feeding. The five distinct phoskill concentrations (ml/L), *viz*; 0.2 ml/5L, 0.4 ml/5L, 0.6 ml/5L, 0.8 ml/5L and 1 ml/5L were fixed and without the addition of phoskill served as a control. Fish deaths were noted at 24, 48, 72, and 96 hours. Throughout the experiment, fish activity was observed together with changes in water temperature, pH, and dissolved oxygen. The test was performed at three times and acute toxicity test's $LC_{50/96}$ hrs mortality values were calculated.

Analysis of Biochemical Composition

The protein, carbohydrate, and lipid-contents of fish were estimated as per the methods of Lowry *et al.* [12], Roe [13] and Folch *et al.*[14] respectively. For protein, the dried samples were precipitated in ethanolic mixture and the samples were kept in undisturbed condition for blue color formation (which was made by biuret reaction). Then the mixture would react with phosphomolybdic - Phosphotungastic of Folin reagent which represents the protein. Then the samples were used for spectrophotometric analyses at 630 nm and Bovine Serum Albumin (BSA) used as standard.

The carbohydrate concentration was estimated with the help of TCA extraction. Primarily, the simple hydrolyzed carbohydrates are being converted as sugars and diluted in hydrochloric acid as hot acetic medium. Then the glucose was dehydrated into hydroxyl-methyl furfural and this mixture was kept in undisturbed condition for the formation of green color (followed by spectrophotometric analyses at 630 nm) and glucose used as a standard. Lipid

was analyzed by chloroform-methanol method. Fish sample (10mg) was taken in test tube having 5ml chloroform and methanol mixture (2:1) and incubated at room temperature for 24 hrs. Filtrated the mixture using filter paper in 10 ml pre-weighed beaker and kept on hot plate. Calculated the beaker weight as prior and end of experiment to know the lipid content present in the sample.

Hematological Analysis

Fishes were collected from the experimental system for haematological analysis. Blood samples were collected from sub-lethal treated individuals at 24hrs intervals. Each treated fish was gently wiped with a dry cloth to remove water. Caudal peduncle was cut with a razor and the blood was collected in a vial containing EDTA (anticoagulant). The blood was mixed well with EDTA solution by using a glass rod and the sample was used for following estimations. Oxygen carrying capacity of blood was calculated by multiplying the haemoglobin content with 1.25, oxygen combining power of Hb/g [15].

Histological Analysis

The major steps involved in histological analysis are; fixation, tissue processing, decalcification and section cutting [16] and staining [17].

Molecular Analysis in Phoskill Treated Fish

Genomic DNA extraction and isolation from phoskill treated fish was carried out as per the protocol of Vemulawada Chakrapani *et al.* [18]. The extracted DNA was analyzed using PCR technique and the PCR amplified product was studied mainly on myostatin gene expression. The DNA extraction, amplification and gene expression studies were carried out in PAR Lab, Tiruchirappalli, Tamil Nadu (India).

Antioxidant Enzymes Study

In phoskill-treated tilapia fish *Oreochromis niloticus*, antioxidant and their enzyme activities were investigated. Total antioxidant capacity was determined in fish samples by Hamed *et al.* [19]. Superoxide dismutase (SOD) activity was determined by its capacity to prevent the formation of a red product from the nitroblue tetrazolium dye's degradation to phenazine methosulphate [20]. Based on the ability of 3,5-dichloro-2-hydroxybenzene sulfonic acid to quickly stop the hydrogen peroxide breakdown reaction catalyzed by CAT and react with the remaining hydrogen peroxide to produce a yellow product, catalase (CAT) was identified [21]. Acetylcholinesterase (AChE) enzyme activity was calculated using the Ellman *et al.* [22] technique. For five minutes in an ice bath, homogenate (50 mg/mL) was produced in 0.1 M phosphate buffer, pH 8.0. As a standard, glutathionine was used. Esterbauer and Cheesman's approach [23] was used to measure the estimation of thiobarbituric acid reactive compounds (TBARS) in order to assess lipid peroxidation.

Statistical Analysis

Results were expressed as mean + standard error of mean (SEM) of three observations. The obtained data were subjected to ANOVA-test using GraphPad Prism through Duncan's Multiple Range Test (DMRT). The differences between the various treatments found at $p<0.05$ have been considered to be significant.

RESULTS

In the quarantine tank, a total of 300 tilapia fishes were stocked. The fishes were 6.1±0.1cm in length and 5.78±0.8 mg in weight (on average). Morphometric characters of the fishes were measured and the findings are presented in Table-1.

Toxicity Experiment

The fishes were subjected to four distinct concentrations (0.1 - 0.4 ml/L) at various time intervals *viz*., 6 hours, 12 hours, 24 hours, 36 hours, 48 hours, 60 hours, 72 hours, 84 hours, and 96 hours (Fig. 1).The fish subjected to 0.1 ml/L pesticide phoskill for 84 hours had slowed their swimming and died in 20% mortality. After 96 hours of exposure, fish under a 0.2 ml/L concentration showed 50% mortality, and their eyes and skin were completely damaged. Gradually, the concentration was raised 0.3 ml/L, the fish's skin and tail began to erode, resulting in 80% mortality. Fish exposed to 0.4 ml/L concentration experienced 100% mortality; the entire body was affected, and the color of gill region changed red to pale yellow. The highest number of fatalities was recorded at concentration 0.4 ml/L. Based on 50% fish mortality rate, the LC_{50} concentration was determined to be 0.2 ml/L.

Biochemical Composition

The protein, carbohydrate, and lipid-contents of muscle samples in phoskill - ingested fishes shown in Fig. 2 and which showed decreased values of 11.53±0.25, 2.17±0.15, 6.10±0.11 mg/gm in relation to control-without phoskill ingested fishes were; 20.45±0.43, 3.46±0.04, 10.3±0.3 mg/gm at different time intervals of exposure; 24, 48, 72 and 96 hrs, respectively. ANOVA results revealed that protein and carbohydrate contents of control fish has significantly different with experimental fish ($P>0.05$), except lipid contents of control fish has not significantly different with experimental fish ($P<0.05$).

Liver protein, carbohydrate and lipids contents were analyzed in control and phoskill ingested fish, *Oreochromis mossambicus* (Fig. 3). The total protein content in liver of normal fish was found to be; 9.82±0.03, 8.2±0.02, 7.27±0.25 and 7.07±0.15 mg/gm and total protein levels were decreased in 50% sublethal treatment fishes were found to be; 7.2±0.26, 6.05±0.51, 5.70±0.15 and 4.80±0.05 mg/gm during 24, 48, 72 and 96 hrs of exposures. The mean control values of carbohydrate content in liver tissues were varied between 9.94±0.04, 8.63±0.15, 8.13±0.15 and 7.10±0.08 mg/gm. Similar trend of results were obtained in LC_{50} of 0.2ml/L phoskill treatment fishes *viz*; 6.80±0.06, 4.78±0.14, 3.28±0.17 and 2.92±0.13 mg/g at 24, 48, 72 and 96 hrs of exposures respectively. Liver total lipid concentration was decreased in phoskill exposed fishes against control fishes. Lipid levels in normal fish were found to be 12.65±0.15, 14.9±0.30, 17.53±0.35 and 20.1±0.26 mg/gm and decreased lipids content in LC50 treated groups were; 14.27±0.31, 12.5±0.2, 10.09±0.27, 8.63±0.15 mg/gm of phoskill treatment at 24, 48, 72 and 96 hrs respectively. ANOVA results shows the protein and carbohydrate contents of control fish has significantly different with phoskill treated fish ($P>0.05$) and lipid contents has not significantly different ($P<0.05$) with phoskill treated fish.

The fish kidney biochemical contents were analyzed (Fig. 4) and depicts the amounts of total proteins recorded were; 6.45±0.03, 5.21±0.02, 4.12±0.15 and 3.42±0.02 mg/gm in kidney tissues of normal fish. Total protein values declined in kidney tissues of LC_{50} treated groups were; 4.3±0.17, 3.52±0.21, 2.65±0.03 and 1.20±0.03 mg/gm at different time intervals of exposures; 24, 48, 72 and 96hrs respectively. Kidney carbohydrates levels were found to be less in experimental fishes *viz*; 5.19±0.04, 4.86±0.02, 3.85±0.02 and 3.48±0.06 mg/gm due to sublethal (LC_{50} value-0.2ml/L) toxicity as against the mean control carbohydrate values *viz*.5.67±0.16, 4.39±0.03, 4.25±0.04 and 3.85±0.04 mg/gm at 24, 48, 72 and 96 hrs of exposures. Kidney total lipids levels were also decreased on the exposure of LC_{50} phoskill toxicity *viz*; 7.46±0.14, 6.05±0.14, 5.21±0.19 and 4.06±0.05 mg/gm, compared with mean total lipids obtained were; 4.83±0.03, 6.4±0.2, 7.83±0.15, 8.2±0.2 mg/gm in kidney of normal fish at 24, 48, 72 & 96 hrs of exposure.

Figure 5 illustrated the phoskill-ingested tilapia fish's biochemical profiles in relation to control fish**.** Protein levels were decreased in LC_{50} treated groups *viz*; 4.66±0.04, 3.56±0.02, 2.43±0.03 and 1.16±0.11mg/gm at 24, 48, 72 and 96hrs of treatment when compared with devoid of phoskill ingested fish. Likewise, gills carbohydrates values decreased in sublethal (LC_{50} value-0.2ml/L) exposed fish *viz*; 4.34±0.05, 3.15±0.04, 2.61±0.06 and 2.12±0.03 mg/gm as compared to control fish gill carbohydrate levels 5.34±0.03, 6.52±0.06, 7.65±0.15 and 8.23±0.03 mg/gm at 24, 48, 72 and 96 hrs exposures respectively. Similarly, lipids contents were decreased in the gills, when fishes treated with LC_{50} (0.2ml/L) phoskill. Gills contained 7.73±0.08, 6.57±0.04, 5.66±0.06 and 5.1±0.2 mg/gm of total lipids on exposure to 0.2ml/L phoskill effect when compared to total lipids in the gills of normal fish 6.33±0.13, 7.03±0.25, 8.43±0.11 and 8.60±0.03 mg/gm at 24, 48, 72 and 96 hrs of treatment. ANOVA results expressed the protein and lipid contents of control has

not significantly different with phoskill treated fishes at $p<0.05$ and carbohydrate mean of control fish has significantly different with phoskill treated fish at $p>0.05$.

Hematological Features

Experimental data obtained on blood characteristics of *O.mossambicus* exposed to 0.2ml/L sublethal concentration of LC_{50} for 24, 48, 72 and 96 hrs are presented in Table 2.

The results showed a decrease in total RBC^{s} with respect to increases in exposure periods(hours).The blood RBC counts (2.18±0.38 x10^{6}/µL) reduced in phoskill (0.2ml/L) exposed fishes at different time intervals (24,48,72and96hrs), whereas control fish have high RBC counts (2.65±0.28 x10^{6}/µL) in blood. Similarly, the least WBC counts (3.58±0.21 x 10^{6}/µL) found in phoskill (0.2ml/L) treated fish blood when compared to control group WBC counts 3.78±1.05 x 10^{6}/µL during 96hrs exposure. The subsequent parameters reduced in phoskill treated fishes were total protein-13.64±0.37%; glucose - 115.4±1.62% & granulocytes - 1.8±0.06% against without treated phoskill fishes.

Histology

In the present study, Tilapia fishes showed five pair of gill arches. In the control group (Fig. 6a), four pairs of slender gill arches form two lines and these two lines are joined to each other at the base by a gill septum. The last pair of gill arches generally transforms into the pharyngeal bone and does not play a role in respiration. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries are separated by pillar cells that run parallel along the surface. Primary gill lamellae consist of centrally placed rod like supporting axis with blood vessels on either side. The secondary lamellae, also termed as respiratory lamellae are highly vascularized and covered with a thin layer of epithelial cells. Blood vessels are extended into each of the secondary gill filaments.

In the treatment group (Fig. 6b), the structure of gill arches was damaged and affected the primary and secondary lamellae. Pillar cells shape and erythrocytes function was altered. On exposure to sublethal concentration (of phoskill), the most significant changes were observed *viz*; lamellar shortening, lamellar necrosis, telangiectasis, lamellar clubbing and the changes observed were architectural loss necrosis, desquamation of epithelial layer hyperplasia and lamellar telangiectasis. In control group (Fig.6c), the liver exhibited a normal architecture with hepatocytes by presenting a homogenous cytoplasm and a large central or sub central spherical nucleus. The important histological changes observed in phoskill treated groups (Fig. 6d) were pyknotic nuclei, clear cell foci, necrosis and also caused heavy damage in glycogen vacuoles and Kupffer cells of the liver samples.

Molecular Analysis of Phoskill Treated Fish

Real-time reverse transcription polymerase chain reaction (RT-PCR) analysis of phoskill treated tilapia, *Oreochromis mossambicus* were studied. Myostatin gene (MSTN gene) and β-actin (as a reference gene) was performed (Elbialy *et al.* 2016) using the following primers for Myostatin gene exon1 forward primer: 5'GCATCTGTCTCAGATCGTGCT3' and reverse primer 5'TGCCATCATTACAATTGTCTCCG3' and β-actin (Pang *et al.,* 2013) forward primer 5'CAGCAAGCAGGAGTACGATGAG3' and reverse primer TGTGTGGTGTGTGGTTGTTTTG. The cDNAs used as the template for Real-time PCR system (Agilent Technologies, Santa Clara, CA, USA). The amplification blots of MSTN gene in muscle of tilapia are shown in Fig.7and observed the expression levels of tilapia fish MSTN gene in muscle tissue are shown in Fig. 8.The myostatin gene expression analytical results are shown in Table-3 and the relative differences in gene expression were calculated using threshold cycle (CT) values that were first normalized to those of *O. mossambicus* β- actin house-keeping gene and by using ΔCT value of muscle as a calibrator using the $2^{-\Delta\Delta CT}$ method as previously described by Livak and Schmittgen (2001).

Antioxidant Enzyme Activities

Total antioxidant activity (TAA) and antioxidant enzymes (Ache, TBARS, CAT & SOD) analysis clearly demonstrated that TAA, Ache and CAT enzyme activities were significantly ($P<0.05$) reduced in phoskill treated

fish while total peroxide (TPX), TBARS and SOD enzymes activities were significantly enhanced (P<0.05) in gill and muscle tissues of phoskill treated fish after exposure to sublethal doses compared with control fish (Table-4).

DISCUSSION

Presently, the freshwater fish, *Oreochromis mossambicus* was subjected to sublethal doses of pesticide phoskill at 96 hrs exposure altered the biochemical nature of biomolecules *viz*; total proteins, carbohydrates and lipids in the selected body organs (muscle, gill, liver and kidney) were studied. The present investigation showed a significant decrease in carbohydrate-content in all the tissues studied. The present study results illustrates the carbohydrate metabolism of *Oreochromis mossambicus* suggest that glycogen being a ready source of energy, reduction in glycogen may probably due to its more rapid breakdown (glycogenolysis) which could release the glucose in circulatory system to meet the increased energy demand in a stressed condition. Another possibility for reduction in the glycogen level may be due to glucose 6-phophate, which is located in the membrane of endoplasmic reticulum as a consequence of phoskill treatment, as opined by Jaroli and Sharma [24].

Many earlier researchers have reported a similar trend of decrease in carbohydrate [25, 26]. On the other hand, some other workers have reported that the sublethal concentration of certain organophosphate pesticides caused glycogenolysis, which caused hyperglycemia in African food fish, *Tilapia mossambica* and the Indian catfish, *Heteropneustes fossilis* [27, 28]. However, Remia *et al.* [29] have reported that the elevation of carbohydrates might be due to stress induced by insecticides as physiology of organisms with the help of corticosteroids. Analysis of such biochemical parameters could help to identify target organs of toxicity as well as the general health status of fishes. It may also provide an early warning signal about the stress on animal such as *Oreochromis mossambicus*. The source of these parameters is the indicators responding to the environmental effects and which can also serve as markers for toxicant exposure and effect in fish.

Proteins are important organic substances required by organisms for tissue building and they can play an important role in energy metabolism [29, 30]. The present toxicity-study showed significant decrease in protein content in the fish tissues analyzed. The percentage of protein content was decreased in liver tissue and such reduction of protein may be due to the proteolysis and increased metabolism under toxicant stress [29]. Kumar and Saradhamani [31] have reported significant decreased protein content in all the tissues of fish, *Cirrhinus mrigala* upon its pesticide exposure. The fish's physiological ability to cope up for pesticide stress, requires a lot of energy (to overcome), is also indicated by the decreases in protein content as seen in current study's phoskill-intoxicated fish. Protein metabolism may have been stimulated by the energy demand. The current study results can be compared with earlier reports such as those of Sastry and Siddiqui [32] in *Channa punctatus*, Sabita Borah [33] in freshwater teleost, *Heteropneutes fossilis*, Rao [34] in *Oreochromis mossambicus*, *Oreochromis niloticus* was studied by Bose *et al*. [35], *Channa punctatus* by Ganeshwade [36], and *Oreochromis mossambicus* by Lakshmanan *et al*. [37] proved the decreased protein content was found in phoskill treated fish, when compared to control group.

The four bodily organs of *Oreochromis mossambicus* chosen for this study viz; the muscle, kidney, liver and gills have shown lower total lipid levels as a result of sublethal toxic doses of phoskill. The reductions in total lipids (mg/gm) might have been brought out by stress, which is mobilized to meet the energy demand, lipolysis, or mitochondrial injury caused by hazardous chemicals that may have been present in phoskill pesticide. The results of previous investigations on *Channa punctatus*, *Clarias batrachus*, and *Gambusia affinis* [38] have all supported the present findings. The toxicity of phoskill may have affected fatty acid metabolism, which is another explanation for the drop in total lipid concentration. For example, Benli *et al.* [39] studied in Nile tilapia *Oreochromis niloticus*, while Mathivanan [40] reported in *Oreochromis mossambicus*, Padmini *et al.* [41] investigated in grey mullet, *Mugil cephalus*. Present study histological analysis of control fish-gills revealed a typical structural organization of the lamellae. But the fish exposed with pesticide phoskill exhibited a number of histological changes like hypertrophy, lifting of the

epithelial linings from the surfaces of secondary lamellae, and in a few locations, lamellar epithelium degeneration, deformations in the gill and liver of tilapia fish.

One of the most widely cultivated fish species in world is the Nile tilapia, *Oreochromis niloticus* [42]. The development of striated skeletal muscle, which makes up 40-75% of body mass in tilapia and other cultured fish species, is crucial [43]. In most teleost fish, white tissue makes up more than 90% of all the muscle tissues. As a result, changes in body mass are mostly caused by variations in the growth of muscle [44]. The majority of fishes expand their tissues through muscular hyperplasia and hypertrophy, which are governed by several regulatory agents like myostatin. Most fish have indeterminate muscular growth that takes place through hyperplasia and hypertrophy [45]. New muscle fibres are recruited to cause hyperplasia, and old muscle fibres are expanded to cause hypertrophy [46]. The size distribution of muscle fibres is altered by variations in the relative contribution of various mechanisms. These adjustments are crucial because genetic and environmental factors have an impact on variances in fibre diameter and quantity, which have an impact on productivity [47].

As a result of environmental factors such as temperature [48], oxygen levels, and diet composition, differences in the distribution pattern of muscle fibre size have been reported between different species [49, 50]. The size of the fibres is also influenced by the stage of development, with bigger diameter fibres being more common in adult Tilapia and smaller diameter fibres being more prevalent in early fish stages [51, 52]. Regulation by growth factors, in particular myostatin, a member of the super family of transforming growth factors (TGF-b), which also contains elements that negatively limit the growth of muscles, is another crucial factor [53]. According to Seiliez *et al*. [53] and Langley *et al.* [54], myostatin regulates the proliferation and differentiation of myogenic precursor cells, which are necessary for the creation of striated skeletal muscle in fish. Contrary to mammals, teleost fishes have at least two paralogs, MSTN-1 and MSTN-2, that are responsible for the transcription of myostatin [55]. In various fish species, myostatin activity has been investigated, and previous research has demonstrated that this ability to suppress growth and increase muscle mass primarily occurs through hyperplasia, hypertrophy, or both [56]. Developmental stage, temperature, and dietary constraints are a few examples of the intrinsic, environmental, and other factors that influence the myostatin gene [57]. In reality, environmental influences may change how some genes express themselves [58]. The expression of various genes for tilapias' adaptability and tolerance to this circumstance may be modulated by high alkalinity stress, according to those researchers. In an effort to control muscle growth, culture-environmental factors may have an impact on myostatin gene expression. Myostatin gene activity, which prevents the development of skeletal muscle was shown to be lower in fish treated with phoskill than in control fish in the current study. The myostatin gene is crucial for the biomass of fish muscles.

Reactive oxygen species (ROS) and oxidative stress are known to be induced by pesticides in fish [59]. The activity of the antioxidant enzymes (Ache, SOD, and CAT) is frequently employed to evaluate the risk of pesticides because they prevent oxidative stress [60]. Oxidative stress affects aquatic organisms when there is an excessive synthesis of reactive oxygen species (ROS), which can degrade biomolecules [61]. In the current study, phoskill pesticide exposure to fish resulted in enzyme activities (TAA, Ache, and CAT) being significantly decreased ($P>0.05$) in phoskill treated fish while total peroxide (TPX), TBARS and SOD enzyme activities were significantly increased ($P>0.05$) in liver, gill and muscle tissues of phoskill treated fish after exposure to sublethal doses compared to control fish. Activity of antioxidant enzymes in fish gill tissue reveals an adaptive response to pesticides. Aquatic species are guarded against oxidative stress by catalase (CAT), a crucial antioxidant enzyme [62]. Pollutant-induced cellular transcription mechanisms or mRNA degradation can also prevent organisms from producing enzymes. In the current study, freshwater fish *O. mossambicus* that have been exposed to pesticide phoskill exhibit substantial changes in non-enzymatic (Ache, TBARS, SOD & CAT) activities as well as biochemical (protein, carbohydrate, and lipid) parameters. Changes in biochemical markers in the current study may offer a useful tool to evaluate the water quality polluted with various pesticides. It is abundantly obvious from the altered antioxidant indicators that pesticide phoskill caused oxidative stress in the fish.

CONCLUSION

Aquatic toxicity-discipline is relatively a new and still evolving discipline, originating from the concern for the safety, conservation and protection of aquatic environments and it is an off-shoot of toxicology field, since degradation of water mass is caused mainly by anthropogenic toxicants dumped into the water bodies by human activities, directly or indirectly. The purpose of this study was to determine how the pesticide phoskill affect the histological, biochemical, and haematological (blood properties) changes in body tissues of the freshwater teleost, *Oreochromis mossambicus*. The fish's respiratory physiology and chemical components (blood parameters and biomolecules) are demonstrated to be disrupted by pesticide phoskill, which causes cell damage (histology) and finally fish mortality. When organisms are exposed to pesticides over an extended period of time, they offer a persistent health danger to people. Hence, consuming these deadly fish would pose a serious threat to human population. Therefore, it is essential to regularly test the levels of pesticide residues in food and people in order to ascertain the population's exposure to these chemicals.

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Table 1. Morphometric Characters of Tilapia Fish

Morphometric Characters	Mean+SD
Total length (cm)	6.1±0.1
Total weight (gm)	5.78±0.8
Standard length (cm)	5.2±0.10
Body width (cm)	1.67±0.21
Eye diameter (cm)	0.5±0.10
Head length (cm)	1.57±0.06
Head width (cm)	1.2±0.10
Caudal length (cm)	0.86±0.03

Table 2. Haematological Parameters of Phoskill Ingested Fish and Control Fish

Parameters	Experimental Fish	Control Fish
RBC Counts ($x10^6$/µL)	2.18±0.38	2.65±0.28
WBC Counts ($x10^5$/µL)	3.58±0.21	3.78±1.05
Total protein (%)	13.64±0.37	16.3±0.95
Total immunoglobulin (%)	18.8±2.06	5.3±1.12
Total lipids (%)	23.4±1.52	12.5±4.36
Glucose (%)	115.4±1.62	164.5±1.64
Lymphocytes (%)	81.25±0.71	74.05±0.32
Monocytes (%)	3.4±0.06	2.77±0.56
Granulocytes (%)	1.8±0.06	7.2±0.54
Neutrophils (%)	18.3±0.04	16.0±0.34

Ig - Immunoglobulin, Mp- Macrophages, Lc - Lymphocytes, Mc - Monocytes, Gc - Granulocytes, Np - Neutrophils

Table 3. Myostatin Gene Expression Studies in *O. mossambicus*

Sample Name	Target Name	Task	RQ	RQ Min	RQ Max	Cт Mean	ΔCт Mean	ΔCт SE	ΔΔCт
Control	B Actin	UNKNOWN				**26.91095**			
Control	MYO	UNKNOWN	1	0.780668	1.280955	27.865784	0.95483208	0.083023	0
Treated	B Actin	UNKNOWN				27.096392			
Treated	MYO	UNKNOWN	1.081034	0.843573	1.385338	**27.938812**	0.84242058	0.083164	-0.11241
NTC	B Actin	NTC							
NTC	MYO	NTC							

Table 4. Antioxidant Enzymes Activities of Gill and Muscle of Tilapia Fish

Antioxidant Enzyme Activities	Gill		Muscle	
	C	E	C	E
Total Antioxidant Activity (TAA)	0.92±0	0.88±0.01	1.01±0.01	0.97±0.03
Total Peroxide (TPX)	1.49±0.02	1.83±0.05	1.62±0.18	1.98±0.03
Ache activity (moles/ml)	4.56±0.04	1.60±0.06	5.82±0.28	3.21±0.04
TBARS Activity (nmol/mg ptn)	4.63±0.04	10.72±0.39	5.19±0.06	8.93±0.04
CAT Activity (nmol/mg ptn)	63.53±0.55	47.63±0.35	40.42±0.70	27.73±0.63
SOD Activity (nmol/mg ptn)	1.44±0.12	5.51±0.45	4.68±0.48	6.95±0.23

C- Control fish (Without phoskill treated fish); E - Experimental fish (Phoskill treated fish)

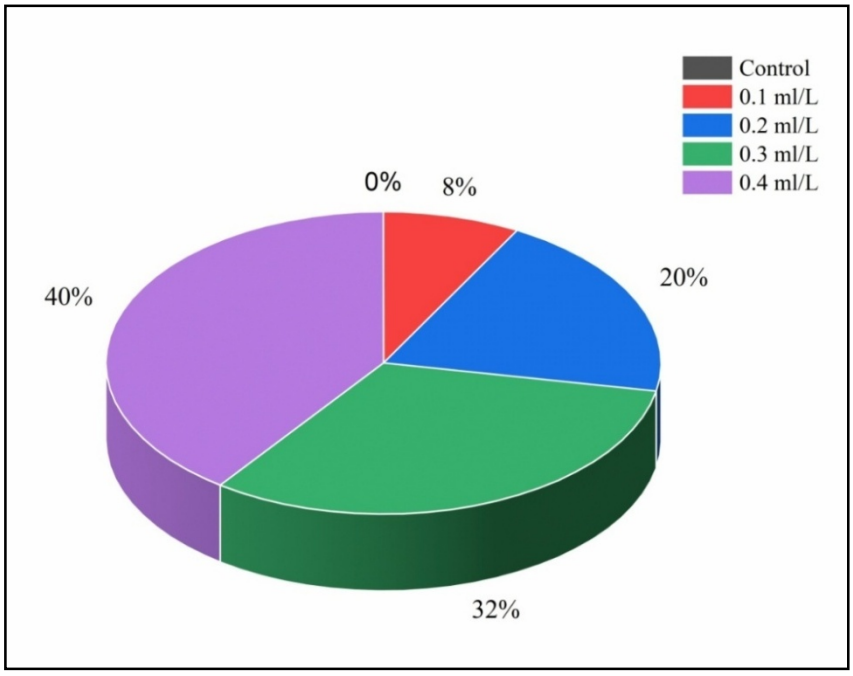


Fig. 1. Effect of Phoskill Ingestion in Tilapia Fish, *Oreochromis mossambicus*. Control was Compared with Different Concentrations of Phoskill Effect (All Values are Mean±SD of Triplicate Samples).

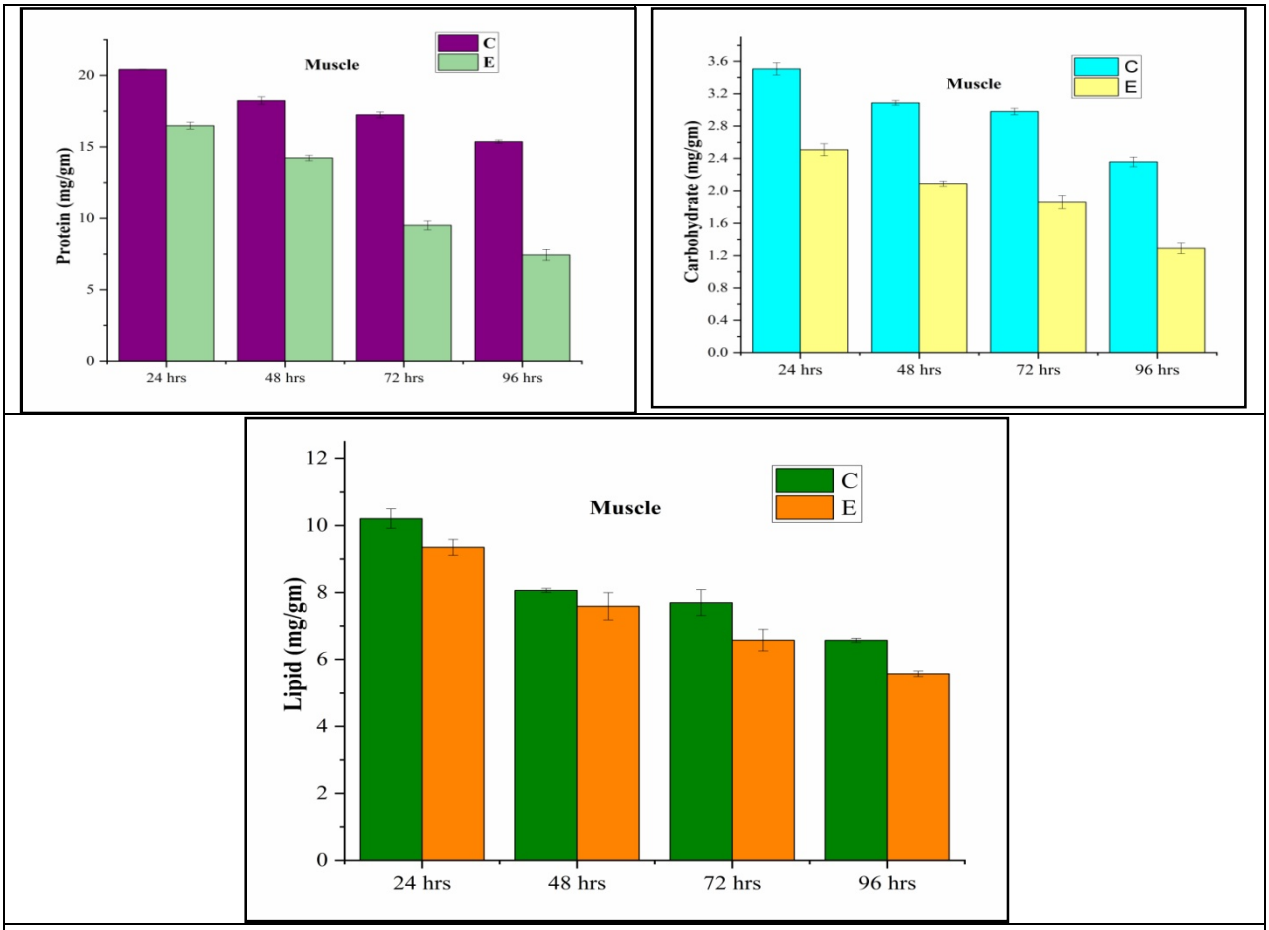
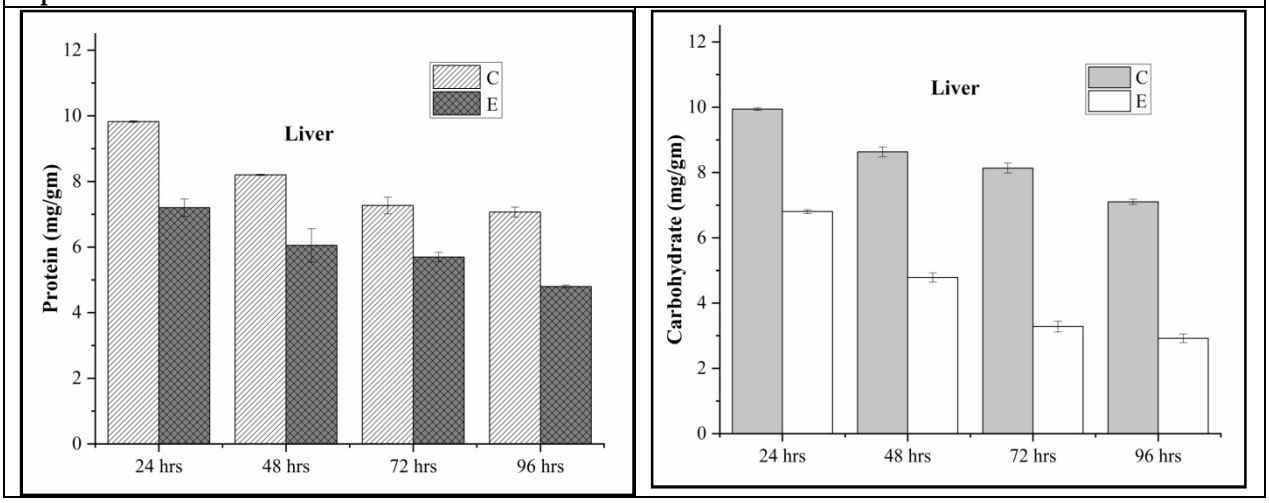


Fig. 2. Biochemical Composition of Muscle Samples of Phoskill Ingested Tilapia Fish, *Oreochromis mossambicus* at Different Time Intervals (C - Control Fish; E - Experimental Fish). All Values were Mean±SD of three replicates.



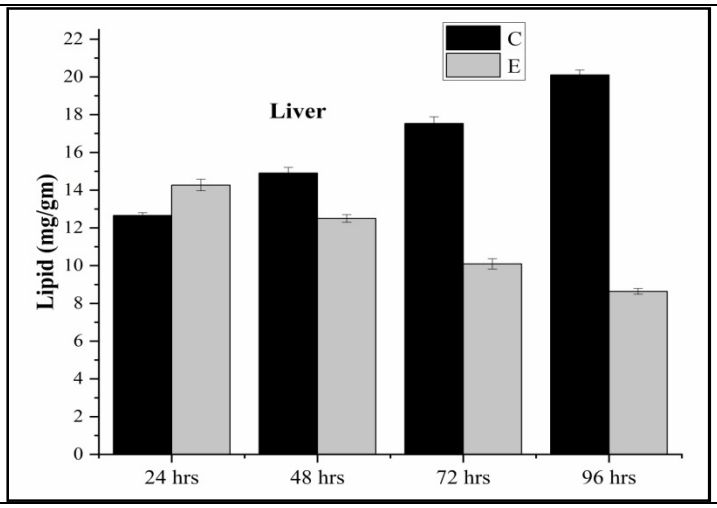
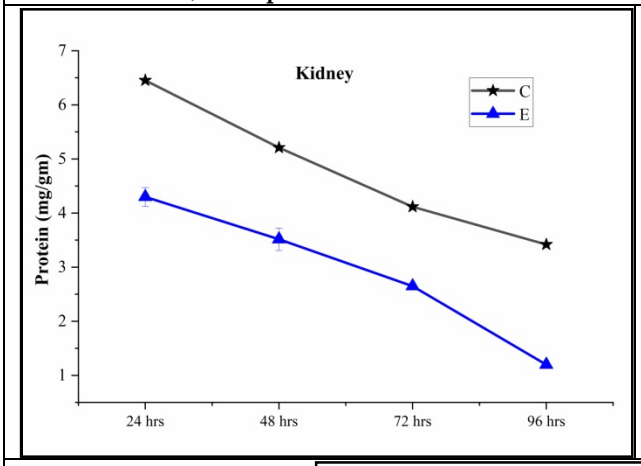
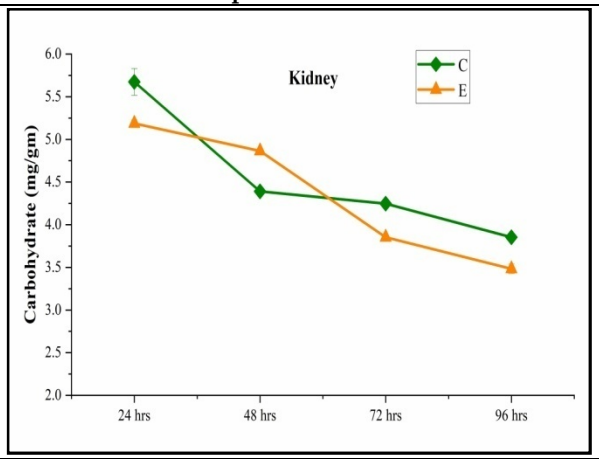


Fig. 3. Biochemical Composition of Liver Samples of Phoskill Ingested Tilapia Fish at Different Time Intervals. C - Control Fish; E - Experimental Fish. All Values were Mean±SD of Three Replicates.





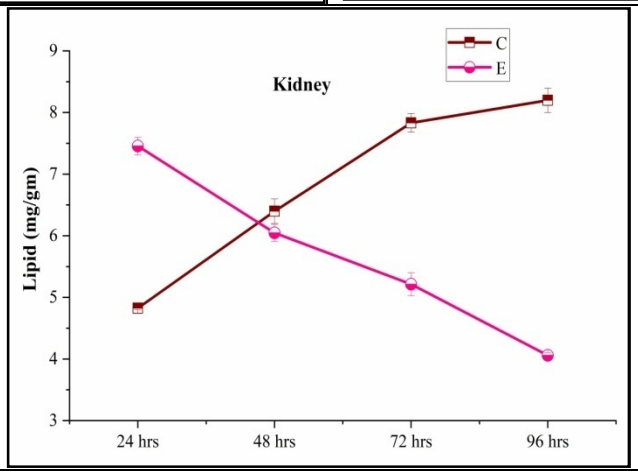


Fig. 4. Biochemical Profiles of Kidney Samples of Phoskill Ingested Tilapia Fish *Oreochromis mossambicus* at Different Time Intervals. C - Control Fish; E - Experimental Fish. All Values were Mean±SD of Three Replicates. Difference between Mean of the Phoskill Treated Fishes with the Control were Assessed by one-way ANOVA Shows a No Significant Difference at p<0.05

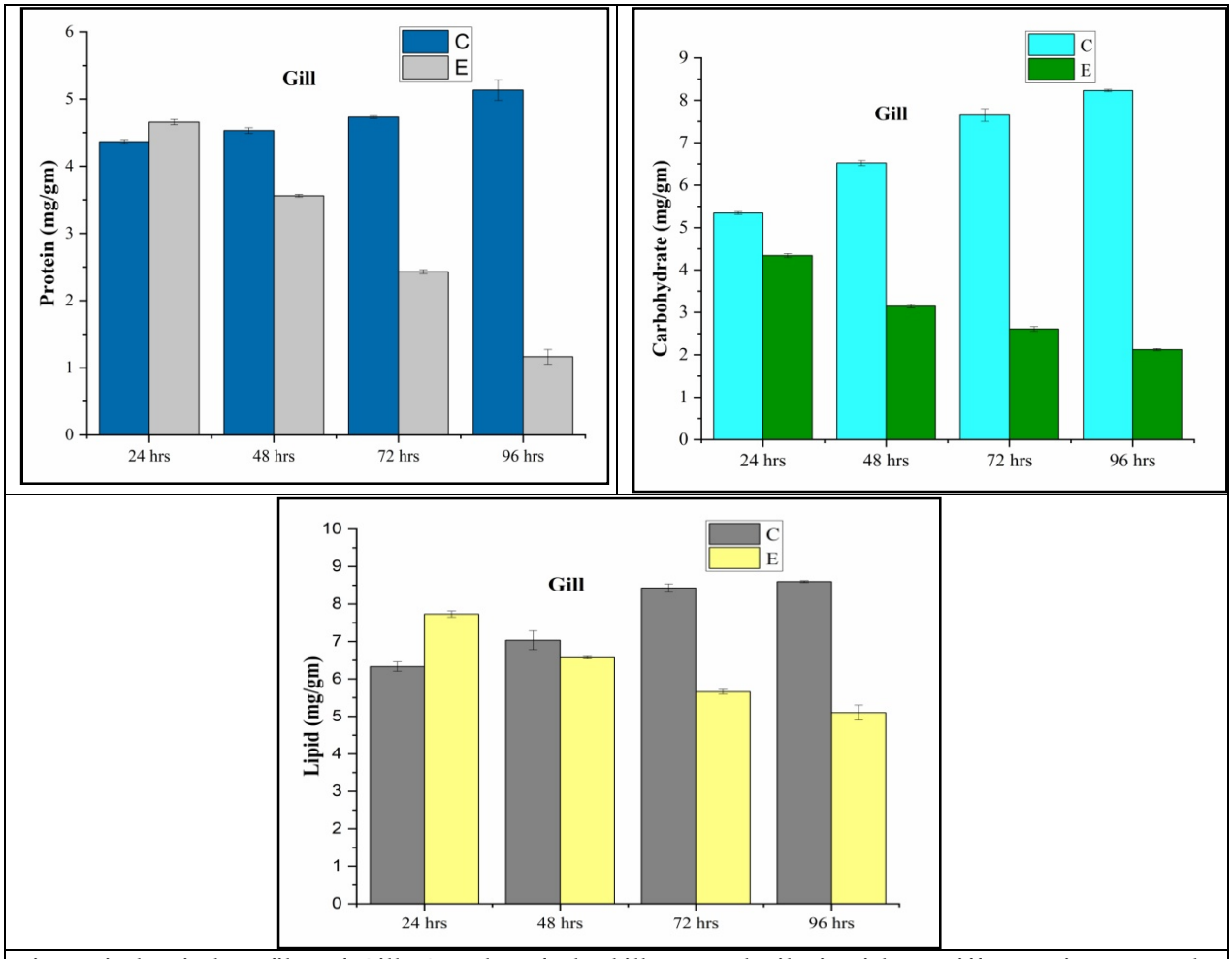
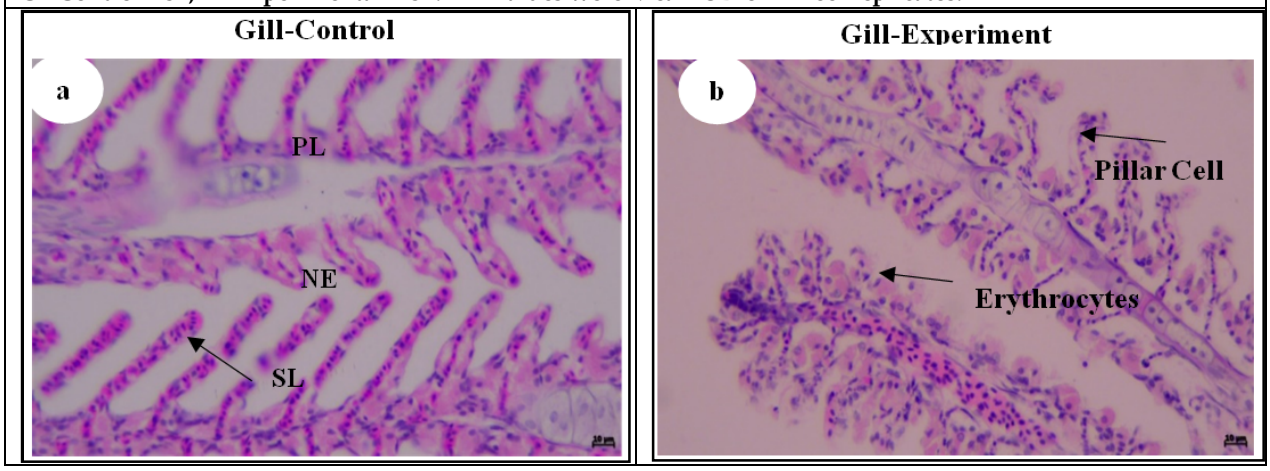
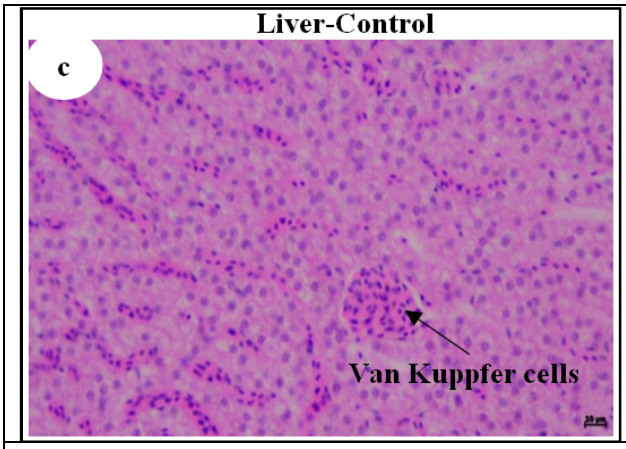


Fig. 5.Biochemical Profiles of Gills Samples of Phoskill Ingested Tilapia Fish at Different Time Intervals. C - Control fish; E - Experimental Fish. All Values were Mean±SD of Three Replicates.





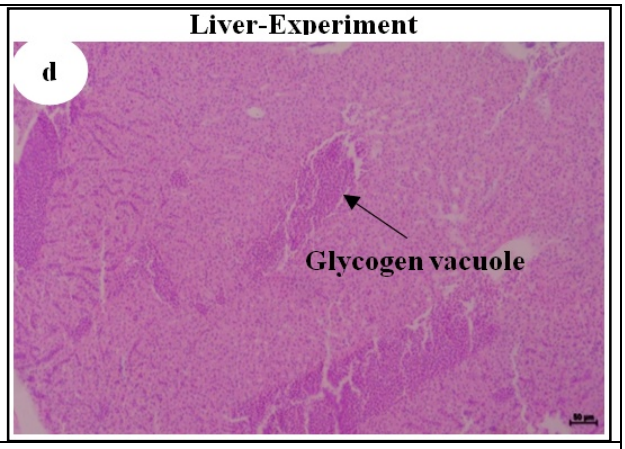


Fig. 6 (a-d). Photomorphographs of Liver and Gills of Phoskill Ingested Tilapia Fish, *Oreochromis mossambicus* Compared with Control. PL - Primary Lamellae; SL - Secondary Lamellae & NE - Normal Epithelium.

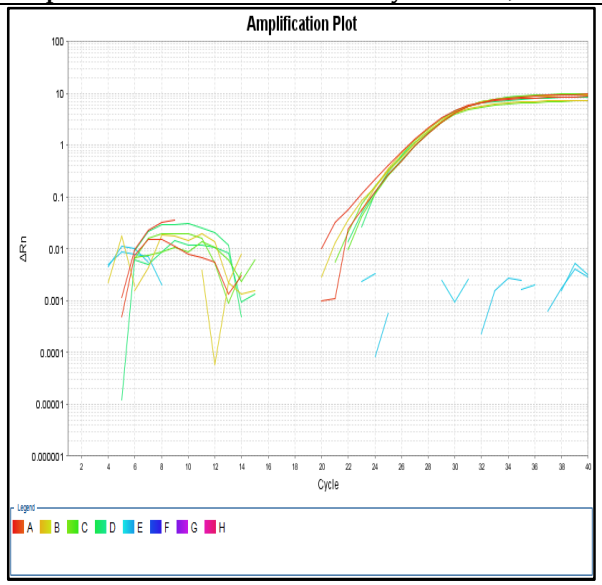


Fig. 7. Muscle DNA Sample Amplified Product in Phoskill Treated Fish Compared with Control

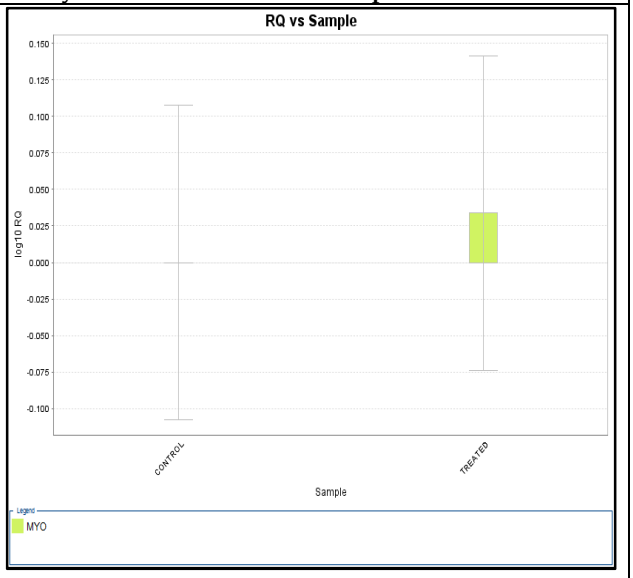


Fig. 8. Myostation Gene Expressed in Phoskill Treated Fish

RESEARCH ARTICLE

Inhalable Nanocarriers for Pulmonary Drug Delivery: Advances, Challenges and Future Prospects

Aniruddha Shejwal[1]*, Ganesh B. Shevalkar[2] and Laxmikant Borse[3]

[1]M.Pharm Student, Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra, India.
[2]Assistant Professor, Department of Pharmaceutics, Sandip Institute of Pharmaceutical Sciences, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra, India.
[3]Principal and Professor, Sandip Institute of Pharmaceutical Sciences, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra, India.

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***Address for Correspondence**
Aniruddha Shejwal
M.Pharm Student,
Department of Pharmaceutics,
Sandip Institute of Pharmaceutical Sciences, Nashik,
(Affiliated to Savitribai Phule Pune University, Pune),
Maharashtra, India.
E.Mail: Aniruddha.shejwal@gmail.com

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ABSTRACT

Pulmonary drug delivery has emerged as an efficient approach for treating respiratory diseases due to its targeted delivery, rapid onset of action, and minimized systemic side effects. Conventional inhalation therapies, including metered-dose inhalers, dry powder inhalers, and nebulizers, often suffer from low drug deposition efficiency, rapid clearance, and lack of sustained release. To overcome these challenges, nanocarrier-based pulmonary drug delivery systems have been developed, utilizing liposomes, polymeric nanoparticles, solid lipid nanoparticles, dendrimers, and inorganic nanoparticles. These nanocarriers improve drug bioavailability, enhance retention in the lungs, and offer controlled drug release. Surface modifications such as PEGylation and ligand functionalization further improve lung-selective deposition while reducing macrophage uptake. However, challenges remain, including mucociliary clearance, alveolar macrophage uptake, formulation stability, aerodynamic properties, and regulatory hurdles. Recent advancements, including stimuli-responsive nanocarriers, biodegradable mucoadhesive systems, and AI-assisted design, are paving the way for personalized pulmonary nanomedicine. Additionally, inhalation device integration with nanocarriers, including dry powder inhalers and nebulizers, is critical for optimizing drug deposition and patient compliance. Future perspectives focus on gene and mRNA delivery, eco-friendly nanocarriers, and the clinical translation of nanotechnology-based inhalable therapeutics. While significant progress has been made, further research

and regulatory standardization are essential for the commercialization of inhalable nanocarrier therapies. This review highlights the advances, challenges, and future directions of nanocarrier-based pulmonary drug delivery, emphasizing its potential in treating chronic respiratory diseases such as asthma, COPD, cystic fibrosis, and lung infections.

Keywords: Nanocarriers; pulmonary drug delivery; inhalable nanoparticles; controlled release; bioavailability; lung targeting.

INTRODUCTION

Pulmonary delivery is a modern and very efficacious method of delivering drugs to ICU patients because of its advantages such as targeted delivery, fast action and minimum side effects to the rest of the body [1]. The drug is quite popular for treating asthma, chronic obstructive pulmonary disease, cystic fibrosis, lung infections as well as pulmonary hypertension [2]. Lungs have a big surface area, which is rich in blood vessels and alveoli, and the barrier between the alveoli and the blood capillaries is rather thin as well; therefore, drug absorption is very efficient, and the effect of drugs is experienced instantly [3]. Pulmonary drug targeting does not undergo first pass metabolism in the liver as is the case with other routes such as oral or intravenous administration; this means that the drug is rapidly made available in the body for its main action. The administered doses of inhaled drugs are relatively smaller compared to those of the systemic route due to the reduced risk of systemic toxicity and better compliance by patients. These make pulmonary drug delivery as one of the most suitable methods of action for localized and systemic illnesses [4]. Nonetheless, there are several drawbacks of conventional inhalable drug formulations that linger and hamper the effectiveness of inhaled therapy [5]. The common pulmonary drug delivery systems include the metered-dose inhalers, dry powder inhalers, nebulizers, and so on that present low drug deposition efficacy [6]. When using MDI to deliver medication a considerable proportion of the drug is deposited in the oropharyngeal region, exhaled or removed through mucociliary clearance leaving little to reach the lower respiratory tract. This inefficient deposition sometimes requires higher doses, which are often repeated, and, as a result, dose-related side effects and treatment load in a patient [7]. Moreover, the small sized drugs are quickly removed from the lungs by macrophages and enzymatic breakdown in the lining fluid of the alveoli reducing the lung tissue concentration and therapeutic effect [8]. Still, there are some drawbacks of conventional pulmonary formulations as there is no controlled and sustained release of the drug and the therapeutic effects are not achieved in the long term and redosing is necessary.

Such challenges necessitate optimization of drug depositions, retention in the lungs and attainment of long term therapeutic effects [9]. Studies on nanocarriers have shown a great promise to overcome the problems associated with conventional inhalable drug delivery systems [3]. Such systems include lipid carriers such as liposomes, polymeric systems like nanoparticles, solid lipid nanoparticles, micelles and dendritic polymers which present some superiority over conventional pulmonary drugs [10]. They possess a small size that enables them to penetrate deep in the lungs and to deposit the drug uniformly on the alveolar surfaces which enhance the bioavailability of the drug and the effectiveness of the administered treatment [3]. This has shown that it is possible to engineer nanocarriers in such a way that they shield the drugs from any enzymatic degradation, increase the delivery time of the drugs within the lungs and also ensure that the drugs are released in a controlled and slow manner [11]. Other factors including PEGylation and ligand targeting can also be used to improve the lung selective deposition of the drug along with reducing recognition by alveolar macrophages [12]. Due to the versatility that is achieved by the availability of properties in the nanocarrier, there is the possibility of delivering small molecules, proteins, nucleic acids and even gene therapies making inhalable nanomedicine useful in the treatment of chronic respiratory diseases and lung infections [13]. This review aims to concentrate on the development of nanocarrier-based pulmonary drug delivery systems which enable the inhalation of the drugs. It gives a detailed over of the various nanocarrier systems, the properties and use in improving drug delivery effectiveness. It also enlists the major struggles associated with

effective design of inhalable nanocarriers comprising of stability of particles, aerodynamic properties, safety factors and regulatory issues. Further, it also discusses about creating new possibilities with the help of adding nanotechnology to inhalation devices and about establishing new horizon in the respiratory disease treatment through developing new drug delivery system. To this end, this review has tried to identify some of the important issues which would help in giving a direction as to how nanotechnology based pulmonary drug delivery could be a game changer in respiratory therapeutics.

Types of Nanocarriers For Pulmonary Drug Delivery

Nanocarriers have highly impacted the delivery of drugs to the lungs by increasing the solubility, stability and release rate of the particular drug [3]. They display attributes such as small size, which allows them to penetrate deep into the lungs, a factor that allows for uniformity of drug dispersion and prevention of drug compounds from being metabolized enzymatically. The nanocarriers that have been investigated for pulmonary delivery include polymeric, lipid-based, inorganic and composite and hybrid systems, with their respective merits and demerits [10].

Polymeric Nanoparticles

Polymeric nanoparticles are preferable for pulmonary drug delivery since they are biodegradable, and their release rate can be controlled while the loading of the drug can be maximized [20]. These nanoparticles are prepared from synthetic or natural polymers for the purpose of improving the stability of the drug as well as developing a controlled drug delivery system [21]. Among all the nanoparticles prepared through various polymers, PLGA-based nanoparticles are more prevalently investigated because of their approval from the FDA, biocompatibility, and the capability to provide prolonged drug release [22]. Chitosan-based nanoparticles, biopolymers of natural chitin, have mucoadhesive character to prolong drug duration in the lungs. Other polymers used include polycaprolackton, poly ethylene glycol, poly (lactic acid) as well as alginic acid; each has its advantages in terms of solubility and bioavailability of the targeted drug [23]. Polymeric nanoparticles prevent drugs from undergoing degradation by enzymes hence making their release duration lengthy leading to lesser dosing. Also, they can be further functionalized with targeting ligands, PEGylation or charge alteration to enhance lung-selective delivery of a drug [24]. Nonetheless, it is important to note that the control of particle size is crucial as the size of particles less than 200 nm is phagocytised by alveolar macrophages and particles with a size of 1-5 μm are more effective in achieving high lung deposition. They include toxicity of the polymers, slow biodegradation of some of the polymers used and careful formulation strategies in order to achieve the set goals of drug delivery [5].

Lipid-Based Nanocarriers

Lipid based nanocarriers are characterize by a number of benefits including biocompatibility, ability to dissolve the drugs and stability in pulmonary drug delivery systems [25]. They replicate the characteristics of natural biological membranes and are biocompatible to be useful in lung delivery of drugs. Of all the lipid based nanocarriers, liposomes and solid lipid nanoparticles (SLNs) are the most investigated systems [26]. Liposomes are membranous structures made of phospholipid double layer and have the ability to accommodate both polar and non polar chemical substances [27]. This is due to their biodegradable nature, non toxic and their efficiency in fusing with cells from the lung epithelial category [28]. Liposomes can be made to release the drug in a controlled and targeted manner by modifying the surface, using polymers, or changing charge characteristics in an attempt to target the lungs specifically [29].The main components of SLNs are stearic acid, triglycerides, and glyceryl monostearate have characteristics of improved drug stability, slow drug release, and controlled drug diffusion. For this reason, SLNs exhibited more increased resistance to enzymatic degradation as compared with the conventional lipid formulations. Thus, the main hindrances in formulation are lipid oxidation and nanoparticle aggregation.Lipid-based nanocarriers can be easily aerosolized using nebulizers, dry powder inhaler, pressurized metered dose inhaler and thus signifying inhalation therapy. They may lack a strong drug-loading efficiency especially for hydrophilic drugs and may require stabilizers to avoid degradation of the drug during storage or transport [30].

Inorganic Nanocarriers

Inorganic nanocarriers have attracted much interest in pulmonary drug delivery because they are more rigid in structure, which would make them more stable within the delivery system, and the surface chemistry can easily be controlled and the drug release kinetics can be well regulated as well [31]. These nanocarriers include high drug stability, size can be changed and functionalized with targeting moieties for site specific drug delivery [32]. Gold nanoparticles have demonstrated outstanding properties such as stability, capability of being functionalized and theranostic properties where they could be used in diagnosis as well as treatment. They can be designed to be non-toxic to the tissues of the lungs and to release the drugs directly to the target site [33]. Silica nanoparticles especially mesoporous silica nanoparticles have been widely used for drug loading and release due to its large surface area and adjustable pore size [34]. The polymeric microparticles can be made to be sensitive to certain physiological stimuli like pH or enzyme activity so that drug is only released in a controlled manner. However, biocompatibility and long-term safety is an issue since inorganic nanoparticles might deposit in the lungs and can cause toxicity. More studies proposing their chemical nature, the way they behave in lung tissues, and their applicability in the formulation of inhalable drugs are necessary [35].

Hybrid Nanocarriers

Biomimetic hybrid nanocarriers combine the features of polymeric, lipid-based, and inorganic systems and can provide multiple functions, including higher stability, sustained release, and better biocompatibility [36]. Such systems are particularly flexible and generic and can be designed for site-specific, active and individualized drug release [37]. Copolymer liposomes are multifunctional particles that combine only sensitivity to lipids with the polymer capacity to control and maintain drug release in the lungs for a longer duration [38]. These nanoparticles are of core shell system in which the polymeric core offers stability and release control and the lipidic shell offers biocompatibility and cellular internalization [39]. Nanomicelles as self-assembled of amphiphilic surfactants are perfect for enhancing dissolution of poorly soluble drugs for pulmonary delivery [40]. They have very low toxicity and reasonable lipophilicity and bioavailability but have very short residence time in lungs which requires further refinement [41]. The stimuli-responsive nanocarriers belong to the hybrid nanocarriers that are capable of responding to external stimuli such as pH conditions, enzyme activity or oxidative stress for drug release [42]. These smart systems improve the effectiveness of therapy minimizing side effects and can be considered as one of the prospects of individual inhalation treatment. Despite these advantages, the formulation of hybrid nanocarriers is not easy, the cost of their preparation is relatively high and there is still a great deal of concern about their stability in the large-scale applicability in clinical settings. The future prospects of developments in this research area are hence concerned with enhancing the efficiency of such nanocarrier formulations, the effectiveness of formulating and delivering the aerosols, and lastly, uncovering the long-term safety of the inhaled drugs [43].

Advances in Inhalable Nanocarrier Technologies

The development of inhalable nanocarrier device in the last few years has marked a new page in pulmonary drug delivery by providing enhancement in drug deposition, targeting and controlled release of drug. Such developments are intended to address the problems pertaining to efficacies of classic inhalation treatments and to enhance therapeutic effects of regimens applied in respiratory diseases [44].

Surface Modification for Improved Retention and Targeting

Modification of the surface of nanocarriers has been identified as one of the most effective ways of increasing the circulation time and targeting of nanocarriers in the pulmonary system [3]. When the surface of nanoparticles is coated with specific ligand such as antibodies, peptides or small molecules target lungs or specific receptors within the lungs can be obtained [29]. It enhances the effectiveness of the administered drugs and also reduces the negative impact within the remainder of the body. For example, functionalization of nanocarrier with transferrin or folate ligands which leads to receptor mediated endocytosis in lung cancer cells thus increasing drug concentration at the tumor site. Also, coating of the surface with polymers such as polyethylene glycol (PEG) may minimise the opsonisation and hence enhance phagocytosis by the alveolar macrophages hence enhancing the residence time of nanocarriers in the lung [45].

Mucoadhesive and Biodegradable Nanocarriers

The advancement in mucoadhesive nanocarriers was proved to be beneficial to enhance the delivery of a drug to the lungs [46]. These nanocarriers are intended to be anchored to the mucus layer that exists around the respiratory tract to ensure prolonged duration of drug presence in the affected area and increased absorption [47]. Chitosan and many of its derivatives have always been preferred because of their mucoadhesive properties owing to their electrically positive charges that enable them interact with the electrically negative charges of the mucosal membranes [48]. In addition, the choice of biodegradable materials for construction of nanocarrier minimizes the potential for chronic inflammation and toxicity after performing its role of drug delivery. This biodegradability is especially crucial when it comes to pulmonary uses because the tissues involved in that human organ are very delicate and may lead to inflammation if they come into contact with materials that the body recognizes as foreign [49].

Smart and Stimuli-Responsive Nanocarriers

Stimuli-responsive nanocarriers give better prospects in pulmonary drug delivery. Such stimuli-sensitive systems can deliver the medicine in response to change in pH, temperature or the presence of specific enzymes which can be active only at the site of the disease [50]. For instance, at the regions of the lungs that have become inflamed, the pH being slightly acidic, it is possible to design nanocarriers that release the anti-inflammatory agents at the right location. Likewise, enzyme-sensitive nanoparticles can also be developed to release the incorporated drug in the presences of matrix metalloproteinases that are up regulated in disease affected lung tissues. This level of control is beneficial in imparting a precise onset and duration of activity with drugs, which increases the therapeutic index and decreases side effects [51].

Controlled and Sustained Release Formulations

It is essential to have controlled and long-term drug release in the lungs to keep the levels high enough to produce sustained drug effects and requiring minimal dosing frequency and compliance [52]. Nanocarrier design has improved, its formulation come with controlled releasing system, so that the drugs can be slowly released. For example, the fusion of drugs into polymeric systems permits the delivery of the therapeutic ingredient dispersed in the matrix as the polymers disintegrate. Also, applying perform coatings, that involves the deposition of the polymer layers of the drug, which gathers drug in the form of layers leads to slow release of the drug. They are quite useful in chronic conditions such as asthma or COPD because it is crucial to maintain constant drug concentration in the bloodstream [53].

Challenges in Pulmonary Nanocarrier Drug Delivery

However, there are challenges that still need to be overcome in order to acknowledge the safety, effectiveness, and translation of inhaled nanocarrier-based pulmonary drug delivery. The complex biological environment which prevails in the lungs, certain problems related to the stability of the formulation, and concerns considered by the regulative authorities are the most critical factors which may restrain the spread of such systems. Alleviating these challenges is critically important in order to achieve the realization of employing nanocarriers in clinical conditions of respiratory diseases [58].

Physiological Barriers

The human respiratory system has developed several barriers in order to safeguard the lungs and other attached tissue from any foreign matter, including drug-bearing nanocarriers [59]. Mucociliary escalator is made up of a layer of mucous and lining cells covered with cilia and helps get rid of particles inhaling the air by moving the mucus constantly. Although this is a natural defense mechanism that is itself highly important for the health of lungs, this reduces the time that nanocarriers can remain in the lungs, thus reducing the time that drugs can be absorbed and hence their effectiveness in treatment. Another type of physiological barrier is alveolar macrophages, which removes the substance. Alveolar macrophage therefore are specialized cells, which are ready to identify and dispose invading particles that arrive at the deep lung. A lot of these nanocarriers have a diameter of below 200 nm and are aggressively taken up by these immune cells, thereby being cleared before reaching the affected area. Although

PEGylation, or ligand coating for instance, would minimize the rates of clearance by macrophages, few more solutions must still be enhanced for lung targeting [60].

Formulation and Stability Issues

It is even more challenging to develop inhalable nanocarriers that remain stable enough during storage and transportation up to the time of administration. Nanoparticles are prone to agglomeration and alteration with time affecting particle size, surface characteristics, and release mechanism of the drug. It can be of vital importance to keep the formulation rather stable and to ensure the physicochemical properties to read out alike all the time [61]. Another issue is the behavior of nanocarriers and lung surfactants, which change the characteristics and deposition profiles of the drug. In the formulation, the drug-polymer interactions as well as drug-lipid interactions may influence the stability and release profiles of the nanocarrier system hence demanding characteristic pre-clinical studies. The formulation of aerosolized nanocarriers must also fit the delivery system eg dry powder inhaler, nebulizers or metered dose inhaler, so that the drug will disperse properly on inhalation [62].

Particle Size Optimization and Aerodynamic Properties

It is noteworthy that the appropriate particle size and the ability of particles to reach deep into the lungs in a timely manner remain critical. In order that nanocarriers reach the depths of the lungs, they must not exceed 1–5 micrometers in diameter. If droplet particles are greater than 5 μm they can impact on the trachea and bronchus, in other cases, particles smaller than 1 μm are expelled before deposition [63]. Besides size, shape and mass density also play a part in determining the efficiency in which particles are deposited in the lung system. It has been found that, in general, both rod-like and porous nanoparticles are taken up better thus have longer half-life in lungs as compared to spherical ones. Besides, through optimization of the surface charge and design of the engineered nanocarriers, their deposition and penetration in deep lung tissues can be further improved. Nonetheless, it continues to be difficult to engineer nanocarriers that have an optimal aerodynamic properties and the ability to support high drug loading [64].

Safety, Biocompatibility, and Toxicity Considerations

Sustainability of the inhalable nanocarriers is an important factor in their medical applicability superseding that safety and biocompatibility are of prominence. It is to be noted that both polymeric and lipid based nanoparticles are generally considered biodegradable and biocompatible; however, serious issue of long term accumulation with non biodegradable inorganic nanoparticles cannot be neglected. Some materials can remain in the lungs for long duration like gold and silica nanoparticles which can cause inflammation or fibrosis [65]. Also, solvents, surface-active agents, and other additives employed in nanocarrier preparations may themselves be toxic to the lungs. As a result, in-vitro and in-vivo toxicity tests must be conducted to determine the impact of nanocarriers on lung cells and their ability to trigger inflammation response, as well as circulation in the bloodstream. Another important consideration is bioactivity of nanocarriers to evade the host's immune system may cause unwanted immune response with undesirable complications. Coating the surfaces and biodegradable polymers enhance the safety features but further research must be carried out before clinical application [61].

Regulatory and Clinical Translation Challenges

Nevertheless, progress has been lacking in the development of nanocarrier-based inhaled drug delivery systems because of problems associated with approval of such systems for clinical application. Several regulatory agencies across the globe possess their guidelinesfor nanomedicine approval such as the FDA and EMA, and they require clinical data on safety, efficacy, and stability of the product. In contrast, the nanocarrier requires other tests slightly different from a conventional drug, which are the nanoparticle distribution, degradation rate, and accumulation of nanocarriers in lung tissue, thus making the approval process lengthy and costly [66].One of the major issues is that it is usually challenging to prepare nanocarrier formulations that can be scaled up and reproduced. Preparation of nanocarriers on an industrial scale entails selecting a very specific particle size, drug encapsulation rate and stability, which can be sometimes hard to accomplish [67]. Third, there may be a concern from the public and therefore it

contributes to low utilization of nanotechnology based therapies that will require more promotion and awareness to be developed [68].

Nanocarrier - Inhalation Device Integration

The successful delivery of nanocarrier-based therapeutics to the lungs hinges not only on the design of the nanocarriers themselves but also on their effective integration with inhalation devices. This ensures that the therapeutic agents get to the target sites in the respiratory system and also increase the surface area on which the production can be done [58].

Overview of Inhalation Devices for Nanocarrier Delivery

Inhalation devices play a very crucial role when it comes to delivering nanocarrier-based drugs to the desired organ, in this case the lungs. These tools consist of DPIs, nebulizers as well as pMDIs. Every device has its techniques or attributes concerning the dispersion of the drug and the choice depends on the properties of the nanocarrier formulation as well as the requirement for the medications to be delivered [69].

Dry Powder Inhalers vs. Nebulizers vs. Pressurized Metered-Dose Inhalers

Dry Powder Inhalers (DPIs): DPIs provide drugs in a powder form and depend on the patient's inspiratory force to expel the powder into smaller particles that the patient can easily inhale. They do not require the hand-breath synchronization, and this makes them breath-actuated. However, DPIs demand sufficient inspiratory flow rate and this may not be in the reach of patients with severe respiratory ailments. In formulating the nanocarriers for DPIs, it is important to factor the flowability and stability of the powder [70].

Nebulizers: Most of the nebulizers produce aerosols from liquid formulations utilization of compressed air, Ultrasonic waves or vibrating mesh. They are most useful where patients can find it hard to use the inhalers such as kids or those in acute respiratory disease. which can make them suseptical to certain nanocarrier formulations wherein nebulizers can achieve delivery of large volumes of medication over duration of time. However, they are normally larger in size, not so mobile and easy to maintain as the portable models [71].

Pressurized Metered-Dose Inhalers (pMDIs): These ones dispense a particular amount of medicine through the use of a propellant in the aerosol technology. They are small in size, can be moved easily from one place to another and administer drugs very quickly. However, it is not that effective or simple to use because the patient must coordinate the hand and breathing during operation. It is for this reason that spacer devices can be employed to address this problem. According to article four and five, formulation of nanocarriers in pMDIs requires compatibility with propellants and achieving of stability of the suspension [69]. All these inhalation devices possess their merits and demerits, which are as follows: Several factors for the selection of the device includes the disease state of the patient, the type of formulation of the nanocarrier and the targeting area of drug delivery within the respiratory tract.

Aerosolization Techniques and Optimization Strategies

Effective aerosolization of nanocarrier formulations is crucial for ensuring that the therapeutic agents reach the target sites within the lungs. Optimization strategies include:

Particle Engineering: Modifying the physicochemical properties of nanocarriers, such as size, shape, and surface characteristics, to enhance aerosolization efficiency and lung deposition.

Formulation Additives: Incorporating excipients like dispersing agents or stabilizers to improve the stability and dispersibility of nanocarrier formulations.

Device Calibration: Adjusting device parameters, such as airflow rates and nozzle designs, to optimize the generation of aerosols with desirable particle size distributions.

Patient Training: Educating patients on proper inhalation techniques to ensure effective delivery of the medication to the lungs. By carefully considering the interplay between nanocarrier formulations and inhalation devices, and implementing appropriate optimization strategies, it is possible to enhance the efficacy of pulmonary drug delivery systems [72].

Future Prospects and Emerging Strategies

The field of inhalable nanocarrier-based pulmonary drug delivery system is gradually expanding, and there are several promising strategies which can further improve the therapeutic result of respiratory diseases. New promising approaches are as follows: nanomedicine for personalization, gene and mRNA delivery, integrating AI, and green approaches, and clinical translation [73,74].

Personalized and Precision Nanomedicine Approaches

The concept of personalized nanomedicine relates to the use of nanoparticles in relation to the individual patient where different parameters are considered, including but not limited to genetic factors and disease manifestation. Getting nanocarriers which could selectively transport particular drug or therapeutic agent to certain parts of the lungs will increase the efficiency and, by the same token, decrease side effects. In this approach, it is essential to produce nanocarriers that will be able to interact with specific molecular indicators present in a patient's respiratory system in order to deliver medication [75].

Gene and mRNA Delivery via Inhalable Nanocarriers

The targeting of selected genes and mRNA to the lung tissues provided a great potential in treating various pulmonary diseases such as genetic disorders, cancers, etc. With such carriers, these nucleic acids can be protected from degrading and can easily target the cells in the respiratory tract. Several investigators have also illustrated that AI constructed nanoparticles could transport mRNA to the target cells at the diseased areas and so advance treatment for lung cancer and cystic fibrosis conditions [76].

Artificial Intelligence in Nanocarrier Design

There is a new trend that AI is being adopted for enhancing nanocarrier design and applied functions. AI systems have the ability to learn from massive data and therefore the course to the appropriate nanocarrier combinations as well as structures suitable for certain therapeutic uses. This way improves the agenda of the nanocarrier based drug delivery systems and also minimizes the time of development. For instance, AI has also been applied in developing nanoparticles for drug delivery which have increased on their efficiency as well as targeting abilities [77].

Sustainable and Eco-Friendly Nanocarrier Development

There is an increase in the interest for green and environmentally friendly nanocarriers in the current world. Scientists are determined to develop methods of using non-toxic and environment-friendly materials for the fabrication of nanocarriers that can biodegrade invariably. This encompasses the use of natural polymers and the development of nanocarriers that have an environmentally friendly decomposing component once they have executed their therapeutic functions [78].

Clinical Translation and Commercialization Trends

There are many barriers associated with the transition of a developed concept from laboratory to clinic such as the approval by the regulatory agencies, production in large scale, and market availability. Newer strategies that are current in this area include attempts made to establish standard procedures on characterization of such nanocarriers so as to allow standardization in production as well as random clinical trials to evaluate the safety and effectiveness of such nanocarriers extensively. Cooperative actions between the professors, representatives from the companies and organizations, and the regulatory bodies are significant in ensuring the commercialization of the I-needs novel inhalable nanocarrier-based therapies [79].

CONCUSION

Nanocarrier-based pulmonary drug delivery systems present a promising alternative to conventional inhalable formulations by enhancing drug deposition, bioavailability, and controlled release. The ability of nanocarriers to penetrate deep into the lungs, avoid enzymatic degradation, and provide sustained release makes them highly suitable for treating respiratory diseases. Various nanocarrier types, including liposomes, polymeric nanoparticles, solid lipid nanoparticles, and dendrimers, have demonstrated potential in improving pulmonary therapeutics. However, challenges such as mucociliary clearance, macrophage uptake, formulation stability, and regulatory approval remain key obstacles. Recent advancements in stimuli-responsive nanocarriers, biodegradable and mucoadhesive systems, and AI-driven nanocarrier design are pushing the boundaries of inhalable nanomedicine. Future directions focus on personalized therapies, gene and mRNA delivery, and eco-friendly nanocarrier development. While significant progress has been made, further research is required to optimize formulation strategies, overcome physiological barriers, and facilitate clinical translation for widespread application in respiratory disease treatment.

ABBREVIATIONS

COPD – Chronic Obstructive Pulmonary Disease; MDI – Metered-Dose Inhaler; DPI – Dry Powder Inhaler; SLN – Solid Lipid Nanoparticles; PLGA – Poly(lactic-co-glycolic acid); PEG – Polyethylene Glycol; AI – Artificial Intelligence.

Authors' Contribution

All authors have equal contribution.

Conflict of Interest

The authors declare no conflict of interest related to this study.

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Table 1: Classification of Inhalable Nanocarriers and their Properties

Nanocarrier Type	Composition	Advantages	Limitations	References
Liposomes	Phospholipids, cholesterol	Biocompatible, protect drugs from degradation, controlled release	Short stability, potential aggregation, high production cost	[14]
Polymeric Nanoparticles	Biodegradable polymers (PLGA, chitosan)	Sustained drug release, targeted delivery, improved lung retention	Risk of polymer toxicity, complex formulation	[15]
Solid Lipid Nanoparticles	Lipids (stearic acid, triglycerides)	Increased drug stability, controlled drug release, biocompatible	Potential for drug expulsion, limited drug-loading capacity	[16]
Nanomicelles	Amphiphilic surfactants or block copolymers	Enhance solubility of hydrophobic drugs, rapid lung absorption	Prone to rapid clearance, requires optimized surfactant selection	[17]
Dendrimers	Highly branched polymers	Precise drug loading, tunable surface chemistry, targeted delivery	High synthesis cost, potential cytotoxicity	[18]
Inorganic Nanoparticles	Silica, gold, carbon-based materials	High drug stability, good pulmonary retention, potential for imaging	Possible accumulation in lungs, safety concerns	[19]

Table 2: Recent Advances In Inhalable Nanocarrier-Based Therapies

Nanocarrier Type	Modification/Feature	Therapeutic Application	Key Benefits	References
Polymeric Nanoparticles	PEGylation for reduced clearance	Lung Cancer Therapy	Enhanced tumor targeting and prolonged circulation time	[54]
Lipid-Based Nanocarriers	Ligand-functionalization for targeted delivery	Asthma Management	Improved drug deposition in inflamed airways	[55]
Inorganic Nanocarriers	pH-responsive release mechanisms	Tuberculosis Treatment	Targeted drug release in acidic environments of infection sites	[56]
Hybrid Nanocarriers	Mucoadhesive surface coating	Cystic Fibrosis Therapy	Increased retention time in mucus-covered lung tissues	[57]

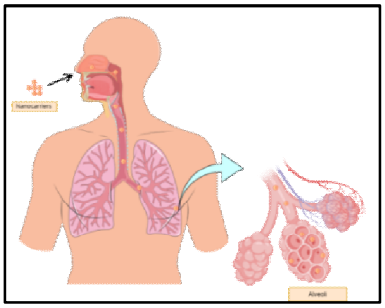


Fig 1: Pulmonary targeted delivery

RESEARCH ARTICLE

Effect of Physical Activity on Heart Rate Recovery Post 6-Minute Walk Test in School-Going Adolescents

Roshani Prafulkumar Tank[1], Meghavi Oza[2]*, Gaurav J. Patel[3] and Savan Kukadia[4]

[1]MPT Scholar, Department of Physiotherapy, Ahmedabad Physiotherapy College, Ghuma, Ahmedabad, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[2]Associate Professor, Department of Physiotherapy, Ahmedabad Physiotherapy College, Ghuma, Ahmedabad, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[3]Principal, Department of Physiotherapy, Ahmedabad Physiotherapy College, Ghuma, Ahmedabad, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[4]Senior Resident Doctor, Department of Palliative Medicine, Gujarat Cancer and Research Institute, India B J Medical College B.J. Medical College, Gujarat University, Ahmedabad, Gujarat, India.

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***Address for Correspondence**
Meghavi Oza
Associate Professor,
Department of Physiotherapy,
Ahmedabad Physiotherapy College, Ghuma, Ahmedabad,
Parul University, P.O. Limda, Waghodia, Vadodara,
Gujarat, India.
E.Mail: meghavi.ozaapc@paruluniversity.ac.in

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ABSTRACT

Physical activity and fitness by age and gender have implications for understanding the etiology of obesity in youth and for suggesting approaches for prevention and treatment. Females are less physically active and less fit than males at all ages. 6MWT is an inexpensive in general submaximal exercise test generally used to quantify the functional exercise capacity in clinical population. The test is frequently used in adults and is increasingly being utilized in children and in adolescents population as well. It has been used in assessment of subject with pulmonary, cardiovascular etc. This study is needed to assess how physical activity influences heart rate recovery (HRR) after a 6-minute walk test in school-going adolescents. Understanding HRR can help evaluate cardiovascular fitness, detect potential health risks early, and promote healthier lifestyles in young individuals. A total of 80Adolescent were taken and they were divided into two groups. Group 1 n=15 subjects (physicallyactive) and Group 2 n=15(sedentary) subjects. Students were instructed to perform six min walk test. Heart rate recovery was calculated between groups. As the data was not normally distributed non parametric Man Whitney U test was used to compare heart rate recovery between both the groups. Result shows that will comparing between groups, physically active children have significant heartrate recovery as compared to sedentary students

(p< 0.004). Present study concluded that physically active students were having faster heart rate recovery as compared to sedentary students thus preventing future cardiovascular risk factors

Keywords: Heart rate recovery, 6 MWT, Physical Activity, Adolescents.

INTRODUCTION

Cardiovascular fitness is a critical component of overall health, particularly during adolescence—a pivotal period marked by significant physical and physiological development. In recent years, there has been growing concern about the increasing prevalence of sedentary behaviours among school-going adolescents, which may predispose them to various cardiovascular risks. Heart Rate Recovery (HRR), defined as the rate at which the heart rate declines after exercise, serves as a valuable indicator of autonomic function and cardiovascular health. An accelerated HRR reflects robust parasympathetic reactivation and overall cardiac fitness, whereas a delayed HRR may signal autonomic dysfunction and heightened cardiovascular risk[9]. The Six-Minute Walk Test (6MWT) is a submaximal exercise assessment widely utilized to evaluate functional exercise capacity across diverse populations. It measures the distance an individual can walk over six minutes on a flat, hard surface, providing insights into their aerobic endurance and cardiovascular efficiency. In paediatric populations, including adolescents, the 6MWT has been employed to establish normative data and assess functional capacity[10]. Engagement in regular physical activity is known to enhance cardiovascular fitness, improve autonomic balance, and facilitate more rapid HRR post-exercise. Studies have demonstrated that individuals who participate in consistent physical training exhibit improvements in HRR, indicative of better exercise tolerance and cardiac health[11]. Conversely, sedentary lifestyles during adolescence have been associated with diminished cardiovascular efficiency and delayed HRR, underscoring the importance of physical activity in promoting cardiac autonomic function. While extensive research has been conducted on HRR in adult populations, there is a relative paucity of data focusing on adolescents, particularly in the context of the 6MWT. Given the unique physiological changes occurring during adolescence, it is imperative to understand how physical activity influences HRR in this demographic. Such insights could inform targeted interventions aimed at enhancing cardiovascular health and mitigating future cardiovascular risks among school-going adolescents.

This thesis aims to investigate the effect of physical activity on HRR following the 6MWT in school-going adolescents. By examining the relationship between habitual physical activity levels and HRR, this study seeks to elucidate the potential of HRR as a non-invasive marker for cardiovascular fitness in adolescents. Furthermore, the findings may contribute to the development of school-based physical activity programs designed to improve cardiovascular outcomes and promote long-term health in this vulnerable age group. In conclusion, understanding the interplay between physical activity and HRR in adolescents is of paramount importance in the context of public health. By addressing this knowledge gap, the present study endeavours to provide evidence-based recommendations for fostering cardiovascular fitness through physical activity, ultimately aiming to reduce the burden of cardiovascular diseases later in life. These data on variations in physical activity and fitness by age and gender have implications for understanding the etiology of obesity in youth and for suggesting approaches for prevention and treatment. Females are less physically active and less fit than males at all ages.

It is generally accepted that the gender difference in fitness is related to body fat, muscle mass, and haemoglobin concentration, but the relatively new data on gender differences in physical activity suggest that the role of physical activity should be considered more carefully. For Males, aerobic power per kilogram body weight remains virtually unchanged throughout youth become less and less active. When physical activity standards for youth are proposed, it must be assumed that activity levels decrease with age. Especially regarding physical activity, one would expect female youth to have higher levels of obesity than males. All other factors being equal, the contribution of physical activity to the risk of obesity would be expected to increase with age as activity decreases in both gender group, but

more so in females. Health benefits of physical activity for children and adolescent, it is vitally important to have accurate information on the epidemiology of physical activity in youth[1]. Here is substantial evidence linking the number of hours of TV viewing and being overweight or obese in children and adolescent. For example, in a representation sample of 7216 children aged 7 to 11 years, TV watching and video game use were risk factors for being overweight(17% to 44% increased risk) or obese(10% to 61%). The authors emphasized the need to examine more than a single sedentary behaviour (ie, TV viewing), particularly because not all sedentary behaviours have been associated obesity[2]. After achieving peak workload, all the patients spent at least two minutes in a cool-down period during treadmill testing at a speed of 2.4 km (1.5mil) per hour and a grade of 2.5 percent. This period was considered the recovery period. The value for recovery of heart rate was defined as the reduction in the heart rate from the rate at peak exercise to the rate one minutes after the cessation of exercise. The mechanisms by which impaired heart rate recovery confers an increases risk of death, even among patients without heart failure myocardial perfusion defects, are not clear. Imai *et al*. examination the physiologic characteristics of heart-rate recovery after exercise in health adults, athletes, and patients with chronic heart failure[5]. they demonstrated that, in all three group, vagal reactivation was the principal determinant of the decrease in heart rate during the first 30 seconds of recovery and that this mechanism was independent of age and the athletes but was blunted in patients with heart failure and was completely abolished by the administration of atropine[3].

MATERIALS AND METHODS

This Prospective Observational Study study was conducted on 80 school going adolescence (N=80). Informed consent was taken to the individuals who were participating in the study. Data was collected from the various centres of Ahmedabad, Gujarat. All the individuals were recruited according to the inclusion and exclusion criteria. Students were divided into two groups. Group 1 n=40subjects (physically active) and Group 2 n=40(sedentary) subjects. Students were instructed to perform six min walk test. Heart rate recovery was calculated between groups

Materials

- Sphygmomanometer
- Pulse oximeter
- Stop watch
- Bottles
- Measure tape
- Pen
- Chalk
- Book
- Chair

The Inclusion Criteria

- Normal Healthy Adolescent
- Age Group between 6 to 18 Years
- Both Male and Female Are Included
- Who wereAble to perform 6 MWT.

The Exclusion Criteria

- Those Students Who were Participating in Other Sports Activity
- Students who had underwent any past surgery since last 6 months
- Students presented with any type of neurological disorder, congenital heart disease etc.,
- Students not welling to participate.

PROCEDURE

Physical Activity Assessment
Physical activity levels will be assessed using
Physical Activity Questionnaire for Adolescents (PAQ-A) – a validated self-report tool.
Pedometer-based step count analysis – to objectively measure daily activity levels.

Participants will be classified into two groups
Active Group: Engaging in at least 60 minutes of moderate-to-vigorous physical activity per day.
Sedentary Group: Engaging in less than 60 minutes of physical activity per day.

6-Minute Walk Test (6MWT)
The 6MWT will be conducted following the American Thoracic Society (ATS) guidelines
- Conducted in a 30-meter indoor corridor with minimal external interruptions.
- Participants will be instructed to walk as far as possible for 6 minutes at a self-paced speed.
- Standardized encouragement will be provided at specific time intervals[8].

Heart Rate Measurement
Resting Heart Rate (RHR): Measured using a digital heart rate monitor after 5 minutes of seated rest.
Post-6MWT Heart Rate: Measured immediately after the test and at 1-minute, to assess HRR.
HRR Calculation: -HRR1 = Peak HR - HR at 1 min post-exercise

RESULT

The result was done from SPSS software 20 version.

Demographic data
In group 1, 18(45%) were male and 22(55%) were female and in Group 2, 26(65%) were male and 14(35%) were female. Table 1 showing data of Demographic. An Unpaired T test was used to compare the pre and post data of individual. The mean ± SD distribution for heart rate recovery (HRR) for the adolescents was physically active (Group A) was 23.95 ± 2.783 and sedentary individuals was 16.07 ± 4.42 (Group B). Shows the data of Group 1 and Group 2 Heartrate Recovery Rate (Figure 1)

DISCUSSION

The present study aimed to evaluate heart rate recovery (HRR) among physically active adolescents and compare it with their sedentary counterparts. The findings revealed a significant difference in HRR between the two groups, with physically active adolescents demonstrating a notably faster recovery ($p < 0.00001$). This suggests that regular physical activity has a substantial impact on autonomic regulation and cardiovascular efficiency. A lowered resting heart rate in active individuals is primarily attributed to an increase in parasympathetic activity and a minor reduction in sympathetic discharge. Regular exercise enhances vagal tone, leading to a quicker return to baseline heart rate post-exercise. The accelerated HRR observed in the physically active group further supports the role of training in optimizing autonomic function. Conversely, sedentary students exhibited a delayed HRR, indicating reduced parasympathetic reactivation and prolonged sympathetic dominance. This imbalance may contribute to long-term cardiovascular risks if not addressed early in life. HRR is considered an essential prognostic marker of cardiovascular health, with delayed recovery being linked to an increased risk of cardiovascular diseases, metabolic disorders, and autonomic dysfunction. Several factors influence HRR, including exercise intensity, cardiorespiratory fitness, autonomic nervous system (ANS) modulation, hormonal changes, and baroreflex sensitivity. Physical training has been shown to enhance cardiac vagal tone, which facilitates quicker HRR. The improved efficiency in

autonomic regulation observed in active adolescents underscores the benefits of regular physical activity in promoting cardiovascular health. These findings highlight the need for structured physical activity programs in schools to enhance cardiovascular fitness and autonomic balance among adolescents. HRR post-exercise can serve as a simple yet effective tool for assessing cardiovascular health, encouraging early interventions to reduce future health risks associated with sedentary lifestyles.

CONCLUSION

The present study concluded that physically active students demonstrated significantly faster heart rate recovery (HRR) compared to their sedentary counterparts, indicating better autonomic function and cardiovascular efficiency. Faster HRR is primarily attributed to enhanced parasympathetic reactivation and reduced sympathetic dominance, which play a crucial role in maintaining heart health. In contrast, sedentary students exhibited delayed HRR, suggesting impaired autonomic regulation that may contribute to an increased risk of cardiovascular diseases in the future. HRR is a well-established prognostic marker of cardiovascular health, with slower recovery post-exercise being associated with higher risks of hypertension, obesity, and metabolic disorders. Regular physical activity strengthens the cardiovascular system by improving vagal tone, increasing baroreflex sensitivity, and enhancing cardiac autonomic balance. These physiological adaptations contribute to a more efficient heart rate regulation, ultimately reducing long-term cardiovascular risk. Given the significant impact of physical activity on HRR, encouraging adolescents to engage in regular exercise is crucial for promoting lifelong heart health. Schools should implement structured physical education programs that emphasize aerobic and endurance training to enhance cardiovascular fitness. Early interventions focused on increasing physical activity levels in adolescents can help mitigate future cardiovascular risks, fostering a healthier generation with improved overall well-being.

Limitation

- More sample size can be administered
- Factors such as diet, sleep pattern was not administrated in the study
- Other outcome measures such as fatigue was not assessed

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Table 1: Demographic Data

Group 1		**Group 2**	
Age	10.55 ± 2.01 Mean± SD	Age	10.77 ± 2.18 Mean ± SD
Gender	Male = 18(45%)	Gender	Male = 26(65%)
	Female = 22(55%)		Female = 14(35%)

Table 2: Data of Group 1 and Group 2

Group 1		**Group 2**	
HRR Mean ± SD	23.95 ± 2.783	HRR Mean ± SD	16.075 ± 4.423
Unpaired T Value	4.6291		
P Value	<0.00001		

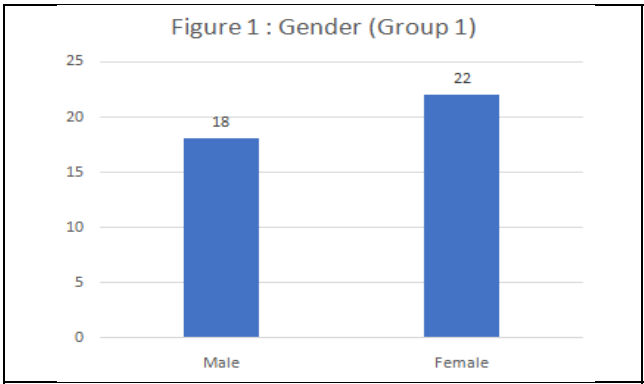


Figure 1 : Gender (Group 1)

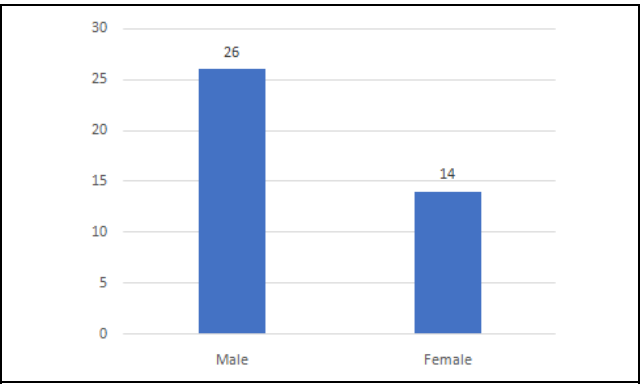


Figure 2 : Gender (Group 2)

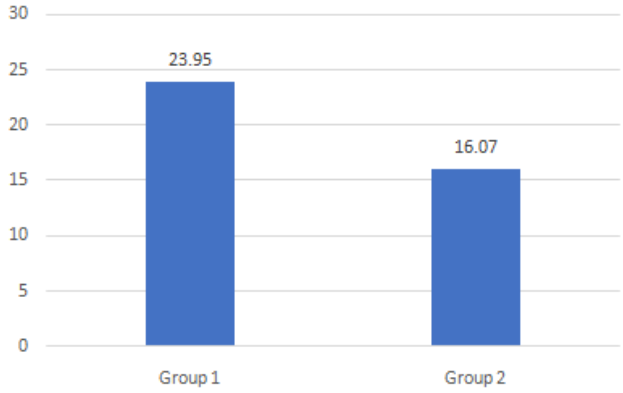


Figure 3 : Mean HRR after 1 minute

REVIEW ARTICLE

Piroxicam a Versatile Non-Steroidal Anti-Inflammatory Agent for Inflammatory Disorders

Manisha M. Rokade[1]* and Vivek V. Byahatti[2]

[1]Assistant Professor, Department of Pharmaceutics, Sandip University, Nashik, Maharashtra, India.
[2]Professor, Department of Pharmacognosy, Sandip University, Nashik, Maharashtra, India.

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***Address for Correspondence**
Manisha M. Rokade
Assistant Professor,
Department of Pharmaceutics,
Sandip University, Nashik, Maharashtra, India.
E.Mail: rokademanisha3131@gmail.com

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ABSTRACT

A powerful member of the oxicam class of non-steroidal anti-inflammatory medicines (NSAIDs), piroxicam has attracted a lot of interest due to its wide range of effectiveness in treating inflammatory conditions. For chronic illnesses such rheumatoid arthritis, osteoarthritis, and ankylosing spondylitis, Piroxicam provides a practical and efficient treatment alternative due to its lengthy half-life and once-daily dosage. As part of its pharmacodynamic profile, it inhibits the cyclooxygenase (COX)-1 and COX-2 enzymes, which lowers the production of prostaglandins that cause fever, discomfort, and inflammation. Piroxicam also shows promising results in postoperative inflammation and acute musculoskeletal injuries. Despite its therapeutic advantages, gastrointestinal and renal adverse effects must be carefully considered, especially when using it for an extended period of time. The pharmacological adaptability of Piroxicam, its therapeutic uses, and the risk-benefit analysis that is essential for maximizing patient outcomes in the treatment of inflammatory diseases are all highlighted in this review.

Keywords: Piroxicam, NSAID, Rhumatoid arthritis, Osteoarthritis

INTRODUCTION

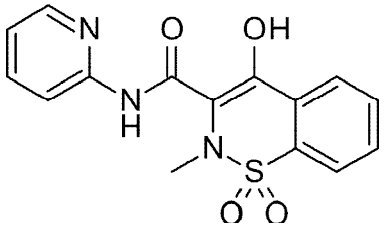
Piroxicam refers to anti-inflammatory agents with anti-aggregating, analgesic, and antipyretic effects [1]. In addition to post-traumatic pain, gout, neuralgia, myalgia, soft tissue inflammation, and inflammatory viral illnesses of the upper respiratory tract, piroxicam is utilised to treat a number of musculoskeletal disorders [2]. Piroxicam works by mechanism inhibiting the COX-1 and COX-2 systems as depicted in figure 1, which are involved in the metabolism of arachidonic acid. Piroxicam is known for its high oral absorption rate and its ability to have an impact for up to one day following the initial dosage. Thus, piroxicam can be given one to two times a day, depending on the manner of dose [3]. New medications being created in contemporary pharmacology based on piroxicam. Additionally, the

mechanisms of action are examined in relation to the age group [4]. Mechanism of Piroxicame [Figure 1]. Piroxicam is an anti-inflammatory drug that is both popular and in great demand. The pharmaceutical dose form is one of the many factors that greatly influence the patient's tolerance, pharmacokinetics, and ease of administration of the medicine. Tolerance and ease of administration also play a significant role in a patient's commitment to therapy. Compliance is therefore essential to the treatment's effectiveness. Since they can make oral administration easier and improve patient comfort, low-soluble or insoluble chemicals in the liquid dose form are important[5]. Piroxicam comes in a number of dosage forms, including pills, suppositories, capsules, and gels for topical application. Seven firms, with their origins in Germany, Poland, Bulgaria, and the Russian Federation, are registered in the CIS nations. It should be highlighted that the limited selection of oral forms provided by these manufacturers is due to their comparable excipient makeup. Fluid forms are now preferred by doctors when prescribing oral dose forms. [6]. The current development of a fluid dosage form of piroxicam presents an essential issue in this respect. With consideration for excipients, patient preferences, and pharmacological factors, this development would expand the selection of piroxicam medication variations. Piroxicam is also utilized as a suspension because it is very insoluble in water [7]. There are a number of characteristics that set suspension apart from other dosage forms. These include the solid fraction's high dispersibility, the therapeutic impact starting sooner than with tablets and other solid dose forms, the therapeutic effect lasting longer than with solutions, and patient simplicity of use. In different dose forms, piroxicam suspensions make up no more than 1.5% of the overall amount of the drug. These low numbers can be explained by the fact that suspensions are generally kinetically and aggressively unstable, which dictates the specifics of suspension preparation and storage. Research into alternative drug delivery mechanisms in the human body is therefore relevant since the effective dose forms of piroxicam that are now accessible are unstable when stored. The purpose of the study is to investigate the gastrointestinal tract's unique piroxicam kinetics in ex vivo tests. An alternate and perhaps more effective method of delivering piroxicam to the gastrointestinal tract lumen during oral administration is to use a theoretically appropriate material, such as pectin and zein-based hydrogels. The study aimed to: [a] examine the kinetics of piroxicam releases from hydrogel-based drug delivery systems in gastrointestinal settings during a rat experiment; [b] compare the experimental outcomes based on the dry matter mass, mass index of piroxicam, and ratio of zein and pectin; and [c] calculate the time interval for which 50% of piroxicam is released in gastrointestinal tract-simulation conditions.

Description

Piroxicam is 4-hydroxy-2-methyl-N-(2-pyridyl)-2H-1,2-benzothiazine-3-carboxamide-l,l-dioxide.

Structure



Appearance, Color, Odor, Taste

Piroxicam is a colourless, odourless, bitter crystalline powder.

Synthesis

Starting with sodium saccharin, the first one uses chloroacetic acid esters to alkylate it at nitrogen. After alkoholysis and a Dieckmann ring closure, the base catalyses the rearrangement of the 5-membered intermediate to produce 3-Acyl-2H-1,2-benzothiazin-4(3H)-one-l, 1-dioxide. When N-Methyl derivative reacts with methyl iodide, it is easily obtained. After that, the product is treated with the proper amine in refluxing xylene to produce the matching carboxamide [8].

Pharmacokinetics

When taken orally or recally, piroxicam is easily absorbed. It builds up after many doses and reaches a stable level in around seven days. With a half-life of around 40 hours in humans, the medication undergoes substantial metabolism to produce metabolites that appear to be inert. Peak plasma levels are reached around two hours following a single oral dosage and 5.5 hours following rectal administration of suppositories or 2U. Because of piroxicam's long plasma half-life, plasma concentrations stay rather constant over the following 24 to 48 hours. 99.3% of piroxicam is bound to plasma proteins at doses ranging from 5 to 30 g/ml. Patients with rheumatoid arthritis can absorb piroxicam through their synovial fluid, where it can reach quantities of around 40% of those seen in plasma. Since piroxicam is so strongly protein-bound, it may displace other medications that are also protein-bound. The simultaneous administration of antacids and aspirin has no effect on its absorption or distribution.

Metabolism

The available information indicates that piroxicam undergoes substantial metabolism in humans through a variety of pathways, including amide hydrolysis, cyclodehydration, glucuronide production, 5'-pyridine ring hydroxylation, decarboxylation, ring contraction, and N-dealkylation. Scheme 3 displays the known piroxicam metabolic routes. The main metabolite in humans is metabolite B, which is created when the pyridyl ring is hydroxylated. It can exist free or in conjugation with glucuronic acid. In terms of prostaglandin synthetase inhibition, the metabolite is at least 1000 times less potent than piroxicam [9]. Within the first eight days following treatment, over 10% of a single oral dosage of piroxicam is found in the urine unaltered. Piroxicam's half-life is prolonged because of its poor clearance rate; in healthy patients, it is typically estimated to be 38 hours, with a range of 31 to 56.7 hours. Neither dosage nor plasma concentration seem to have any impact on half-life. The rat appears to hydroxylate the pyridyl ring at the para-position to form metabolite B, whereas the dog's primary mechanism is the synthesis of the cyclodehydrated metabolite [A]. Up to 60% of a daily dosage of the latter molecule is found in urine and faeces, making it the primary human metabolite, whether free or conjugated with glucuronic acid. In addition, laboratory animals produce ring contraction, deamidate, and decarboxylate in different proportions. These latter mechanisms appear to have little influence on human behaviour. Laboratory animals show no discernible anti-inflammatory effects from any of piroxicam's metabolites. Together with their extremely low plasma concentration at or below the limits of analytical detection], the metabolites' lack of activity supports the theory that piroxicam is inherently active and that its metabolites have no role in that activity. It appears that the special mix of functional groups that piroxicam has is necessary for its effect. Piroxicam can be formulated in various novel dosage form as shown in figure 2 and table 1.

Sublingual tablet system using low doses of Piroxicam

In this technique, piroxicam and a bioadhesive substance are applied to water-soluble carrier particles during dry mixing. In theory, the pill breaks down rapidly into the ordered units made up of the bioadhesive component, piroxicam, and carrier. At first, these units stick to the mucosa. Piroxicam dissolves with the water-soluble carrier particles as they progressively disintegrate. This method combines the bioadhesive retention of the medication in the oral cavity with the best possible exposure of the active ingredient to the dissolving solution. The rate of dissolution in the gastrointestinal (GI) tract frequently regulates the oral absorption rate of poorly soluble, highly permeable (class II) medications, such as piroxicam. Thus, in addition to permeability, a drug's solubility and dissolving behaviour are important factors that determine its oral bioavailability. Because of the prolonged interaction between the medicine and the GI mucous, this undesirable quality may further increase the amount of GI damage. Consequently, it is a perfect fit for evaluating the MFD tablet's potential for dissolving stability.

Transdermal Drug Delivery System

A popular and effective non-steroidal anti-inflammatory medication, piroxicam has the potential to be delivered topically. Using a Keshery Chein diffusion cell, the penetration of piroxicam from a reservoir-type transdermal gel formulation based on proniosomes over excised rat abdomen skin was examined. The flow was significantly better than with the control gel composition. The size of the generated niosomes and the entrapment effectiveness of the lipid vesicles were assessed. It was shown that formulations based on Span 60 generated the smallest and most efficient vesicles, whilst those based on Span 80 generated the least efficient vesicles. Lecithin's addition improved

entrapment efficiency even further. The traditional method of making proneosomes used sorbitol and maltodextrin as the basis. We used scanning electron microscopy to examine the proniosomes' morphology. The maximum flow of 35.61 g/cm2/h was attained, and the transdermal system based on proniosomal gel was 7.39 times more effective than the control gel. Anti-inflammatory research showed that piroxicam might be delivered using a transdermal drug delivery method based on proniosomes. When compared to the control, the carrageenan-induced rat paw inflammation was significantly reduced. S4 and S6, the niosomes made with spans, exhibited the highest percentage of entrapment, at 90.4% and 94.8%, respectively. Niosomes from S4 were found to be bigger than those from S6, according to the particle size study. Because they tend to fuse more easily, vesicles of a smaller diameter are thought to penetrate the skin more effectively. Compared to S4, the niosomes of S6 were smaller, showed greater entrapment effectiveness, and had a larger surface area. Due of their limited entrapment effectiveness, S2 niosomes were not pursued for more research. Span 40 niosomes were found to create bigger niosomes, however piroxicam vesicles from Span 60 had a greater entrapment effectiveness and were therefore chosen; this was likely due to its low permeability and high transition temperature [24]. Additionally, lecithins were attempted to be included. Drug entrapment was discovered to be improved by the addition of egg lecithin. Since lecithin improves permeability, its inclusion is likewise warranted. By adding lecithin, the percentage of drug entrapment increased to 96.1%. Lecithin increases hydrophobicity, which causes vesicles to become smaller when it is incorporated. Drug leakage is likely prevented by the creation of a more compact and well-organised bilayer. The anti-inflammatory trials' findings demonstrated that lecithin vesicles based on span 60 (S6L) significantly decreased paw oedema. The percentage inhibition was greater than that of the carbopol gel containing piroxicam. *In vitro* investigations show a delayed release, which can be explained by the short fall found with formulations based on sorbitol and maltodextrin. It has been investigated and assessed how well piroxicam penetrates proniosomes of different kinds and compositions of nonionic surfactants in vitro. High effectiveness piroxicam entrapment was achieved within the vesicles' lipid bilayers. Based on the experimental results, it is possible that surfactants promote penetration or that the vesicles fuse with the stratum corneum's intercellular lipid and transport the medicine from the vesicles to the skin. The presence of lecithin most likely facilitates the process. Proniosomes can therefore transport piroxicam and most likely additional medications.

Self-microemulsifying drug delivery system (SMEDDS)

SMEDDS promotes the absorption of lipophilic medicines in vivo and increases their dissolution in vitro. The droplet size of the SMEDDS system ranges from 20 to 200 nm. The purpose of the solubilisation capacity was to look at SMEDDS for piroxicam. Due to differing intermolecular interactions between the medication and SMEDDS components, different medicines in the current investigation had varied stable SMEDDS formulations. SMEDDS's tiny droplets improve the pace at which medications dissolve and their bioavailability. The simplicity of production and scalability are two other significant benefits of SMEDDS [25]. A solid self-microemulsifying drug delivery system was created by filling a hard gelatin capsule with a liquid self-microemulsifying drug delivery system that included Neusilin US2. The optimised formulation contains 27.58 percent Transcutol-P, 44.16 percent Cremophor EL, and 28.26 percent Capmul MCM. The findings demonstrated that piroxicam's drug release profile from the self-microemulsifying drug delivery formulations was superior to that of piroxicam powder in its pure form. Piroxicam was quickly and completely released. It is anticipated that solid self-microemulsifying drug delivery systems, once packed in hard gelatin capsules, will be a viable method of delivering a liquid formulation in solid dosage form. Formulations were assessed for drug release experiments conducted in vitro. In comparison to pure drug, it demonstrated an improvement in the rate of drug release. An indicator of better solubility and dissolution rate of the pure medication is the enhanced in vitro dissolution from the S-SMEDDS. Consequently, S-SMEDDS is a promising piroxicam system [26].

Nanosponge Formulation

PXM nanosponge (PXM-NS) formulations were made with β-cyclodextrin and carbonyldiimidazole as a cross linker. Three ratios of β-cyclodextrin to crosslinker and three drug to nanosponge ratios were investigated. The particle size, zeta potential, physical compatibility, and in vitro release of piroxicamnanosponge formulations were all evaluated. For the best recipe, stability tests were conducted at three different temperatures. Lastly, research on the ideal

formula's pharmacokinetic characteristics and in vivo analgesic effectiveness was done. Particle), polydispersity index, zeta potential, and %EE were shown by the optimised PXM-NS formula (PXM-NS10). When compared to the unformulated medication, the dissolving testing revealed a significantly higher quantity of PXM dissolved. The nanosponge demonstrated acceptable stability for ninety days at four and twenty-five degrees Celsius, according to stability tests. Studies on in vivo pain management confirmed that mice's analgesic response to PXM was significantly improved, and that PXM-NS10's relative bioavailability was 1.42 times higher than that of commercial tablets. An promising method for improving Piroxicam's solubility and, consequently, its bioavailability is to create nanosponge under ideal circumstances [27]. The addition of PXM to β-cyclodextrinnanosponge improved its analgesic efficacy. To find the best nanosponge formula for PXM, twelve PXM-nanosponge formulations were created utilising three ratios of β-cyclodextrin to cross linker and three ratios of drug to nanoponge. The ideal ratios were discovered to be 1:2 for the medication and 1:8 for the β-cyclodextrin to crosslinker. Using these formulation parameters significantly enhanced the PXM in vitro solubility and dissolution testing. In comparison to commercial tablets, the optimal nanosponge formulation of PXM demonstrated a significant increase in analgesic effectiveness and in vivo pharmacokinetic bioavailability. The study demonstrated that a nanosponge prepared under ideal circumstances can increase Piroxicam's analgesic action [27].

Ionic Liquids Nanoemulsion Loaded with Piroxicam for Drug Delivery System

In order to create the ionic liquid-in-oil nanoemulsions (IL/o NEs) system, two different ionic liquid types were used: 1-butyl-3-methylimidazolium hexafluorophosphate [Bmim][PF6] and 1-hexyl-3-methylimidazolium chloride [Hmim][Cl], with varying mass ratios with Tween-80/Span-20 1:1, 1:2, 2:1, and 2:3. To obtain the optimal outcome for the NEs system, they underwent stability testing prior to characterisation, rheology behaviour, and release investigation. High stability against separation, creaming, sedimentation, and flocculation is demonstrated by the high content of Tween-80 in the NE formulation. The pH value, zeta potential, and droplet sizes for each formulation were taken into consideration within the ranges of 4.72 to 5.50, -37.3 to -55.3 mV, and 100 to 500 nm, respectively. Images of the nanoemulsions obtained by transmission electron microscopy showed spherical droplets. Rheological analyses revealed that both ionic liquids exhibited non-Newtonian shear thinning behaviour at low shear rates up to 14 S-1 of NE. The dialysis bag approach was utilised to examine the in vitro drug releases by the insertion of Piroxicamnanoemulsions. The drug permeation showed that the ionic liquid had 93% of the drug released, and the optimal surfactant ratio was 2:1. It is determined that the NEs made from ionic liquids have high promise as a Piroxicam medication delivery vehicle [28]. Piroxicam, a medication that is only weakly soluble in water, can be administered using IL/o NEs. Using hydrophilic IL, and hydrophobic IL, it was possible to successfully substitute water in nonaqueous emulsions. The optimal ratio of combination surfactant Tween-80/Span-20 is 2:1, according to the formulation of NEs for both ionic liquids. Additionally, stability experiments demonstrate that IL/o NEs have high chemical and physical stability. The outcomes of the encapsulation efficiency test showed that both ILs were capable of dissolving the medication and encapsulating it in NE micelles. Apart from this, both ILs exhibit outstanding physicochemical characteristics, which produced the greatest penetration of Piroxicam in the in vitro release assay. Shear thinning for both ILs was shown by IL/o NEs for the rheology investigation. But in terms of stability, physicochemical characteristics, and in vitro release analysis, the formulation produced the best results. IL/o NEs are therefore seen to have promising potential for Piroxicam medication delivery.

Nanoemulsifying Drug Delivery System

Based on the solubility research and pseudo-ternary phase diagram, the optimised formulation of liquid SNEDDS (L-SNEDDS) was chosen. It was made up of soybean or coconut oil/Tween 80/Transcutol HP (12/80/8%w/w). Following their adsorption onto adsorbents, optimised L-SNEDDS and liquid supersaturatable (L-sSNEDDS) preparations were created as immediately compressed tablets. The creation of a nanoemulsion and the presence of the drug in an amorphous state with hydrogen bond interaction between the drug and SNEDDS components may be the cause of the enhanced drug dissolution rate in the solid supersaturatable preparation (S-sSNEDDS). The oral bioavailability of piroxicam from S-sSNEDDS was much better than that of the pure drug (PP) and its commercial counterpart (Feldene®) (CD), according to in vivo pharmacokinetic experiments conducted on eight healthy human volunteers. In comparison to PP or CD, S-sSNEDDS had a relative bioavailability of around 151.01% and 98.96%, respectively.

The findings obtained confirm that S-sSNEDDS is a potential drug delivery method to increase piroxicam's oral bioavailability. An approach that shows promise for increasing piroxicam's oral bioavailability and dissolving rate is the new self-nanoemulsifying drug delivery system (SNEDDS). HPMC-E15's addition to liquid SNEDDS and subsequent transformation into solid dosage form successfully prevent drug precipitation and provide an extra option in the quest to enhance product design, manufacturing, performance, and viability. Its benefits also include quick drug dissolving, simple preparation, and the use of non-toxic excipients. S-sSNEDDS has also been shown to increase the drug's oral bioavailability in in vivo pharmacokinetic experiments. This might be because of the combined mechanism of nanoemulsion dispersion with a greater surface area. As a result, the newly created formulation (S-sSNEDDS) would be beneficial in terms of its quick beginning of action, particularly in painful situations when an immediate analgesic impact is needed [29].

Piroxicam Encapsulated Niosomes

Compared to alternative medication delivery methods, niosomes provide a number of benefits. The stability problem, which is a major disadvantage for niosomes, is yet unclear. The stability of niosomes was the main focus of this investigation. Niosomes were loaded with piroxicam, the medication that was selected as the model. Different versions of the original niosome formulation were made, and niosomes were made using the thin-film approach. For three months, the samples were kept at either 25°C±2°C/60% RH±5% RH or 5°C±3°C. Niosomes were observed under an optical microscope, and the drug leakage percentage, particle size and distribution, zeta potential, and drug release patterns were ascertained. Piroxicam was released continuously thanks to niosome composition. At a level of 1.56–6.63%, drug leakage from stored niosomes was noted. But when niosomes were stored, their particle size grew. Neither physical shape nor time had any impact on the zeta potential values. Drug release patterns and quantities were quite comparable for the original formulation and all niosome forms; however, there was a modest reduction in drug release levels with time. This suggests that when niosomes age, they become stiffer. Although lyophilisedniosomes at 5±3°C were shown to provide the best storage conditions in this investigation, the use of an appropriate cryoprotectant and an optimisedlyophilization procedure should be further assessed [30].

GellanNanohydrogels: Novel Nanodelivery Systems for Cutaneous Administration of Piroxicam

Gellan conjugated with either riboflavin or cholesterol was used to test the viability of employing gellannanohydrogels (Ge-NH) as a delivery mechanism for the cutaneous administration of piroxicam (PRX). A 50% w/v Transcutol aqueous solution, a saturated aqueous drug solution, and a commercially available PRX plaster were used as controls in the in vitro skin penetration tests into the human epidermis. To shed light on the Ge-NHs permeation process, confocal microscopy, ATR-FTIR spectroscopy, circular dichroism, and a dynamometer-assisted extrusion experiment were used. According to skin penetration experiments, Ge-NHs slow down the permeation process in comparison to the controls while also improving PRX retention in the epidermis. NHs have the ability to pierce the stratum corneum before slowly dissolving and spreading across the viable epidermis to reach the spinosum layer. To sum up, NHs offer a fresh approach to addressing the epidermis's weakly permeable substances, which will enhance the treatment of cutaneous diseases. According to the findings of this study, Ge-NHs may be helpful in transporting and improving the skin penetration of insoluble and weakly permeable pharmaceutical ingredients. The data acquired indicates that they can enter intact in the upper layers of the skin and undergo a progressive disassembly process by diffusion in the deeper layers of the epidermis, albeit further research is necessary to fully understand their mode of action. The potential for internalisation in epidermal cell lines and the capacity of GeRfv-NHs to localise the medication in the epidermis make these nanosystems useful for developing novel therapeutic approaches for the treatment of dermatological diseases. It is important to note that these formulations may be the last dose form used for pharmacological molecules to be delivered topically. In fact, we have shown in a prior study that it is possible to deliver additional nanocarriers—namely, ultraflexible liposomes and nano-emulsions—by a straightforward spray. Since the nanogel solution may be supplied as is, this method should be beneficial as it avoids dilutions that could lower its concentration or cause the loaded medicine to diffuse outside the nanocarrier. In fact, the process of preparation produced a suspension where the drug is both free in the suspension medium and loaded in the nanogel, resulting in a saturated state that prevents drug leakage from the nanocarrier. The in vitro skin permeation experiment employing the saturated solution shows that, in the particular

case of PRX, the penetration pattern of PRX-loaded Ge-NHs is significantly greater than the penetration of the solubilised fraction through the human epidermis [31].

Drug Interactions

Few studies have been conducted expressly to ascertain how piroxicam interacts with other commonly used medications; however, piroxicam has not been demonstrated to interact pharmacokinetically with digoxin, aspirin, or antacids that contain aluminium hydroxide either by itself or in combination with magnesium hydroxide. The anticoagulant action of acenocoumarin has been shown to be enhanced by piroxicam, as demonstrated by changes in the Quick test in four out of two participants. A patient using 20 mg of piroxicam per day was reported to have had toxicity due to elevated plasma concentrations of lithium. When piroxicam was stopped, the concentration of lithium dropped from 1.5 to 1.0 mmol/L. After starting piroxicam two weeks before his third round of treatment for lymphocytic lymphoma, a patient experienced acute uric acid nephropathy [32].

Piroxicam in therapy

Piroxicam has been shown in clinical trials, some with large patient populations, to be an effective analgesic and anti-inflammatory treatment for rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute pain in musculoskeletal conditions and episiotomy. Piroxicam has been found to be a good substitute for aspirin, aloxiprin, indomethacin, phenylbutazone, ketoprofen, diclofenac, and sulindac in rheumatoid arthritis and for aspirin, naproxen, fenbufen, indomethacin, diclofenac, and sulindac in osteoarthritis, according to comparative studies. These trials demonstrate that it is generally effective and tolerable enough to compare well with such medications. Piroxicam appears to be beneficial in treating acute gout, according to open research, yet it is unclear how it compares to other regularly used medications. The half-life of piroxicam is greater than that of other non-steroidal anti-inflammatory medications, and it works best when taken once daily, which has been demonstrated to increase patient adherence to the medication. Typically, the medicine is well tolerated at doses of 20 mg or less per day; nevertheless, when used for extended periods of time, the gastrointestinal system is less tolerant of larger quantities [40 mg per day). Not all individuals can benefit from the newly developed non-steroidal anti-inflammatory medications, even though they are typically better tolerated than aspirin, indomethacin, or phenylbutazone. Now a well-known member of this class, piroxicam ought to be taken into account in addition to other medications of its kind when determining which medication is best for each patient [33].

DISCUSSION

Piroxicam, a non-steroidal anti-inflammatory medication (NSAID) that belongs to the oxicam class, has shown notable effectiveness in treating a number of inflammatory conditions. Its pharmacokinetic profile, which includes a lengthy half-life of roughly 40 to 50 hours, makes once-daily dosage easier and improves patient compliance, especially for chronic illnesses like osteoarthritis and rheumatoid arthritis.According to clinical research, piroxicam, when taken daily at a dose of 20 mg, is as effective as other NSAIDs such as aspirin, indomethacin, and naproxen in treating osteoarthritis and rheumatoid arthritis. 844 individuals with a range of inflammatory disorders participated in a Phase IV study conducted in India, and Piroxicam showed a good response rate of 82% to 96% across a number of measures, including pain, tenderness, and edema [34].Piroxicam's non-selective suppression of cyclooxygenase enzymes may result in gastrointestinal (GI) adverse effects despite its therapeutic advantages. According to a Norwegian study, the upper gastrointestinal tract—including peptic ulcers and hemorrhages—was implicated in 73 out of 83 likely adverse responses to Piroxicam. However, despite being linked to a higher risk than meloxicam, a worldwide meta-analysis of 75 randomized clinical trials revealed that Piroxicam did not significantly vary from other NSAIDs in terms of serious GI events. This also Co-administration with gastroprotective medications such proton pump inhibitors or H2 antagonists is advised to reduce GI risks, particularly in patients with a history of GI issues. To reduce negative effects, cautious patient selection and monitoring are also essential. In summary, piroxicam is still a useful NSAID for treating inflammatory diseases since it provides convenient once-daily dosing together with efficient symptom control. Although gastrointestinal side effects are a worry, treatment

results can be maximized with careful patient selection and preventative measures. Its safety profile could be improved and its therapeutic efficacy increased with more investigation into other formulations and delivery methods [35]. An effective treatment for both acute and chronic inflammatory conditions is piroxicam, a common NSAID that belongs to the oxicam class. Once-daily dosing is made possible by its lengthy elimination half-life (about 40–50 hours), which enhances patient compliance, a crucial aspect of managing chronic conditions like osteoarthritis and rheumatoid arthritis. Piroxicam's therapeutic effectiveness is widely established. In comparison to other NSAIDs such as ibuprofen, naproxen, and diclofenac, comparative studies have demonstrated that it offers comparable or better symptom management (JAPI, 1989). Additionally, it has shown successful in treating a variety of rheumatic illnesses, musculoskeletal injuries, and postoperative pain.

CONCLUSION

Although piroxicam is still a powerful and adaptable NSAID for inflammatory diseases, new drug delivery technologies can increase its therapeutic usefulness. These developments give patients who need long-term anti-inflammatory treatment a safer option in addition to better therapeutic results. For these formulations to be validated and incorporated into standard therapeutic practice, future clinical trials will be essential.

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Table 1: Literature shows Piroxicam can be formulated in different dosage form with lots of advantages and applications:

Sr. No.	Novel Delivery System	Description	Advantages	Applications	Reference
1	Nanoparticles	Piroxicam encapsulated in polymeric/lipid nanoparticles	Enhanced bioavailability, sustained release	Targeted delivery, chronic inflammation	10
2	Niosomes	Non-ionic surfactant-based vesicles containing piroxicam	Improved permeability, reduced side effects	Topical and transdermal delivery	11
3	Microspheres	Biodegradable polymer-based microspheres	Controlled release, site-specific delivery	Injectable formulations, intra-articular delivery	12
4	Liposomes	Phospholipid bilayer vesicles encapsulating piroxicam	Biocompatibility, improved solubility	Topical, oral, and parenteral routes	13
5	Solid Lipid Nanoparticles (SLNs)	Piroxicam incorporated into solid lipid matrix	High stability, controlled drug release	Oral and topical anti-inflammatory therapy	14
6	Transfersomes	Ultra-deformable vesicles enhancing skin penetration	Enhanced transdermal delivery, reduced GI toxicity	Transdermal patches, arthritis treatment	15
7	Proniosomes	Dry formulations converted into niosomes upon hydration	Improved stability over niosomes	Easy storage, used in transdermal systems	16
8	Hydrogel-based Delivery	Piroxicam loaded into hydrophilic polymer networks	Controlled release, improved skin retention	Topical applications for localized pain relief	17
9	Mucoadhesive Buccal Films	Thin films applied to buccal mucosa	Bypasses first-pass metabolism, rapid onset	Buccal drug delivery for systemic anti-inflammatory effect	18
10	Microemulsions	Piroxicam solubilized in oil-water mixtures stabilized by surfactants	Enhanced solubility and absorption	Topical and oral formulations	19
11	Ethosomes	Soft, malleable vesicles with high ethanol content	Improved skin penetration, suitable for deep dermal delivery	Transdermal systems, arthritis pain relief	20
12	Cubosomes	Nanostructured liquid crystalline particles	High drug-loading capacity, sustained release	Oral, topical, and ocular delivery	21
13	Dendrimers	Branched, tree-like nanocarriers	High drug loading, targeted delivery potential	Intravenous delivery, anti-inflammatory applications	22
14	Floating Drug Delivery Systems	Low-density formulations that float in the stomach	Prolonged gastric residence, sustained drug release	Oral delivery, especially for gastric inflammation	23

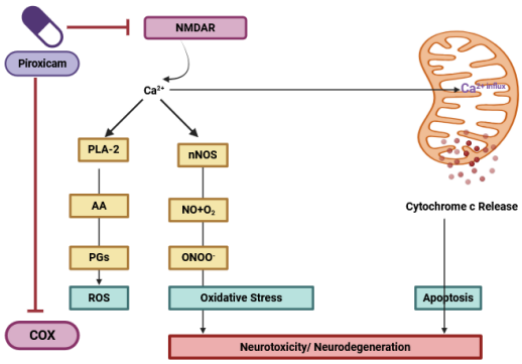


Figure 1: Mechanism of Piroxicame

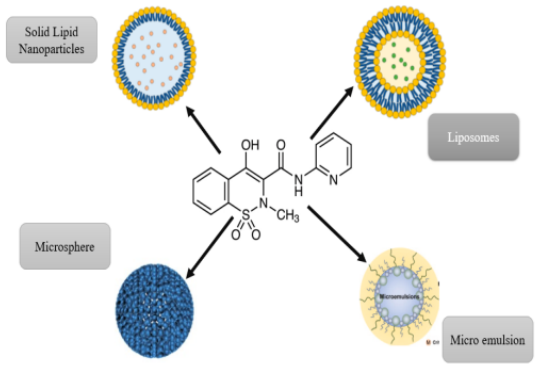


Figure 2: Piroxicam formulations in novel dosage forms

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RESEARCH ARTICLE

A Comparative Study on Impact of Lecture Classes and Audiovisual Teaching Method based on the Topic Kala Sharira

Dayana H[1], Bandapalle Dattu Narayanrao[2]* and Shyny Thankachan[3]

[1]Ph.D Scholar (Reg.No:. 200200402004), Department of Rachana Sharir, Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat, India.
[2]Professor and HOD, Department of Rachana Sharir, Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat, India.
[3]Ph.D Scholar (Reg.No.: 180200402011), Department of Rachana Sharir, Parul Institute of Ayurveda, Parul University, Vadodara, Gujarat, India.

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***Address for Correspondence**
Bandapalle Dattu Narayanrao
Professor and HOD,
Department of Rachana Sharir,
Parul Institute of Ayurveda, Parul University,
Vadodara, Gujarat, India.
E.Mail: drdayanah@gmail.com

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ABSTRACT

Kala Sharira is a significant topic in the curriculum for first-year BAMS students, thoroughly elaborated in the *Sushruta Samhita*. References to this concept are also found in other classical Ayurvedic texts such as the *Charaka Samhita*[1], *Ashtanga Hridaya*[2], *Ashtanga Sangraha*[3], *Bhavaprakasha*[4], and *Sharngadhara Samhita*[5]. Despite its importance, students often struggle to comprehend the concept of *Kala Sharira*. In the context of *Sharirasthana*, the term *Kala* refers to the membrane that separates *Dhatu* (body tissues) from its corresponding *Ashaya* (body cavities or spaces). The revised NCISM syllabus emphasizes innovative teaching methodologies alongside traditional classroom lectures. This study explores ways to enhance the effectiveness of classroom teaching and improve students' understanding of *Kala Sharira*. Traditional classroom lectures involve structured verbal explanations, discussions, and supplementary written materials like notes or handouts. In contrast, audio-visual teaching incorporates multimedia tools such as videos, slides, and animations to make learning more engaging and impactful. The findings reveal that integrating audio-visual aids with traditional lectures significantly improves students' comprehension and retention of the subject matter. By combining auditory and visual elements, concepts are illustrated more effectively, making the lessons more relatable and easier to grasp. This approach highlights the value of incorporating multimedia tools to create more dynamic and enjoyable learning experiences in Ayurvedic education.

Keywords: *Kala Sharira*, Lecture, Animation, Audio-Visual

INTRODUCTION

Acharya Sushruta, regarded as the pioneer of *Rachana Sharira* (anatomy), introduced the concept of *Kala Sharira* in the fourth chapter of *Shaareerasthana*. This principle is a unique contribution to Ayurvedic science. *Kala* refers to a membrane or boundary that separates *Dhatu* (tissues) from their corresponding *Ashaya* (spaces or cavities). *Dhatu*includes not only the seven primary tissues but also the *Tridosha* and *Trimala*, as they contribute to the structural and functional integrity of the body. *Ashaya* denotes the specific spaces or regions where the *Dhatus* are located and supported.[6] The *Kala* in the body are arranged in layers, resembling the concentric rings of a tree. There are seven distinct types of *Kala*: *Mamsadhara Kala*, *Raktadhara Kala*, *Medodhara Kala*, *Shleshmadhara Kala*, *Pureeshadhara Kala*, *Pittadhara Kala*, and *Shukradhara Kala*.[7] Each type of *Kala* plays a crucial role in maintaining the structural equilibrium of the body. Beyond serving as structural boundaries, these *Kala* also perform specialized functions, making them an integral yet often underappreciated aspect of Ayurvedic physiology. Education involves imparting knowledgeand cultivating wisdom in learners through a structured learning process. Teaching aids play a vital role in enhancing the teaching-learning experience. The chalk-and-board method has long been the most commonly used traditional approach to teaching. In this method, key points and sentences are written on the blackboard, and relevant diagrams are illustrated using chalk to support the lecture. In recent times, PowerPoint presentations have also become a widely accepted component of traditional teaching methods. These presentations tend to engage learners more effectively compared to the chalk-and-board approach, as their visual appeal captures attention and encourages active listening. The advancement of technology has revolutionized teaching and learning, introducing innovative dimensions to the educational process. Integrating technology into the classroom starts when educators design lessons that incorporate digital tools in purposeful and engaging ways. Effectively combining teaching aids with appropriate methodologies enhances the overall learning experience, creating a more dynamic and enriching educational environment.[8] Audio-visual learning extends teaching beyond traditional classroom settings and moves away from the conventional chalk-and-talk approach. These aids enhance the learning process by engaging multiple senses, creating more stimulating and effective educational experiences by integrating elements such as images, animations, videos, sounds, text, and other interactive features. There by audio-visual tools offer diverse and dynamic presentations. Animations, in particular, provide the capability to visually represent microscopic entities like bacteria, viruses, atoms, and molecules. This innovation transforms the teaching and learning process, allowing abstract concepts and intangible phenomena to be vividly illustrated and easily understood by learners. Even though non-lecture activities are given more importance in the revised syllabus, they are mainly limited to group discussions, self-learning, seminars, and practical classes. Therefore, this study aims to evaluate the extent to which the audiovisual teaching method enhances students' understanding of the topic when integrated with classroom lectures.

MATERIALS AND METHODS

The study employed a cross-sectional design. A video was created using a suitable application, focusing on the concept of *Kala Sharira*. Initially, a traditional lecture using PowerPoint presentation was delivered to a group of 54 first-prof students of BAMS degree. After the session, feedbacks were collected to assess their understanding of the topic. After a period of 6 months, the animation video of the topic was provided for the same group. The students were asked to give feedback regarding the effectiveness in understanding the topic. The responses from both the feedbacks were evaluated and compared to measure the effectiveness of each teaching method.

Study sample

54 students of first professional B.A.M.S degree from ayurveda colleges in Kerala.

RESULTS

This study was designed to assess the effectiveness of integrating audiovisual aids into the learning process. The findings reveal a noticeable improvement in understanding the topic. This indicates that audio-visual classrooms significantly enhance knowledge acquisition when incorporated with conventional textbook-based learning.

Statistical analysis

The response graph from 54 participants were analysed. The graph shows that the understanding and retention level improved when the study tool is incorporated in the teaching. The students found the class as more interesting with the study tool.

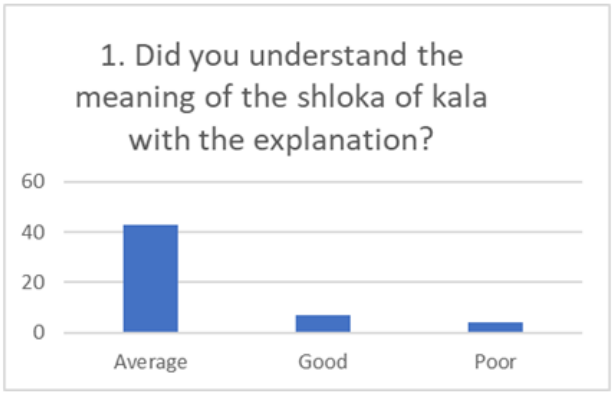
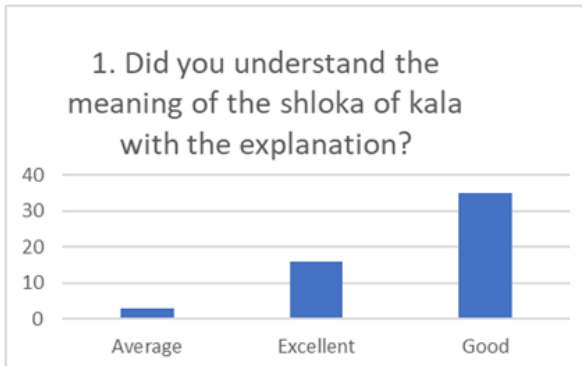
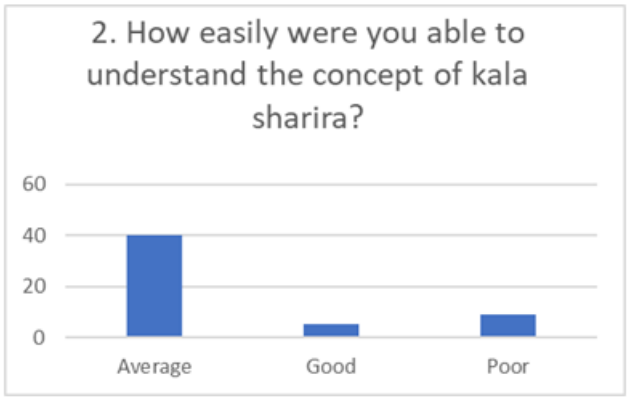
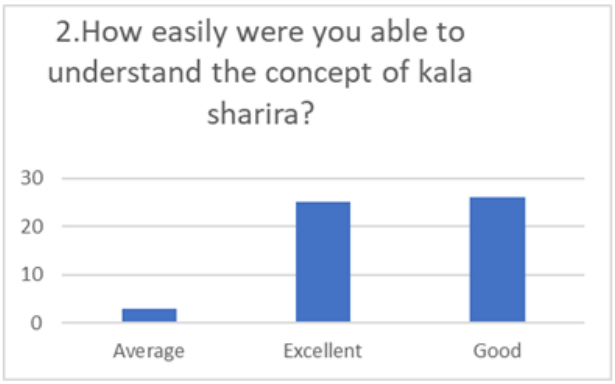
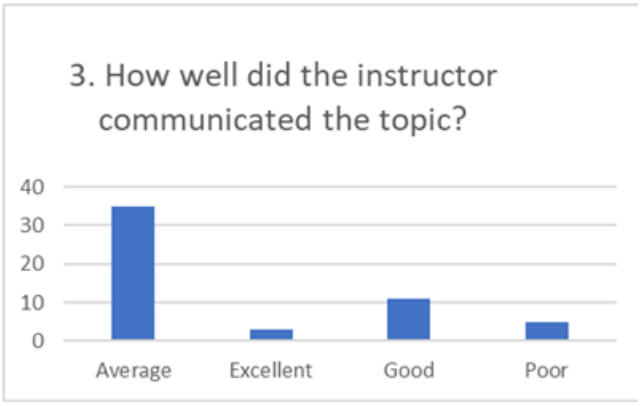
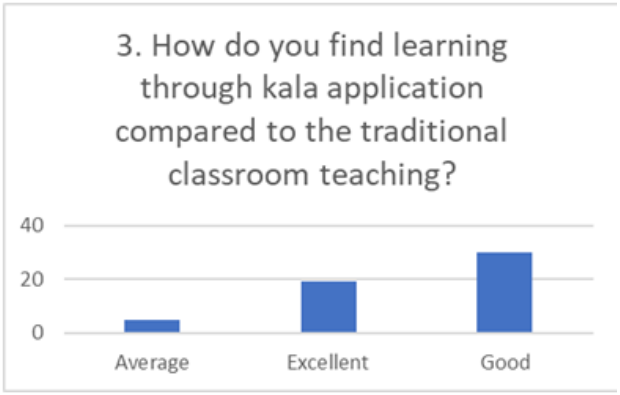
DISCUSSION

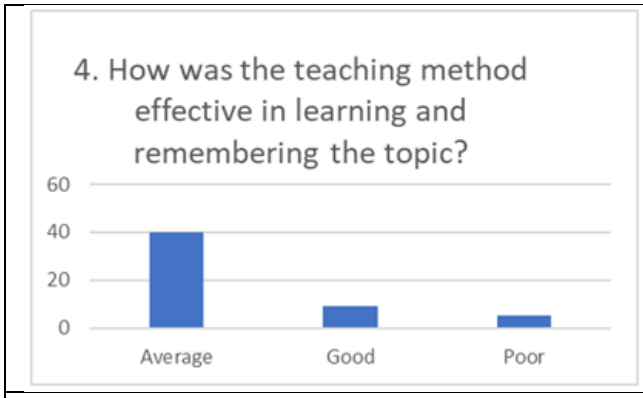
Kala Sharira is a crucial topic in Ayurvedic anatomy (*Rachana Sharira*), but understanding the concepts solely through the traditional study of Samhitas can be challenging. While the texts provide various similes to aid comprehension, elaborating on these through conventional lectures often falls short. Effective teaching involves employing diverse strategies to enable the students to grasp complex ideas and develop essential skills. The choice of teaching methods greatly influences learning outcomes. When teachers employ appropriate methods, they enhance the teaching-learning process, promote better understanding, and facilitate desired changes in students. Incorporating technology into the curriculum offers innovative approaches to teaching and promoting higher-order thinking skills. Audiovisual tools, for instance, support visualization and better interpretation of abstract concepts, making them valuable in explaining topics like *Kala Sharira*. This study highlights the importance of adopting technology-driven methods to optimize classroom learning experiences. The concept of *Kala Sharira*, as explained in the Samhitas through various similes, is often difficult for students to comprehend using traditional classroom methods such as lectures, blackboard teaching, or simple PowerPoint presentations. However, the use of audiovisual tools has been found to significantly improve students' understanding and retention of the topic. These tools facilitate a more engaging and effective delivery of complex concepts. The application of audiovisual aids in teaching *Kala Sharira* demonstrates their potential to enhance not only this subject but also other areas of study, making classroom teaching more effective overall. By encouraging an interactive and visually enriched learning environment, audiovisual tools play a pivotal role in modern education, bridging gaps in traditional methods and fostering deeper comprehension among students.

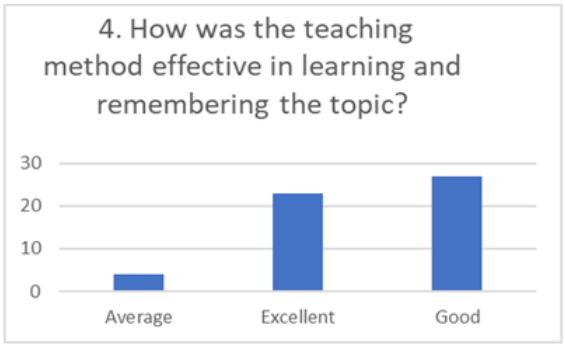
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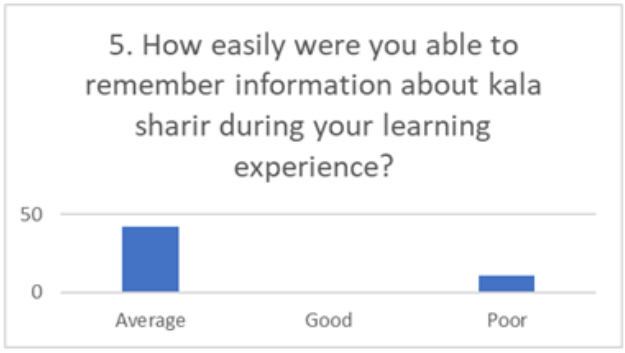
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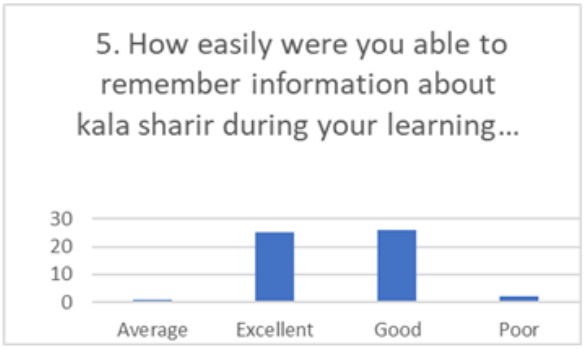
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RESPONSE -1	RESPONSE -2
	
	
	


4. How was the teaching method effective in learning and remembering the topic?
60
40
20
0
Average
Good
Poor

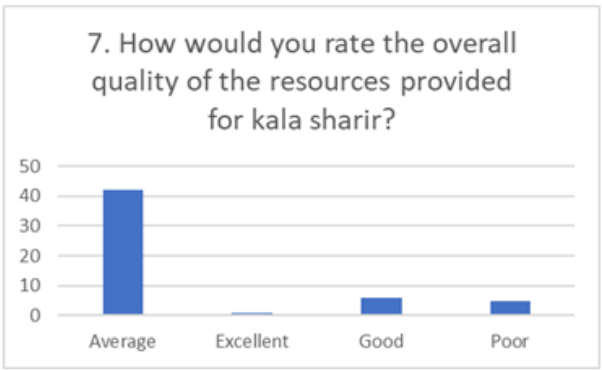

4. How was the teaching method effective in learning and remembering the topic?
30
20
10
0
Average
Excellent
Good

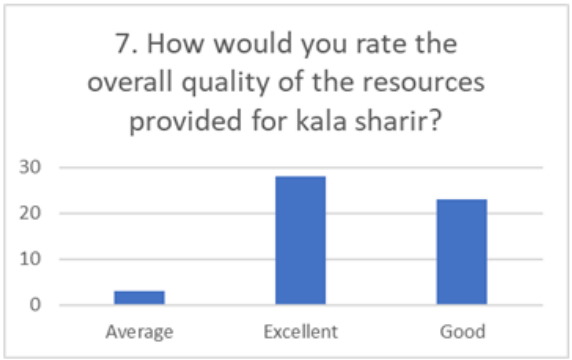

5. How easily were you able to remember information about kala sharir during your learning experience?
50
0
Average
Good
Poor


5. How easily were you able to remember information about kala sharir during your learning...
30
20
10
0
Average
Excellent
Good
Poor


6.How satisfied are you with your entire learning experience?
40
30
20
10
0
Average
Good
Poor


6.How satisfied are you with your entire learning experience?
30
20
10
0
Average
Excellent
Good
Poor


7. How would you rate the overall quality of the resources provided for kala sharir?
50
40
30
20
10
0
Average
Excellent
Good
Poor


7. How would you rate the overall quality of the resources provided for kala sharir?
30
20
10
0
Average
Excellent
Good

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RESEARCH ARTICLE

Mental and Intellectual Health Needs among Medical Students : A Structured Narrative Review and Thematic Analysis

Dhara Patel[1]* and Vd. Shraddha N Dhundi[2]

[1]Associate Professor, Department of Samhita and Sidhanta, Sumandeep Ayurved Medical College and Hospital, Sumandeep Vidyapeeth (Deemed to be University), Waghodia, Vadodara, India.
[2]Professor and H.O.D, Department of Rasshastra and Bhaishajya Kalpna, Sumandeep Ayurved Medical College and Hospital, Sumandeep Vidyapeeth (Deemed to be University), Waghodia, Vadodara, India.

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***Address for Correspondence**
Dhara Patel
Associate Professor,
Department of Samhita and Sidhanta,
Sumandeep Ayurved Medical College and Hospital,
Sumandeep Vidyapeeth (Deemed to be University),
Waghodia, Vadodara, India.
E.Mail: dhara.patel@sumandeepvidyapeethdu.edu.in

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ABSTRACT

Medical students face significant psychological and intellectual challenges due to the high demands of medical education. These challenges often manifest as stress, burnout, insomnia, depression, and reduced cognitive performance, which can impact both academic success and future clinical competence. This review aims to examine the mental and intellectual health needs of medical students by analysing existing literature and traditional health frameworks, including Ayurvedic principles. A narrative review was conducted using structured searches in PubMed, Google Scholar, and institutional reports. Classical Ayurvedic texts such as Charaka Samhita and the Bhagavad Gita were also reviewed. Thematic synthesis was employed to categorize findings into mental health challenges, intellectual development, stressors, and preventive strategies. High levels of psychological distress, including anxiety (up to 43.7%) and depression (17–25%), are common among medical students. Academic pressure, sleep deprivation, and competitive environments are major contributing factors. Intellectual wellness, often neglected, is essential for cognitive growth, problem-solving, and academic performance. Ayurvedic perspectives emphasize holistic mental well-being through self-discipline, mindfulness, and balance in lifestyle. There is an urgent need to integrate mental and intellectual wellness frameworks into medical education. Promoting self-awareness, stress management, and intellectual development can enhance academic performance and personal well-being among medical students.

Keywords: Medical Students, Psychological and Intellectual Challenges, Medical Education, Ayurvedia, Pubmed, Charaka Samhita, Bhagavad Gita

INTRODUCTION

The World Health Organization defines health as a state of complete physical, mental, and social well-being not merely the absence of disease or infirmity. In traditional Indian medical systems, Acharya Sushruta similarly emphasizes the holistic nature of health, describing a healthy person as one whose physiological systems are balanced, whose mind and senses are content, and whose soul is at peace. Mental health, in modern terms, refers to emotional, psychological, and social well-being. It affects how individuals think, feel, and behave, as well as how they cope with stress, relate to others, and make choices. Intellectual health, while less frequently addressed, is equally vital. It involves maintaining cognitive functioning, engaging in creative pursuits, and continuously developing knowledge and skills for effective decision-making and lifelong learning. Medical students represent a uniquely vulnerable population due to the intense academic, emotional, and professional pressures they face. Research has consistently shown a high prevalence of depression, anxiety, burnout, and sleep disorders among this group. For example, studies have reported that up to 17% of medical students experience moderate to severe depression, while nearly half exhibit signs of academic burnout. Such mental health challenges are further compounded by intellectual fatigue, poor time management, and a lack of structured coping mechanisms. The modern medical curriculum, though rigorous, often neglects the mental and intellectual well-being of students. While institutions may provide academic support, structured programs addressing holistic development including emotional resilience, mindfulness, and cognitive wellness are rare. Additionally, classical Indian philosophical and Ayurvedic teachings offer timeless insights into self-regulation, balanced living, and the cultivation of intellect, which remain underutilized in modern education. This narrative review aims to critically explore the mental and intellectual health needs of medical students, synthesizing data from biomedical literature, educational surveys, and classical Indian knowledge systems. The objective is to provide a comprehensive framework for understanding these challenges and to offer integrative solutions that may inform educational policy and wellness programming.

METHODS

Design and Approach

This study was conducted as a structured narrative review, synthesizing both contemporary literature and classical Indian philosophical texts to examine the mental and intellectual health needs of medical students. While not a systematic review, this narrative approach employed transparent and structured methodology to ensure analytical rigor and thematic coherence.

Sources of Data

A comprehensive search was performed across the following sources:
Electronic Databases: PubMed, Google Scholar, ScienceDirect
Keywords Used: "mental health in medical students", "burnout", "academic stress", "intellectual health", "Ayurveda and mental well-being", "student wellness in medical education"
Institutional Reports: Council of Medical Education, WHO mental health publications
Classical Texts: Selections from Charaka Samhita, Bhagavad Gita, and other authoritative Ayurvedic sources relevant to mental and intellectual well-being
The search included English-language publications from the past 15 years and key classical Sanskrit translations with verified interpretations.

Inclusion Criteria

- Studies involving undergraduate or postgraduate medical students
- Articles or reports discussing psychological stress, burnout, cognitive impairment, or wellness programs in medical education
- Classical Ayurvedic texts discussing mental and intellectual wellness

Exclusion Criteria

- Articles without relevance to either mental or intellectual health
- Studies focusing solely on physical health outcomes without correlation to cognitive or emotional domains

Data Extraction and Synthesis

Relevant literature was reviewed in full and thematically coded into four core domains:

1. Mental health challenges among medical students
2. Intellectual well-being and cognitive development
3. Contributing stressors and risk factors
4. Preventive and integrative solutions, including traditional knowledge

A thematic analysis approach was employed to group findings under conceptually meaningful subheadings, and a synthesis was performed to identify recurring patterns and integrative frameworks.

RESULTS

Thematic analysis of literature and classical sources revealed five major domains relevant to the mental and intellectual health of medical students: (1) psychological morbidity, (2) academic stress and lifestyle patterns, (3) intellectual health and cognitive resilience, (4) traditional perspectives on mental wellness, and (5) existing and proposed interventions.

Psychological Morbidity in Medical Students

A significant body of literature underscores high levels of psychological distress in medical students. The prevalence of depression ranges from 17% to 30% in Indian and international studies, while anxiety disorders affect 28–43% of medical students globally [1–3]. Burnout defined by emotional exhaustion, depersonalization, and reduced personal accomplishment affects up to 49% of medical students by mid-training [4,5]. A 2021 meta-analysis by Puthran *et al*. found that 33.8% of medical students globally experienced depressive symptoms [6]. The risk of suicidal ideation was reported to be significantly higher among medical students than in the general population, with some reports suggesting they are up to 3 times more likely to die by suicide [7]. Sleep disorders are also highly prevalent. Up to 88.6% of students report sleep disturbances, including pre-, mid-, and post-insomnia [8]. Sleep deprivation due to late-night study and continuous academic load contributes to mood disorders and poor cognitive function.

Academic Stress and Contributing Lifestyle Factors

Major stressors identified include:

- Academic overload
- Performance pressure and fear of failure
- Time constraints and poor time management skills
- Sleep deprivation
- Lack of extracurricular or recreational breaks
- Peer competition and lack of faculty support

Studies from Russia and India consistently show that academic stress peaks during exam sessions and clinical rotations [9,10]. One Indian cross-sectional study found that 38% of students reported chronic fatigue, and over 33% slept fewer than six hours per night during exam periods [11].

Substance misuse and **eating disorders** have emerged as maladaptive coping mechanisms in some students, further worsening mental health and disrupting intellectual performance [12].

Intellectual Health and Cognitive Challenges

Intellectual health, though often neglected, is critical to effective learning, problem-solving, and academic success. Poor intellectual wellness manifests as

- Inability to concentrate

- Memory lapses
- Poor academic confidence
- Burnout-related cognitive dulling

Research shows that high levels of stress impair executive functioning and working memory, both essential for mastering complex medical subjects [13,14]. Moreover, the fear of judgment, intense competition, and lack of emotional support can lead to imposter syndrome, adversely impacting cognitive growth. Despite the emphasis on cognitive performance in medical training, structured interventions to enhance intellectual wellness (e.g., curiosity, lifelong learning, meta-cognition) remain largely absent.

Ayurvedic and Philosophical Insights on Mental and Intellectual Health

Classical Indian texts offer profound insights into balanced living, mental purity (sattva), and intellectual discipline (buddhi). In the Charaka Samhita, Acharya Charaka highlights the unity of mind and body

"मनसोज्ञानविज्ञानवैराग्यस्मृतिसमाचरिभिः" – Mental stability, knowledge, wisdom, and memory are integral to health. The Bhagavad Gita also addresses mental resilience through detachment, self-discipline, and self-awareness. The concept of Sattva is seen as the purest mental state one that fosters clarity, wisdom, and emotional stability. Ayurveda emphasizes that imbalances in doshas (Vata, Pitta, Kapha) affect mental faculties. Practices like meditation, pranayama, sattvic diet, and dinacharya (daily routine) are prescribed to maintain psychological equilibrium.

Preventive and Supportive Strategies

Modern evidence and traditional knowledge converge on several strategies for promoting mental and intellectual health

Mindfulness meditation: Reduces cortisol levels and improves attention span [15]
Yoga and pranayama: Improve mood, reduce anxiety, and enhance cognitive flexibility [16]
Time management training: Leads to better academic performance and reduced burnout [17]
Peer and faculty mentoring programs: Improve emotional well-being and academic engagement [18]
Inclusion of wellness modules: Institutions incorporating resilience training and emotional intelligence report improved outcomes [19]

DISCUSSION

This review synthesizes findings from contemporary literature and classical health systems, highlighting the urgent need to address the mental and intellectual well-being of medical students. The evidence demonstrates that the prevalence of psychological distress including depression, anxiety, burnout, and sleep disorders is consistently higher in medical students than in the general population or other academic groups [1,2].

Psychological Distress: An Overlooked Epidemic

The demanding nature of medical education creates a fertile ground for psychological morbidity. In line with the meta-analysis by Rotenstein *et al*., which reported depressive symptoms in over one-third of medical students globally [7], Indian studies have found similar trends, with additional cultural and institutional stressors such as academic hierarchy, parental pressure, and stigmatization of mental illness [4,5]. Furthermore, the risk of suicidal ideation and self-harm behavior is concerning. Studies by Dyrbye *et al*. and others have shown that the suicide risk in medical students is two to three times higher than in the general population [1,20]. This alarming statistic calls for comprehensive institutional mental health policies, early screening, and access to care.

Intellectual Health: The Silent Variable

Intellectual health, encompassing curiosity, analytical thinking, creativity, and self-directed learning, is often overlooked in traditional academic metrics. However, this review finds that cognitive burnout is as critical as emotional burnout. Stress impairs the prefrontal cortex, reducing executive functions such as decision-making,

attention, and working memory [21,22]. A study by Durning *et al*. emphasized that cognitive overload during clinical rotations directly affects medical reasoning and memory retrieval [23]. Moreover, the "hidden curriculum" implicit pressures and norms within medical schools often discourages open intellectual inquiry and creativity, fostering a culture of rote learning and performance anxiety [24].

Bridging Tradition and Modern Mental Health

Ayurvedic concepts offer a holistic framework for balancing mind, intellect, and behavior. The principle of *Sattva* promotes clarity, mindfulness, and equanimity mental qualities shown to buffer against stress-related cognitive impairment [25]. Mindfulness-based interventions rooted in Eastern traditions have been shown to reduce cortisol, improve attention, and mitigate symptoms of anxiety and depression [26,27]. Similarly, the concept of *Buddhi* (intellect) aligns with modern neurocognitive constructs. The cultivation of *Buddhi* through structured routine (*Dinacharya*), mindful discipline (*Yama-Niyama*), and *Swadhyaya* (self-study) enhances metacognition and self-regulation [28,29].

Institutional and Preventive Interventions

Numerous studies highlight the effectiveness of evidence-based interventions to address these challenges:
Mindfulness-based cognitive therapy (MBCT) improves concentration and reduces depression recurrence [30].
Yoga and physical exercise have been associated with better academic performance and emotional balance [31].
Resilience training and **emotional intelligence workshops** are increasingly adopted by forward-thinking medical schools [32,33].
The success of programs like Vanderbilt's "Mind-Body Medicine Elective" and Saint Louis University's "Wellness Curriculum" demonstrates the feasibility and scalability of such interventions [34,35]. Moreover, peer-support groups, faculty mentorship, and stress-reduction modules have all shown positive outcomes in terms of student engagement, retention, and satisfaction [36].

The Need for Policy Integration

Despite overwhelming evidence, wellness remains peripheral in most medical curricula. A review by Slavin *et al*. found that fewer than 20% of medical schools had structured, longitudinal wellness programs [35]. There is a need for

- Mandatory mental health orientation in the first year
- Embedded counseling access without stigma
- Scheduled "wellness breaks" and digital detox hours
- Integration of Ayurvedic mindfulness or lifestyle electives

The National Medical Commission (NMC) in India has the opportunity to lead in this regard by mandating psychological health modules under the Attitude, Ethics and Communication (AETCOM) program [37].

Limitations of This Review

This narrative review, while structured, is limited by its reliance on secondary data. No primary or survey-based data was collected. Although thematic synthesis and diverse source triangulation were used, systematic bias may be present in the selection of articles or interpretations of traditional texts.

CONCLUSION

Mental and intellectual well-being are indispensable components of a medical student's overall health and academic success. The high prevalence of psychological disorders such as depression, anxiety, burnout, and sleep disturbances among medical students globally and in India specifically underscores a systemic failure to prioritize mental wellness in medical education. Equally concerning is the widespread neglect of intellectual health, including cognitive resilience, critical thinking, and sustained curiosity, which are crucial for both personal growth and professional competence. This review has demonstrated that medical students operate in environments fraught with academic

overload, emotional stress, and insufficient psychosocial support. It has also highlighted the underexplored yet powerful potential of traditional health systems especially Ayurvedic concepts like *Sattva* and *Buddhi* in restoring balance and promoting holistic wellness. There is a clear and urgent need to integrate structured mental health screening, cognitive wellness programs, mindfulness practices, and emotional resilience training into the medical curriculum. Policy reforms at institutional and national levels, including incorporation of mental health education under modules like AETCOM, are critical steps toward cultivating a generation of physicians who are not only clinically competent but also emotionally intelligent and intellectually thriving. A healthy medical student is more likely to become a compassionate and capable physician. Ensuring their mental and intellectual well-being is not merely an academic concern it is a public health imperative.

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Table 1. Prevalence of Key Mental Health Issues in Medical Students (Selected Studies)

Mental Health Issue	Reported Prevalence	Source/Study	Country
Depression	17% – 33.8%	Puthran *et al*., 2021 [6]	Global
Anxiety	28% – 43.7%	Zaid *et al*., 2018 [3]	UAE
Burnout	35% – 49%	Ishak *et al*., 2013 [4]	USA
Insomnia/Sleep Deprivation	33% – 88%	Kalmbach *et al*., 2019 [8]	USA/India
Suicidal Ideation	Up to 11%	Rotenstein *et al*., 2016 [7]	Global

Table 2. Ayurvedic Concepts and Their Relevance to Mental and Intellectual Health

Ayurvedic Concept	Description	Modern Interpretation	Relevance
Sattva	Mental purity, clarity, peace	Emotional balance, mindfulness	Promotes resilience and cognition
Buddhi	Intellect, decision-making	Executive function, rational thinking	Essential for learning and judgment
Ahar-Vihar	Diet and lifestyle	Nutritional psychiatry, lifestyle medicine	Supports neurocognitive functioning
Dinacharya	Daily routine	Circadian hygiene, sleep-wake cycle	Reduces fatigue, improves focus
Mano-nigraha	Control over the mind	Cognitive control, emotional regulation	Prevents impulsivity and distraction

REVIEW ARTICLE

A Review of Siddha Medicines for the Management of *Valippu Noi* (Epilepsy)

Madheshwaran .K[1]*, H. Nalini Sofia[2] and H. Vetha Merlin Kumari[3]

[1]PG Scholar, Department of Pothu Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Guindy), Chennai, Tamil Nadu, India.
[2]Associate Professor, Department of Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Guindy), Chennai, Tamil Nadu, India.
[3]Professor and Head, Department of Pothu Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Guindy), Chennai, Tamil Nadu, India.

Received: 23 Jun 2025 Revised: 18 Jul 2025 Accepted: 15 Aug 2025

***Address for Correspondence**
Madheshwaran .K
PG Scholar, Department of Pothu Maruthuvam,
National Institute of Siddha, Tambaram Sanatoruim,
(Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Guindy),
Chennai, Tamil Nadu, India.
E.Mail: madheshwaran100@gmail.com

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ABSTRACT

The Siddha system, one of the oldest streams of Indian medicine, provides a unique and in-depth approach to managing neurological disorders such as epilepsy, referred to in classical texts as Valippu Noi. In contrast to modern treatments that primarily aim to control seizures, Siddha therapies seek to correct the underlying imbalance among the three vital energies Vatham, Pitham, and Kabam. This review was carried out to systematically gather and document Siddha based formulations and therapeutic methods used for Valippu Noi. Data was compiled through the study of classical Siddha texts from various well-established libraries in Tamil Nadu. A total of 59 internal medicines and 14 external therapies were identified, including preparations such as kudineer (decoctions), chooranam (powders), mathirai (tablets), and Ennai (medicated oils). Many of these are attributed to notable Siddhars, reflecting the deep traditional roots of the system. External treatments largely involve medicated oil applications and fomentation techniques aimed at restoring balance in the humors. The review aims to provide a foundation for integrative neurological care and promote further clinical validation of these traditional formulations.

Keywords: Siddha Medicine, Valippu Noi, Epilepsy, Herbal Formulations, Neurological Disorders

INTRODUCTION

The Siddha medical tradition is a time-honoured healing system with deep roots in Tamil culture, developed and enriched by generations of Siddhars. Unlike modern healthcare, which often focuses on diagnosing and treating symptoms, Siddha medicine prioritises disease prevention and maintaining equilibrium among the three bodily humors Vatham, Pitham, and Kabam. It integrates herbal and mineral medicines, dietary practices, external therapies, and lifestyle adjustments to promote long-term health. Among the conditions discussed in classical Siddha literature, Valippu Noi, closely resembling epilepsy, is considered a significant neurological disorder. Terms such as Isivu, Iluppu, and Muyal Vali are used to describe seizure-like events, often linked to an imbalance in Kabam, usually along with disturbances in Vatham and Pitham(1). Factors such as stress, hereditary tendencies, prolonged illness, and fatigue are believed to contribute to the condition. Common symptoms described include sudden loss of consciousness, limb convulsions, foaming at the mouth, and facial distortions (2). In modern neurology, epilepsy is understood as a chronic brain disorder marked by recurrent seizures due to abnormal electrical activity. Causes may vary from genetic and metabolic issues to head trauma or infections. While Siddha and modern approaches differ in theory and practice, Siddha medicine offers comprehensive care by strengthening the nervous system and addressing root imbalances. However, many Siddha remedies for epilepsy remain unexplored in scientific literature. This study aims to compile and classify such formulations for future research and potential therapeutic application.

MATERIALS AND METHODS

A detailed literature search for the formulation of valippu noi was done in the library of the National Institute of Siddha, Library of Govt. Siddha Medical College, Palayamkottai, Government Siddha Medical College, Chennai, Siddha Central Research Institute Library, Chennai, The Connemara Public Library, Chennai, The Anna Centenary Library, Chennai, and Saraswathi Mahal Library, Thanjavur. In these libraries the keywords used to collect the formulations are Ilupu, Isivu, Valippu Noi, vali, and muyal vali. In that formulations were collected and analysed with the help of MS Office.

RESULTS

The study results showed that there are 32 Siddha literature evidences dealing with valippu noi. Mostly these books were written by Agasthiyar in his Agasthiyar Vaithiya Rathina Churukkam, Agasthiyar 2000, and Agasthiyar Vaithiya Pillai Tamil. From our documentation, 59 internal medicines were classified under various forms, such as kudineer – 4, chooranam – 1, kirutham – 2, rasayanam – 1, ilagam – 2, ennai – 11, maathirai – 10, mezhugu – 4, pathangam – 1, chenduram – 10, parpam – 7, kattu – 2, chunam – 1, karuppu – 2, and karpam – 1. The external medicines are 14 and were classified under the form, such as thailam – 9, anjanam – 1, nasiyam – 2, pugai – 1, and kallikam – 1. The formulation of internal and external medicines for Valippu Noi is detailed in Table 1 & 2.

DISCUSSION

This review comprehensively analyzed Siddha medical formulations traditionally used in the management of Valippu Noi (epilepsy). The vast array of internal and external medicines compiled from classical Siddha literature highlights the depth of traditional knowledge in treating neurological disorders, particularly seizure-related conditions. The classical classification of Valippu Noi into different types like Kakkai vali, Muyal vali, and Muppini vali aligns with modern classifications of epilepsy such as generalized, reflex, and partial seizures. This correspondence provides a useful framework for exploring integrative approaches to epilepsy care. A total of 59 internal formulations and 14 external therapeutic options were identified, spanning various dosage forms such as kudineer (decoction), mathirai (tablet), mezhugu (wax-based), ennnai (medicated oil), chooranam (powder), pathangam, chenduram, parpam, and karpam. The multi-herbal and mineral compositions in these preparations

reflect a sophisticated system of formulation that balances the three vital humors—Vatham, Pitham, and Kabam. This tridoshic approach is rooted in the Siddha principle that neurological disturbances often stem from Kabam derangement, compounded by Vatha-Pitha imbalance. Notably, several herbal ingredients used across these formulations—such as Bacopa monnieri (Brahmi), *Withania somnifera* (Amukkura), *Centella asiatica* (Vallarai), and *Acorus calamus* (Vasambu)—are pharmacologically validated for their anticonvulsant, neuroprotective, and anxiolytic properties. These plants exhibit multiple CNS activities including modulation of GABAergic, serotonergic, and cholinergic systems, supporting their inclusion in antiepileptic regimens. Studies cited in this review demonstrate antioxidant, adaptogenic, and nerve-stabilizing effects that could reduce neuronal excitability and seizure frequency. The inclusion of formulations containing minerals and metals (e.g., Parpam, Chenduram, Karuppu, Kattu) reflects Siddha's unique Rasa pharmacology. However, these metallic preparations warrant careful toxicological evaluation and standardization to ensure safety, especially when transitioning into clinical research and integrative healthcare models. The external therapies—especially medicated oils like Kasthuri ennai, Maantha ennai, Kalingathi ennai, and nasal applications (Nasiyam)—serve dual purposes. They not only address localized symptoms but are believed to assist systemic balance via olfactory and transdermal absorption. Pugai (fumigation) and Anjanam (collyrium) offer additional therapeutic avenues for reflex epilepsy and behavioral manifestations. Despite the extensive documentation of these formulations, there is a notable lack of systematic pharmacological studies and clinical trials validating their efficacy and safety. Most existing evidence is anecdotal or derived from traditional practice. Bridging this gap requires multidisciplinary collaboration involving pharmacognosy, phytochemistry, toxicology, and clinical pharmacology. Another challenge is the standardization of raw materials, preparation methods, and dosage forms. Variability in these aspects can affect therapeutic outcomes and reproducibility. Adopting PLIM (Pharmacopoeial Laboratory for Indian Medicine) standards for quality control and Good Manufacturing Practices (GMP) can greatly enhance the reliability and acceptability of Siddha formulations in contemporary settings. Furthermore, the integration of traditional diagnostic concepts with modern clinical assessment could provide more individualized and holistic treatment protocols. Understanding patient constitution (Udaliyal), humor imbalance (Uyir Thathukkal), and seasonal variations (Kaala Maruthuvam) remains essential in tailoring Siddha-based epilepsy management.

CONCLUSION

This review highlights the therapeutic richness and diversity of Siddha medical formulations in managing Valippu Noi (epilepsy). Drawing upon classical texts and categorizing numerous internal and external preparations, the study reaffirms the historical depth of Siddha knowledge in neurological care. Many of the herbal ingredients have demonstrated pharmacological actions such as anticonvulsant, neuroprotective, and antioxidant effects. Additionally, the Siddha system's tridosha-based pathophysiology aligns meaningfully with modern understandings of seizure subtypes. However, the absence of standardized protocols and rigorous clinical trials remains a barrier to mainstream integration. There is an urgent need for further scientific exploration including preclinical, toxicological, and human studies to evaluate efficacy and safety. By bridging the traditional formulations with modern research methodologies, Siddha medicine can evolve as a complementary approach in epilepsy care. The documentation presented here serves as a foundation for advancing that integration.

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Ethical Approval

As the present study is a Literature review, it does not involve the use of human or animal subjects and therefore, ethical clearance is not applicable.

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Conflict of Interest
The author(s) declared no potential conflicts of interest with respect to the research,

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Table 1: Formulations of Internal Medicines For *Valippu Noi* (Epilepsy)

INTERNAL MEDICINE	PREPARATORY MEDICINE	INDICATION
Kudineer	*Vallipukukudineer[5]*	Valippu noi (Epilepsy)
	Kunmam, vaanthi, valippu, vaaineerooralukukudineer[5]	*Vaanthi* (Vomiting), *Kumatal* (Nausea), *Valippu noi* (Epilepsy) *Gunmam* (Gastric ulcer),*Vaai neerooral*(Hypersalivation)
	Muyal valippu kudineer[5]	*Muyal valippu* (Reflex epilepsy)
	Sethuma sura kudineer[3]	*Kaasam*(Cold),*Erumal*(Cough), *Suram*(Fever), *Valippu noi* (Epilepsy), *Kuralkammal* (Hoarseness of voice)
Chooranam	*Kakkai valippu neengachooranam*[24]	*Kakkai valippu* (Grandmal epilepsy)
Kirutham	*Maha mega kirutham*[9]	*Kalladaipu*(Renalcalculi) *Muyal valippu* (Reflex epilepsy), *Perumbadu* (Menorrhagia), *Vellai noi* (Leucorrhoea), *Neerkiricharam*(Urinary tract infection), *Kshayam*(Tuberculosis), *Erumal* (Cough)
	Kandangathiri kirutham[7]	*Muppini vali* (Partial adversiveseizure),*Gunmam*(Gastric ulcer)
Rasayanam	*Chithiramoolarasayanam*[15]	*Paandu* (Anaemia), *Kuttam* (Leprosy), *Valippu noi* (Epilepsy), *Meganoi* (Syphilis), *Gunmam*(Gastric ulcer)

Ilagam	*Kandangathiri legiyam*[3]	*Gunmam*(Gastric ulcer), *Valippu noi* (Epilepsy), *Peenisam*(Sinusitis), *Megavettai*(Gonorrhea)
	Mulli muti legiyam[7]	*Kudarpuzhu*(Worm infestation), *Valippu noi* (Epilepsy)
Ennai	*Neikottaanpalathennai*[12]	*Muppini vali* (Partial adversive seizure)
	Kasthuri ennai[12]	*Valippu noi* (Epilepsy), *Karapan* (Eczema), *Iraippu*(Asthma)
	Kaalakini thailam[12]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Maanthaennai[3]	*Valippu noi* (Epilepsy), *Seriyamai* (Dyspepsia)
	Viluthiennai[3]	*Valippu noi* (Epilepsy), *Kudarpuzhu* (Worm infestation)
	Kalingathiennai[3]	*Valippu noi* (Epilepsy), *Gunmam*(Gastric ulcer), *Karapadhasoolai* (Peripheral neuritis)
	Anjennaithailam[15]	*Valippu noi* (Epilepsy), *Neerandam* (Hydrocele), *Sanni* (Delirium)
	Thalagathailam[16]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Sagalavalippukusudarthailam[10]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Nimba ennai[10]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Aegamulithailam[25]	*Kuttam* (Leprosy), *Meganoi* (Syphilis), *Megavettai* (Gonorrhea), *Muyal vali* (Reflex epilepsy), *Perumbadu* (Menorrhagia), *Peenisam* (Sinusitis), *Sanni* (Delirium), *Kandamalai* (Cervical lymphadenopathy), *Madhumegam* (Diabetes mellitus)
Maathirai	*Vishnu chakaramathirai*[6]	*Sanni* (Delirium), *Valippu noi* (Epilepsy), *Vikkal*(Hiccups)
	Karrupuvishnuchakaramathirai[8]	*Valippu noi* (Epilepsy), *Neerandam* (Hydrocele), *Gunmam* (Gastric ulcer), *Pakkavadham* (Hemiplegia), *Keelvayu* (Arthritis), *Suram* (Fever)
	Yogarasamaathirai[6]	*Kaasam* (Cold), *Erumal*(Cough), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Gunmam* (Gastric ulcer), *Kshayam* (Tuberculosis)
	Suriya prabaimaathirai[6]	*Sanni* (Delirium), *Valippu noi* (Epilepsy), *Uppusam*(Bloating), *Suram* (Fever), *Seriyamai*(Dyspepsia)
	Korosanaimaathirai[3]	*Valippu noi* (Epilepsy)
	Swarna chinthamanimaathirai[12]	Kshayam (Tuberculosis), *Erumal* (Cough), *Valippu noi* (Epilepsy),*Meganoi*(Syphilis)
	Panchikarnamaathirai[15]	*Vaanthi* (Vomiting), *Kuralkammal* (Hoarseness of voice), *Gunmam* (Gastric ulcer), *Kamalai* (Jaundice), *Peruvayiru* (Ascites), *Valippu noi* (Epilepsy), *Kshyam*(Tuberculosis)
	Maanthathoosakuligai[15]	*Seriyamai* (Dyspepsia), *Valippu noi* (Epilepsy), *Suram* (Fever)
	Navaspooramaathirai[19]	*Seriyamai*(Dyspepsia), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Suram* (Fever)
	Lagu Ayari maathirai[21]	*Keelvayu*(Arthritis), *Kakkai vali* (Grandmal epilepsy)
Mezhugu	*Veera mezhugu*[26]	*Valippu noi* (Epilepsy), *Karappan*(Eczema), *Araiyappu*(Lymphadenopathy), *Sirangu* (Scabies), *Mugavadham*(Facial palsy)
	Uruthiravayumezhugu[22]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Kanaga linga mezhugu[19]	*Meganoi* (Syphilis), *Megavettai* (Gonorrhea), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Valiazhalkeelvayu* (Rheumatoid arthritis)
	Laguvanamamezhugu[32]	*Suram* (Fever), *Kshyam*(Tuberculosis), *Kuttam*(Leprosy), *Gunmam* (Gastric ulcer), *Valippu noi* (Epilepsy)
Pathangam	*Kommatikaaipathagam*[9]	*Valippu noi* (Epilepsy), *Valippu noi* (Rheumatoid arthritis)
Chenduram	*Lagu kaadikarachenduram*[12]	*Sanni* (Delirium), *Valippu noi* (Epilepsy), *Kazhichal*(Diarrhea), *Suram* (Fever)
	Chandamarutha chenduram[12]	*Muyal vali* (Reflex epilepsy)

	Satrasa chenduram[12]	*Suram* (Fever), *Kshayam*(Tuberculosis), *Peruvayiru*(Ascites), *Kuttam* (Leprosy), *Neerandam* (Hydrocele), *Araiyappu* (Lymphadenopathy), *Valippu noi* (Epilepsy), *Manneralveekam* (Spleenomegaly), *Powthiram* (Fistula)
	Vangarasanjeevi chenduram[13]	*Muyal vali* (Reflex epilepsy), *Meganoi* (Syphilis), *Kamalai* (Jaundice)
	Santhirothayam chenduram[13]	*Venkuttam* (Leukoderma), *Powthiram* (Fistula), *Gunmam*(Gastric ulcer), *Kshyam* (Tuberculosis), *Iya vali* (Febrile fits), *Paandu* (Anaemia), *Suram* (Fever), *Kaasam* (Cold)
	Vatharatchasan chenduram[13]	*Kshayam* (Tuberculosis), *Iraippu* (Asthma), *Muyal vali* (Reflex epilepsy), *Suram* (Fever), *Valippu noi* (Epilepsy)
	Linga chenduram[27]	*Suram* (Fever), *Valippu noi* (Epilepsy)
	Raguramapana chenduram[19]	*Suram* (Fever), *Valippu noi* (Epilepsy), *Seriyamai*(Dyspepsia), *Kazhichal*(Diarrhea)
	Panja paadana chenduram[32]	*Suram* (Fever), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Meganoi*(Syphilis), *Gunmam* (Gastric ulcer), *Kuttam* (Leprosy), *Venkuttam*(Leukoderma), *Powthiram* (Fistula), *Araiyappu*(Lymphadenopathy), *Neerandam* (Hydrocele), *Karapan* (Eczema)
	Lingam chendurum[23]	*Muyal vali* (Reflex epilepsy), *Muppini vali* (Partial adverse seizure), *Kakkai vali* (Grandmal epilepsy)
Parpam	*Udumburasa parpam*[20]	*Pakkavadham* (Hemiplegia), *Mugavadham* (Facial palsy), *Valippu noi* (Epilepsy), *Kakkai vali* (Grandmal epilepsy), *Gunmam* (Gastric ulcer)
	Rasa parapam[7]	*Valippu noi* (Epilepsy), *Gunmam* (Gastric ulcer), *Suram* (Fever)
	Thanga parpam[3]	*Valippu noi* (Epilepsy), *Moolam* (Hemorrhoids), *Keelvayu*(Arthritis)
	Aarupuseiyanerparpam[18]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Asta karporaparpam[19]	*Suram* (Fever), *Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Veera parpam[19]	*Valippu noi* (Epilepsy)
	Velvanga parpam[17]	*Kakkai vali* (Grandmal epilepsy), *Kandamalai*(Cervical lymphadenopathy)
Kattu	*Paasanakattu*[28]	*Kakkai vali* (Grandmal epilepsy)
	Saathilingakattu[15]	*Muyal vali* (Reflex epilepsy), *Puppini vali* (Partial adverse seizure), *Kakkai vali* (Grandmal epilepsy), *Saithiya noi* (Psychosis)
Chunam	*Sandarasachundam*[20]	*Neerandam* (Hydrocele), *Valippu noi* (Epilepsy), *Suram* (Fever), *Seriyamai*(Dyspepsia), *Gunmam*(Gastric ulcer)
Karrupu	*Periya pattu karrupu*[29]	*Suram* (Fever), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Padhavanmeegam*(Elephantiasis), *Meganoi*(Syphilis)
	China pattu karrupu[29]	*Suram* (Fever), *Sanni* (Delirium), *Valippu noi* (Epilepsy), *Paadhavanmeegam*(Elephantiasis), *Meganoi*(Syphilis)
Karpam	*Vembukarpam*[16]	*Valippu noi* (Epilepsy), *Vali azhalkeelvayu* (Rheumatoid arthritis), *Sanni* (Delirium)

Table 2: Formulations of External Medicines For *Valippu Noi* (Epilepsy)

EXTERNAL MEDICINES	PREPARATORY MEDICINE	INDICATION
Ennai	*Kaduguennai*[15]	*Saithiya noi* (Psychosis), *Valippu noi* (Epilepsy)
	Alinjil thailam[25]	*Valippu noi* (Epilepsy)
	Sambirani thailam[25]	*Sanni* (Delirium), *Valippu noi* (Epilepsy)
	Sannikuthailam[30]	*Sanni* (Delirium), *Valippu noi* (Epilepsy), *Mugavadham*(Facial palsy)
	Poonagathailam[5]	*Kakkai vali* (Grandmal epilepsy)
	Aathirathithailam[5]	*Vali azhalkeelvayu* (Rheumatoid arthritis), *Valippu noi* (Epilepsy), *Irappu* (Asthma)
	Kakkaivalithailam[11]	*Kakkai vali* (Grandmal epilepsy)
	13 sannikuthailam[3]	*Valippu noi* (Epilepsy)
	Iyennaithailam[9]	*Mugavadham* (Facial palsy), *Valippu noi* (Epilepsy), *Sanni* (Delirium)
Anjanam	*Kuthiraivalikkuanjanam*[14]	*Valippu* (Epilepsy)
Nasiyam	*Vallipukunasiyam*[31]	*Saithiya noi* (Psychosis), *Muyal vali* (Reflex epilepsy)
	Soothagasani,vallipukkunasiyam[4]	*Muyal vali* (Reflex epilepsy)
Pugai	*Janni pugai*[6]	*Kakkai vali* (Grandmal epilepsy)
Kalikkam	*Sarvaanga kallikam*[16]	*Saithiya noi* (Psychosis), *Valippu* (Epilepsy)

Table 3: Commonly Used Plants In Management Of *Valippu Noi* (Epilepsy)

S.No	Plant Name	Botanical Name	Actions	Activity
1	Brahmi	*Bacopa monnieri*	Nervine tonic, diuretic, laxative	Antiepileptic, anti-convulsant [33]
2	Amukkura	*Withaniasomnifera*	Tonic, soporific, diuretic, sedative	Antiepileptic, neuroprotective[34]
3	Vallarai	*Centella asiatica*	Nervine tonic	Anticonvulsant, neuroprotective [35]
4	Vasambu	*Acorus calamus*	Nervine sedative,	Anticonvulsant [36]
5	Karunjchirakam	*Nigella sativa*	Neuroprotective, antioxidant	Antiepileptic [37]
6	Ginger	*Zingiber officinale*	Anti-inflammatory, antioxidant	Anticonvulsant [38]

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RESEARCH ARTICLE

An Eclectic Survey on Current Technical Innovations in Malware Analysis and Classification

S.Divya*

Research Scholar (Register No : Ph.D- FT- 2123115), Department of Computer Science and Engineering, Indian Institute of Information Technology, Ministry of Education, Government of India, Sethurapatti, Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
S.Divya
Research Scholar (Register No : Ph.D- FT- 2123115),
Department of Computer Science and Engineering,
Indian Institute of Information Technology,
Ministry of Education,
Government of India, Sethurapatti,
Tiruchirappalli, Tamil Nadu, India.
E.Mail: divyakrish5798@gmail.com

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ABSTRACT

Malicious software often known as invader malware, is metamorphic and polymorphic by its nature, suggesting that it may modify the program as it escalates. Malicious programs that steal users' personal information, send data to elicit deductions, utilize a surge in privilege to manage the control, and so on, inflict severe harm on end users. Furthermore, the diversity in the number of their variations significantly impairs the efficiency of existing defenses, which mainly utilize signature-based approaches and are unable to identify previously discovered malicious executables. Malware group variations have common behavioral traits that identify their source and intent. Malware detection is critical to computer security. For extracting harmful traits, modern machine learning algorithms have been based on domain expertise. Furthermore, many possible traits can be employed, and manually identifying the optimal attributes is time-consuming and complex, especially considering the various nature of malware. Due to inadequate security design as well as the unique properties of IoT devices, such as processor architectural heterogeneity, IoT malware detection has quite complexities, particularly in identifying cross-architecture IoT malware. As a result, in recent years, the security community has focused its research on this issue of IoT malware detection. This study provides an outline of malware detection techniques, the types of malware, and the advantages of malware analysis. The systematic review will provide a thorough comprehensive index of strategies to assist in the conflict over malicious information.

Keywords: Malware analysis and detection, IoT malware, Android system, Machine Learning, Hypervisor

INTRODUCTION

Malware is an unauthorized, harmful initiative intended to cause chaos for the user and the system it is installed on. Viruses, Trojans, backdoors, spyware, crypto-lockers, and ransomware are examples to name a few. Malware assaults have risen substantially in recent years, demonstrating that many businesses, from huge multinational firms with high-security budgets to low businesses with individuals, are unable to safeguard themselves from the attackers adequately [12]. Android is an open-source mobile OS software that runs on a wide range of devices. According to Google, 1.2 million Android devices are enabled every day. As reported by Cisco [1], 98 percent of malware is targeted at Android users. In general, the Android OS is vulnerable to security flaws or threats. Spyware, Trojans and phishing apps are among the malware types putting the user's data and device in danger. The Internet of Things (IoT) [23] is a technology in which a collection of interconnected devices exchange data via a wireless connection without the need for human involvement. Monitoring device actions [5] to develop behavioral fingerprints or profiles is one method that has gained attraction in detecting malware-infected devices. Machine Learning (ML) [29] is the earliest and most plausible solution to the malware categorization challenge, and it has been used to solve various attacks. A classifier model is built using pattern recognition to understand the malware behavior and propagation techniques. ML has traditionally been used in anomaly-based detection approaches in which the training and detecting or testing phases [19] are the most important steps to detect malicious behavior. Cyber security [38] is critical in defending against the attacks that arise in secure computing. Virtual Machine Introspection (VMI) technique [37] is another way to solve cyber security problems. In Virtual Machine Monitor (VMM) the security firewalls are paralyzed to get the key information of the host running machine, the semantic perspective of an OS in the host virtual machine could be assembled using low-tier contemplation semantics. Malware detection procedures using VM are illustrated in Fig.1. Cloud Computing [10] Cyber security researchers deal with attacks that exploit legitimate cloud services in attempts to access sensitive data. Cloud malware attacks cloud computing-based systems with malicious code and service.

Preliminaries

Introduction to Malware

Malware (also known as malicious software) is software that is meant to harm users, businesses, computers, and communications systems. Malware can disable internet access, harm an operating system, steal a user's password, and other personal information, and encrypt vital documents on a computer before demanding a ransom. Most antivirus engines detect and classify malware by scanning files regularly and matching their signatures to those of known malware. Human antivirus professionals (also known as malware defenders) analyze the collected malware samples to produce malware signatures. Malware signatures [18] might be filenames, text strings, or byte code regular expressions. Signature-based approaches, on the other hand, can only detect traditional malware that doesn't differ much. Categories of malware are classified into three types, as indicated in Fig.2.In a piece of fiction, malware plays a crucial part. The fiction could be in the form of audio, video, game, or something else entirely; malware, like viruses, plays a vital role. In fiction, the hackers find several infections some of which are described in Table 1.

Malware analysis is usually classified into one of three categories [11].

- Static Malware Analysis
- Dynamic Malware Analysis
- Hybrid Malware Analysis

Malware Types

Malware encompasses a wide range of harmful software including viruses, worms, rootkits, Trojan horses, backdoors, botnets, spyware, adware, and ransomware. Malware versions are categorized depending on certain characteristics such as propagation mechanisms, infection types, and so on. Table 2. Depicts the types of malware.

Patterns of Malware attacks

Despite harmful software may be grouped into numerous categories, malicious software is difficult to review. The following are examples of generic malware attacks [18]

- Infiltration of currently active operations.
- Mutates are created as well as acquired.
- Monitoring the stability of the network.
- System services can be started and stopped.
- Re-modelling of the executables.
- Re-modelling of the root path.

Android Malware Detection Analysis Techniques

Android malware is malicious software that attacks mobiles and wireless-enabled devices which results in system crashing and private details being destroyed or exposed. According to the survey, Android malware detection analysis methodologies are classified into three types: static analysis, dynamic analysis, and hybrid analysis. Numerous sorts of Android Malware Detection Techniques are shown in Fig 3. In malware threats, the major purpose of static analysis is to investigate permissions, its application's software, components, resources, and signatures. The APK file contains all the data about the application. The APK file is used for static analysis. The permissions, resources, code, services, and other data are taken and evaluated properly from the APK file. For static analysis [1], there are several tools available including jd-gui, aapt, apktool and dex2jar.

Classification of Malware by Malicious Behaviors

Malware uses certain characteristics to carry out destructive operations, but it also uses other behaviors to evade detection or hide its presence. Focusing on malware behavior rather than malware kind is more significant for analytical reasons. It should be highlighted that after studying binary code and knowing about its behavior, it is much simpler to categorize it into the appropriate malware category and describes the behavior of malware [12].

Study on Static and Dynamic Malware Analysis

Malware analysis involves the study of determining how a suspicious file or a URL behaves and what its aim is. The analysis output aids in detecting and mitigating the possible threat. In this section, a full comparison of different malware analyses is presented from the perspectives of IoT Malware, Android System, Machine Learning, and Hypervisor. Evanson *et al*. [24] proposed an employing technique of Haralick picture texture characteristics with ML classifiers such as Random Forest (RF), Naive Bayes (NB), and K-Nearest Neighbor (KNN) to analyze and identify IoT malware. Code compression, Code encryption, IP identification, User interaction, Data identification, Environmental awareness, Code obfuscation, and timing are the popular IoT malware evasion strategies [23]. With a focus on IoT malware detection, Abawajy *et al*. [8] mentioned the feature selection issue like a quadratic programming challenge and then investigated the effectiveness of frequently adopted filter-based datasets. Rey *et al*. [5] discuss the opportunities that federated learning opens up in terms of IoT malware detection, as well as the security challenges that come with the proposed solution. To ensure model authenticity and quality, Yulei *et al*. [22] propose a unique user Local Neural Network (LNN) that enables local access and a smart sensing signed agreement that allows integration through the blockchain network. Sercan *et al*. [35] explain how to categorize Android malware samples into groups. Prerna *et al*. [2] proposed a generic malware detection technique. The proposed method [31] elaborates on the difficulties that can be encountered while using existing tools and techniques. As a result, a robust obfuscation-resistant malware detector is required. Qing *et al*. [42] discuss the Android app composition, analyses the source of static features, and present an analysis of Android malware static detection. Yue Liu *et al*. [21] provide a thorough examination of the use of Deep Learning (DL) in Android malware investigation. The research also looks at the state of DL-based Android malware countermeasures.

Machine Learning

ML is used to conduct a statistical examination of detection procedures. Malware detection strategies [32] can be classified in a variety of ways based on mobile malware-based techniques, classical, ML-based approaches, deep

learning-based techniques, and PCs. Daniele *et al*. [4] explain how ML works on malware research using Windows systems, specifically for the study of Portable Executables and their goals. Neil *et al*. [15] claimed that the experiment findings showed that the strings feature set had attained a high level of accuracy for this dataset and that these detectors execution times looked tractable making them appropriate for application in large-scale malware analyzers. This research reviews on ML-based android malware detection algorithms [19]. Welsh's t-test [20] is used to determine the uniqueness of spectrograms and DWT coefficients from diverse malware. Preeti *et al*. [3] introduce VMShield, a contemplation stateful firewall for safeguarding virtual domains within cloud-based portals that aim to detect malware in cloud architecture. The suggested AMD framework [10] functionality has been tested in the cloud with the most recent malware datasets. Finally, this examines the proposed framework's ability to meet VM security criteria.

IoT Malware

DEAM or Depthwise Efficient Attention Module [36] is a novel as well as simple Convolution Neural Network (CNN) focus module that is introduced and coupled including a DenseNet to create an innovative malware detection and family heuristic approach. Nazar *et al*. [13] propose a methodology for aggregating numerous platform-independent models with non-overlapping statistics combined into a single model continuously. As devices work on well before network infrastructure [33], such as the Mirai Malware Attack, the scope for cyber-attacks on IoT Infrastructure was immense. A novel automation algorithm-based technique [25] for identifying Mirai is presented and performed. For reliable and rapid malware detection, Zhaoqi *et al*. [27] proposed a unique and low-cost technique to extract features, as well as an efficient deep neural network architecture.

Android System

To identify malware from trusted programs a unique detection method was suggested. The proposed work interpreted each sequence like a phrase in the syntax and then built a signaling on the Long Short-Term Memory (LSTM) classifier [16] asserting that system call patterns like high-level languages, include semantics. Following that, two similarity scores were produced using these models. DroidEvolver[7] seems to be an Android malware detection strategy which can update itself instantly and persistently while identifying malware without the need for human interaction. This paper proposed a quick malware detection system for Android combining a variety of features: FAMD is an acronym for Fast Android Malware Detector [9]. Yucming *et al*. [28] reveal how contrastive learning was used to enable obfuscation-resistant malware analysis. PermPair[26] a novel detection approach was developed to produce and analyze networks across malicious as well as regular extracts by retrieving access matches out of a user's folder.

Machine Learning

Malware detection relies on a combination of Changed Registry Keys, Summary Information, APIs and DLLs. Cuckoo sandbox [11] is used for dynamic attack detection and provides high accuracy. This research presents a dynamic malware detection method that uses memory investigations to retrieve destructive artifacts in memory and integrate those using data acquired throughout malware processing. Pre-modeling methods [40] are employed for classifiers before the validation set which consists of the data set on ML algorithms. Zhixin *et al*. [6] use ML to investigate a promising strategy for hardware-assisted malware detection. The most serious threat to Cyber-Physical Systems (CPS) is being infected with malware that turns them into botnets. Following botnets are the two new types of malware that have been identified: DoS and advanced persistent threats. The utmost malware strikes within those modules, the most enticing processes for detecting attacks in cyber-physical frameworks or the prospective cables of investigations only with maximum effect about the near future [17]. This evaluates several reflective posts and summarizes the results acquired, as well as the most familiar cyberattacks in these modules are classified. Matthew *et al*. [30] collected information across multiple permission levels within another operating system to better identify possible variations.

Hypervisor

This study suggested retaining a list of the features in storage access patterns at the hardware level by applying window-based trait computation [14]. This research paper proposes a new dataset for cloud IDS implementation based on VM Introspected data. Using the Virtual Machine Introspection (VMI) approach [38], the dataset was produced on virtualization by combining malware behavior with benign trial activation artifacts. Jian *et al*. [37] propose a new malware detection approach in order to increase virtual machine privacy and ensure the overall security infrastructure Using Memory Forensics Analysis (MFA) and Virtual Machine Introspection (VMI) technologies, this study extracts a variety of data from virtual machine core, the hypervisor layer and the hardware overlay. Leon *et al*. [39]introduce a method for analyzing malware within the context of the operating system.

Literature Review on Hybrid Malware Analysis

Roopak *et al*. [34] suggested a novel hybrid malware detection method that relies on Tree Augmented Naive Bayes (TAN) that exploits implicit interrelations between crucial static and dynamic characteristics these were essential by a platform's execution. To ensure the effectiveness and productivity for large-scale Android malware detection, the research work presents a hybrid approach based on CNN and Deep Auto-Encoder (DAE) [41].
Comparative Analysis on Static and Dynamic Analysis

Comparative Investigation for Static and Dynamic Analysis

Detailed Comparative Investigation for Static Analysis on IoT, Android, Machine Learning and Hypervisor are tabulated in Table 3, Table 4, Table 5 and Table 6 respectively. Similarly, Detailed Comparative Investigation for Dynamic Analysis on IoT, Android, Machine Learning and Hypervisor are tabulated in Table 7, Table 8, Table 9 and Table 10 respectively.

Reverse Engineering

Reverse engineering is a process of analyzing a malicious code to determine its structure components, elements, and behavior. The advantage of reverse engineering is that it enables one to observe the hidden behavior of the file under analysis, which is unable to be done by simply running it. In static malware analysis, commencing analysis of any given problem by identifying the characteristics related to it and analyzing the behavior from those features without directly involving the analysis in execution. The benefit of static analysis is that, without actually executing it, it provides us with a clear understanding of how it will impact the environment. Static analysis of malware can be aided by a variety of tools, including decompilers, disassemblers, source code analyzers, and other tools that support extracting vital features from malicious code. It is not required to have a controlled environment to do static analysis on malware. Static analysis examines the malware code to nearly predict its characteristics and features. To mitigate static analysis, the malware author applied methods like binary obfuscation and packing. In a dynamic analysis, the malware's complete activity and impact on the environment are monitored as it runs. Debuggers, registry monitors, file system monitors, network sniffers, and API call tracers are tools that contribute to the dynamic analysis of malware. The code that needs to be executed in the case of dynamic analysis is corrosive and uncertain. The reversing environment must be isolated from the other hosts on the network. Due to the strong separation between the guest operating system in a virtual machine and the host operating system, even if the virtual machine is infected with malware, it has no impact on the host operating system while reverse engineering is worked out in a VM.

CONCLUSION

This study examined core characteristics/parameters and several malware analysis methodologies. The proposed work makes three major contributions. First, malware analysis preliminaries are suggested, which comprise malware and its classifications, malware attack forms, significance, and issues related to malware categorization and composition analysis. Second, a Literature review on static and dynamic malware analysis from the perspectives of the Android system, Machine Learning, the Internet of Things, and the Hypervisor. Third, a comparative evaluation is conducted for current malware classification and composition analysis methodologies, arranging them according

to the suggested taxonomy. This work highlighted the advantages of these findings based on the mechanism, detection study, and processing time, which can be employed to enhance future work efficiency.

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Table 1: Malware by Fiction

Sl. No.	Name of the Malware	Function
1.	Digimon Fusion	A bunch of kids is transported to another digital domain and given the duty of saving the digital society from wickedness
2.	Digimon Data Squad	
3.	Digimon D-Cyber	
4.	Digimon Adventure	
5.	Digimon Adventure 02	
6.	Digimon Adventure tri	

Table 2: Types of Malware

Type	Function	Example
Ransomware	Until the ransom is paid, the victim's access to data is disabled	RYUK
Spyware	without the users' knowledge, gathers information on user activities	DarkHotel
Adware	promotes intrusive ads	Fireball
Trojans	conceals itself to be desired code	Emotet
Rootkits	allows hackers to take control of a victim's device remotely	Zacinlo
Bots	unleashes a barrage of attacks	Echobot
Keyloggers	tracks the keystrokes of individuals	Olympic vision

Table 3: Static Malware Analysis on IoT

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[8]	To assess and classify malware in IoT, Texture features and conventional machine learning classifiers are used.	For IoT malware, the roc curve and PR analysis are used tocritique the Haralick texture features for each picture	Haralick images and Machine Learning Classifiers	Open Data Institute node-Trento	VirusTotal	95\% accuracy	Haralick features have been used to evaluate trained models
2	[23]	Beaucoup strategies are used to mitigate a black hole assault in IoT malware detection	Malware was separated into fundamental structure, low-level activity, and high-level behavior	Trust-based scheme and HCP	AMPds Datasets	Not Mentioned	Not Mentioned	Various malware evasive techniques were discussed
3	[22]	To detect IoT malware a quadratic programming problem is solved using a filter-based	The characteristic ensemble selection Approaches are presented employing filter-based features extraction	Filter-based feature selection methods	AndroZoo and VirusShare	WEKA 3.8 and VirusTotal	More than 95 %	The best selections of features are used to minimize the space and time complexity of classification

		feature selection algorithm	approaches					
4	[5]	A framework is presented to employ federated learning to detect malware that affects IoT devices	N-BaIoTFederated Learning methodology is used in the proposed technique.	MINIBATCH AGGREGATION and MULTI-EPOCH AGGREGATION	N-BaIoT datasets	VirusTotal	97 % Accuracy	Following the federated approach, it had been proved that data privacy was ensured without sacrificing model performance
5	[35]	To aggregate many user-trained networks using non-overlapping into one model, a blockchain-based aggregation approach is utilized	Decentralized Learning using Blockchian is used in the proposed IoT multi- feature deep learning model	Compose deep learning scores of the public blockchain and DroidBot Emulator	Bluestack, DroidKin dataset and Andro - MalShare	Not Mentioned	Federated Block chain achieves 98.62% accuracy	When uploading and downloading programmes from the Internet,to assess if it is harmless or malicious, a blockchain request is used

Table 4: Static Malware Analysis on Android

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[2]	To Classify Android malware samples into their family	AndMFC is Android Malware Family Classification Framework technique is used recognize families of android malware	A set of classifiers was used to detect malware based on a few identified features	AMD Dataset and Drebin	Apktool v2.3.4, Smali language	93.63 % - AMD and 86.54% - Drebin	The proposed system performs static malware analysis and extracts a large number of features from package contents, some of which are useful for malware characterization
2	[31]	A generic malware detection technique which performs static overview of the console to detect malware more correctly while using less time, power, and resources.	Improved Sensitivity attribute approach for identifying Android Malware in network.	Sensitive feature analysis	Android Malgenome Project, Google Android Market	Obfuscapk tool	98\% - Malware families	Static analysis-based detection approaches are more time, power, and resource intensive.
3	[42]	To detect android malware using obfuscation techniques and installing light-weight malware detectors.	Conduct static analysis on exe files in the Android environment to interpret malware.	Droid detective approaches	Android Drebin Project	Apktool v2.3.4	More than 95%	Automated Droid Variant Taint Detects android Malware from Native Code Variant.
4	[21]	To analyze android malware	It identifies Android malware employing	Intensive feature	Baidu Application	VirusTotal	98.1% - Baidu	Android environment

		static detection with Android app composition, analyses the source of static features.	connection structures and retrieves a weighted situational API schema as program semantics by erecting features.	Engineering	Market			detection for complex flow is efficient.
5	[32]	To critique the performance of static analysis deep learning technique is used in Android malware investigations.	The Droid Deeplearner technology is used to identify harmfulSMiShing assaults. SMS and MMS assaults are examples of SMiShing attacks.	A framework based on Contrasting Permission Pattern	Pandaapp, Third party Android Application Markets.	Obfuscapk tool	More than 90%	End-to-end detection for Malware attacks is achievable on the Mobile application.

Table 5: Static Malware Analysis on Machine Learning

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[4]	To conduct a statistical examination of detection procedures especially for PC malware using Machine Learning.	The set of various attributes which infects IOT with the MIRAI malware by exploiting default credentials.	Tracing and Parsing of System Calls using Introspection.	Drebin Dataset	PExplorer and Peview	92% accuracy	The implementation is basic and straightforward. With the help of irrelevant datasets, NB can do extremely simple tasks.
2	[15]	Portable executable files are used to process malware research in Windows system.	The Real-time applications for the network and host operating system to find the potential vulnerabilities.	3NN, Perception, RF and anomaly detection	Microsoft Malware Classification Challenge (BIG 2015) dataset	Not mentioned	Accuracy score is 0.915	It is possible to update at quite a cost. It's incredibly simple to put into action. There is no need to make assumptions about the datasets. It does not necessitate any type of training.
3	[19]	To attain high level of string features set six different ML classifiers are designed and implemented.	Sequence and PE preamble feature extraction are categorized in order to identify malware.	Stacked LSTM Network and Control flow integrity	APT1 Dataset	PeId and IDA pro	97.3% accuracy	This algorithm reduces the risk of overfitting. It ensures that the model is quite accurate.
4	[38]	To detect malware a ML based algorithm which encompasses four-step convergence scheme is implemented.	DANdroid is a malware detection framework that employs deep learning that identify applications and process malware detection tools and strategies driven by machine learning.	Anomaly Detection and Hierarchical DBSCAN	Kaggle	dell precision tower 7910	89.9 % accuracy	Missing values might be done manually. It is not necessary to scale the features. Normalization is indeed not necessary.

5	[20]	A fusion of 1D-STFT and 2D-DWT on spectrograms of EM end probes is used to recover fine-grained rate of different malware.	An ML intrusion detection systems approach feature valuing that optimizes both weight-mapping and classifierscharacteristics at same time.	KNN, KNN+DTW, SVM, RF, NN and signature based detection	Drebin Dataset	XGradiebt Boost detector	Accuracy score is 0.91	It works well with a wide variety of datasets. It is possible to deal with a wide range of data features.

Table 6: Static Malware Analysis on Hypervisor

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[3]	To identify malware in cloud infrastructure it is described an introspection-based approach for defending artificial regions in cloud-based portals.	Focus on providing a cloud-based access control and repair platform on Android phones with ongoing scientific study to identify network misdeeds.	In-VM Process Catalogue Generation, Process Catalogue Validation, Virtual Machine Process Validation and Out-VM Process Catalogue Generation.	UNM Dataset and BareCloud Dataset.	Python 3.6.2 version	More than 97.66%	Only high-risk VMs are subjected to continuous monitoring, lowering total computing costs and enhancing scalability.
2	[11]	A signature-based detection technique is used to detect anomaly malware in hypervisor.	Presents a semantic- based malware classifier system for detecting zero-day malware as well as novel variations of existing malware families.	Signature Based technique, Classifier Accuracy-based Fitness Function (CAFF) and Anomaly Detection techniques	Kaggle, ClaMP Integrated and ClaMP Raw.	Cloud testbed	More than 95\%	The intended AMD framework is secure since it is executed with ultimate capabilities, making it hard to compromise.

Table 7: Dynamic Malware Analysis on IoT

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[10]	To provide a unique malware detection and family categorization model a module of CNN is introduced and integrated with a DenseNet framework.	Depthwise Efficient Attention Module (DEAM) technique is used to detect IOT malwares.	DepthwiseSpatial Attention (DSA), DenseNet architecture, Improved Efficient Channel Attention (IECA).	MalImg dataset and BIG 2015	categorical cross-entropy, TensorFlow 2.0 framework and Adam optimizer	99.3\% overall accuracy	In terms of malware detection and group categorization, the suggested strategy works expertly.
2	[36]	LNN malware detection is used to train on local	A cross deep learning framework is	Droid Emulator andDroidBotEmulator are used to generate the	DroidKin dataset	Bluestack emulator, python	98.62% of accuracy	The suggested methodology was efficiently

		distribution, and enables aggregation processes across block chain platforms to ensure model validity and quality.	designed that supports many elements of the network node while also providing security to IOT devices.	Dynalog file.				identifying malware, resulting in increased security for IoT devices.
3	[13]	To perform classification and detection technique for MIRAI malware attack network forensic is implemented.	Random Forest Algorithm is derived to detect MIRAI malware in IoT devices	Random forest [Gini Index], Tree Importance Decision.	Scan attack dataset	PT-838 Provision devices	95.01% of accuracy	The selection of the appropriate features furthermore aids in producing high detection accuracy.
4	[33]	To identify MIRAI Malware a unique Automation Algorithm-based strategy is implemented using python.	The evaluation of various models, including reliable model that gives accuracy in identifying malware and a classifier that generates huge TP data and a high F-1 value for technique to work.	Artificial Neural Network, Multi-layer perceptron	BIG 2015 Dataset	Matlab 2018b and python	92.8% of accuracy	The model comparison yielded a precise estimation with high precision for classifying malware.
5	[25]	Including an efficient deep neural network architecture for accurate and speedy malware detection, a unique and low-cost technique is adapted.	Gated-CNNs is implemented which allows the selection of important and relevant information with language tasks, it competes against recurrent classifiers.	Hash functions, Deep Neural Network	SecureAge Dataset	Cuckoo sandbox and Virtual Machines	98.80% of accuracy	Every API call's wide hash characteristics must be transformed, multiple gated-CNNs are used.

Table 8: Dynamic Malware Analysis on Android

Sl. No	Paper Ref. No	Aim	Proposed Methodology / Algorithm/ Techniques	Methodology / Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[27]	To distinguish malware from trusted applications, a categorization on the	LSTM model-based classifiers are used to demonstrate the effectiveness of the proposed system.	LSTM Classifier, Maline model to generaten-gram model	Not mentioned	Android Framework and Real mobile Devices.	93.7% of accuracy	Extensive trials were carried out in order to assess the proposed algorithm.

		Long Short-Term Memory (LSTM) autoencoder is used.						
2	[16]	An Android malware detection solution which can update itself dynamically while detecting malware without the need for human intervention.	DroidEvolvertechnique is used to detect android malware.	Regularized Dual Averaging (RDA), DroidEvolver: Passive Aggressive(PA), Adaptive Regularization of Weight Vectors (AROW).	VirusTotal	Apktool kit	92.9% of accuracy	DroidEvolver does not require retraining or true labels to update it.
3	[7]	To create a framework which combines variety of feature set to detect android malware	FAMD : Fast Android Malware Detector is a frame work used to detect Android malware.	Integrate features permission with Dalvik opcode features from various operating systems, CatBoost classifiers	Android Drebin Project	Androguardplatform	97.40 % of accuracy	Employing CatBoost as classifier, the outcome significantly increased accuracy.
4	[9]	A contrastive learning is used to enable obfuscation-resistant malware analysis.	IFDroid a novel contrastive learning- based robust and interpretable android malware classification system.	Grad-CAM++, SupCon Loss function	Genome project and FalDroid	ResNet-18	98.2% of accuracy	The extensive testing results show that IFDroid outperforms seven cutting-edge Android malware classification systems.

5	[28]	A detection technique that creates and analyses representing malware and regular datasets derived from access sets retrieved from an application's root folder.	PermPair creates and examines visualizations for malware and regular samples exporting from an application's root folder.	Graph merge algorithm	Genome, Drebin and Koodous	Apktool kit	accuracy of 95.44 %	The proposed method outperformed eleven popular mobile anti-malware apps, according to the results.

Table 9: Dynamic Malware Analysis on Machine Learning

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[24]	To create a malware detection framework which relies on a combination of various Cuckoo sandboxes, This is employed in the study of dynamic malware.	To analyze malware, a variety of machine learning algorithms were used. To identify malware, the ROC curve in dynamic analysis employing the Gradient Boosting Algorithm is applied.	Bagging classifier and AdaBoost classifier	Baidu Application Market	Cuckoo sandbox, python	94.64 % accuracy rate	To detect the virtual environment, the tricky malware executes APIs.
2	[26]	To use memory forensics in	This integrated malware	10-fold cross validation,	Drebin Dataset	WEKA tool	98.5% accuracy rate	Combining relevant characteristic

		dynamic malware detection approach to retrieve malicious artifacts from memory.	detection technique that uses computer analytics to recover harmful artifacts from memory then integrates those in a dynamic analysis with data acquired while malware activation.	ML classifiers				s from storage with features acquired by static response enhances the recognition rate.
3	[40]	A strategy for on-chip network traffic monitoring and hardware-assisted malware detection employing an embedded trace buffer is implemented.	A framework for hardware trace collection and malware detection based on the ZC702 critique kit is implemented.	Choosing hardware performance metrics and developing the ML network are two critical steps in HPC-based malware detection.	GARNET2.0	gem5 simulator and Xilinx evaluation kit	Existing HPC is efficient than proposed ETB	Using Counters for hardware performance, embedded trace buffers, and on-chip network traffic analysis, Machine learning might be useful in detecting malware.
4	[6]	To evaluate the most representative malware attacks in cyber physical system.	A proof of concept is used to illustrate a form of DOS which is possible across a cyber physical system.	SVM, RF and n-grams	Koodous Dataset	APIMon	More than 98\% accuracy	The best classification results were obtained by mixing user details with that at the Kernel program, resulting in greater accuracy
5	[17]	To compare data at the	To understand	AdaBoost classifier	Not mentioned	Cuckoo sandbox	93.58% accuracy	The user level approaches

		kernel and user levels during dynamic malware analysis	in a simulated sandbox environment, the malware's file systems are caught through a user-level trap.	and KNN				are vastly popular, The findings revealed that kernel datasets performed analytically higher than all other categorizatio n techniques.

Table 10: Dynamic Malware Analysis on Hypervisor

Sl. No	Paper Ref. No	Aim	Proposed Methodology/ Algorithm/ Techniques	Methodology/ Algorithm/ Techniques Used	Dataset Used	Software Tools Used and Platform	Performance Metrics	Advantages
1	[30]	To detect ransomware using machine learning and procure storage access through live-forensic hypervisors.	To discern ransomware from a benign application, storage access through live-forensic hypervisors from WaybackVisor and machine learning models are devised and scrutinised.	RF, SVM and KNN	WaybackVisor	Scikit-learn version 0.20	98% accuracy	The latter simulation employing five-dimensional features yields a higher F-measure score.
2	[37]	To assess data for intrusion detection using virtual machine Monitor.	For cloud-based IDS, a collection centered on Virtual Machine Introspected (VMI) results is developed.	RF, RIPPER, NB, KNN and SVM	UNM Dataset	WEKA Tool, Shell script	96.3 % accuracy	ML algorithms are implemented to each dataset, and the outcomes are examined obtained from the experiments as well as purpose of effective.
3	[18]	To identify malware via dynamic multi-feature detection. At Hypervisor, Ensemble	Various forms of dynamic information within virtual machine data are recovered employing virtual	VMI library (LibVMI), AdaBoost M1 classifiers, Voting combination strategy and	LID Dataset	Xentrace and perf tool	0.9999 accuracy achieved	The detection rate and speedup of the overall classifiers were effectively improved by

		Learning is deployed.	machine introspection (VMI) technology in combination with memory forensics analysis (MFA) technology.	memory forensic analysis framework				using the hardware layer features.
4	[14]	To analyze dynamic malware using hypervisor in Operating System.	For a hypervisor-based system, a hardcoded monitoring tool is inoculated into the address space of a guest OS for dynamic malware analysis.	RF, SVM and VM Techniques	Drebin Dataset	Apktool kit	More than 95% accuracy	It has been discovered that efficient detection of zero-day attacks is possible.

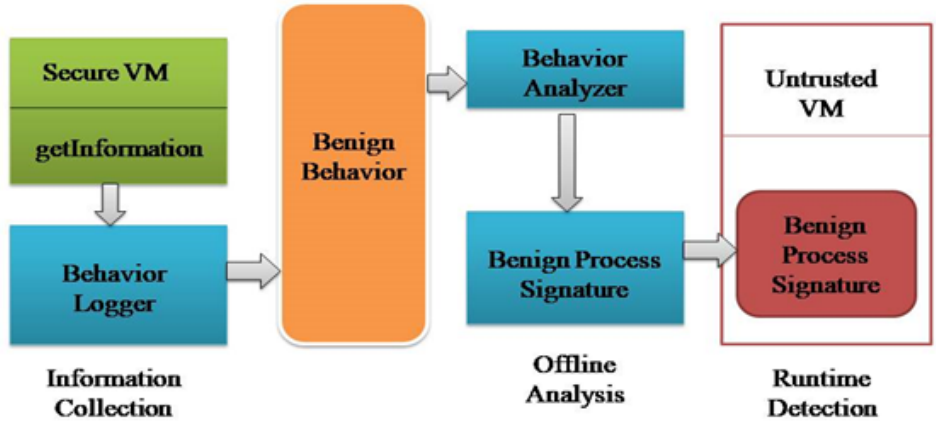


Fig. 1. Malware Detection Procedure

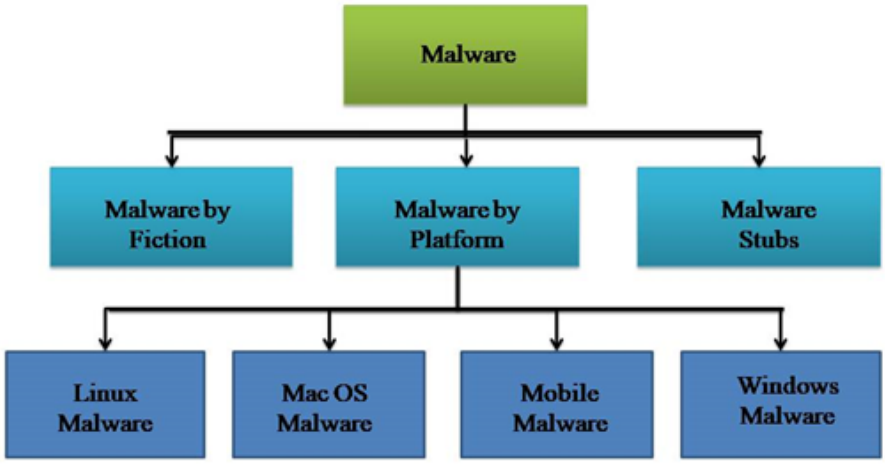


Fig. 2. Categories of Malware

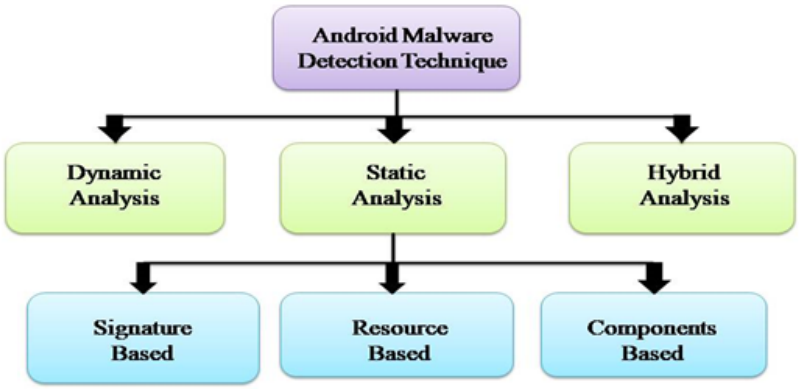


Fig. 3. Android Malware Detection Technique

REVIEW ARTICLE

Regulatory Requirements for Conducting Clinical Trials in Europe : A Comprehensive Checklist and Review

T. Ankitha[1]* and Koushik[2]

[1]Student (M.Pharamacy), Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.
[2]Associate Professor, Department of Regulatory Affairs, Chalapathi Institute of Pharmaceutical Sciences, (Affiliated to Acharya Nagarjuna University), Guntur, Andhra Pradesh, India.

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***Address for Correspondence**
T. Ankitha
Student (M.Pharamacy),
Department of Regulatory Affairs,
Chalapathi Institute of Pharmaceutical Sciences,
(Affiliated to Acharya Nagarjuna University),
Guntur, Andhra Pradesh, India.
E.Mail: Ankithatungala03@gmail.com

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ABSTRACT

This review paper provides a comprehensive overview of the legal requirements Performing clinical studies in Europe, with an emphasis on the rules According to EU provisions Clinical Trials Regulation. (EU CTR) No.536/2014.The process for applying investigator responsibilities, safety reporting, data protection, ethics committee approval, and other important regulatory issues are discussed. Along with providing practical guidance for achieving compliance, it illustrates the difficulties stakeholders encounter while negotiating the regulatory landscape. According to EU law, the paper is a helpful resource for researchers, Study funders and regulatory experts.

Keywords:

INTRODUCTION

Role of the Checklist

So that assist sponsors, researchers, and other interested parties in navigating the significant legal requirements and procedures associated with Implementing clinical trials in Europe, this checklist was created. This ensures compliance with EU legislation, facilitates a seamless approval process, and ensures participant safety and transparency. Furthermore, in order to facilitate clinical trial management and monitoring Within the EU, the

checklist will assist in guaranteeing compliance In compliance with the Clinical Trials Regulation (EU) No. 536/2014 and other pertinent regulations.

Overview Related to clinical trial Regulations in Europe

The main regulation governing Clinical studies in Europe is Regulation (EU) CTR stands for Clinical Research Regulation, commonly known as No. 536/2014.The Clinical Research Directive (2001/20/EC) was superseded by this rule on January 31, 2022. The new rule seeks to speed up and simplify the approval process, increase participant safety, and make clinical trial data more widely accessible.

Among The clinical investigations Regulation's primary characteristics are

Single Application Process: Companies can Utilize the Clinical Trials Information System (CTIS) to submit an application for clinical trials authorization in a number Within EU member states and countries in the European Economic Area (EEA).

Improved Safety Surveillance: Safety reporting requirements for adverse occurrences Including serious adverse events are tightened by the CTR.

Transparency: Sponsors are obligated to register clinical trial outcomes so that the public can access important study data.

Enhanced Cooperation: The regulation promotes closer coordination between national authorities and The European Agency for Medicines(EMA), increasing the efficiency of study authorization and supervision.

Regulatory Authorities

The European Agency for Medicines(EMA): Pharmaceutical oversight, monitoring, and scientific reviews are carried out by The European Agency for Medicines, a decentralized organization Within the European Union. It is crucial for creating safety regulations, marketing authorization protocols, And the clinical research study guidelines, among other elements of the clinical studies approval process.

- As part Regarding the Clinical Trials Regulation (CVR), the EMA is responsible for ensuring the quality, safety, and efficacy of pharmaceuticals, including those being studied in clinical studies.

National Regulatory Authorities: Clinical studies carried out inside the borders of each The Economic Area of Europe country and EU member state are overseen by a National Competent Authority (NCA). NCAs oversee the evaluation and approval of clinical studies applications, supervise trial conduct, and Ensure conformance to national and EU regulations.

- Another responsibility of NCAs is to make sure that trials follow ethics and safety regulations, including Good Clinical Practice (GCP).

Clinical Trial Authorization (CTA) A clinical study's formal approval from the appropriate regulatory bodies To initiate a clinical studies in a given country is known as a clinical studies authorization (CTA). The European Clinical Trials Act (CTA) Guarantees that clinical studies are conducted in the EU meet All applicable regulatory standards, including those pertaining to safety, scientific quality, ethics, and legal obligations. The CTA process provides thorough trial data for assessment by the trial's host country's National Competent Authorities (NCAs) and competent Ethics Committees (ECs).

Ethical Approval

Institutional Review Boards and Independent Ethics Committees (IEC and IRB, respectively)

Institutional Review Boards (IRBs) and Independent Ethics Committees (IECs) are the independent boards in charge of reviewing, approving, and overseeing Clinical studies to verify their implementation ethically and prioritizing the participants' well-being and security. Where you live, the terms "IEC" and "IRB" may have distinct names, even if they are used interchangeably. Although IEC is the most commonly used term in the EU, certain countries, like the US, use IRB more frequently.

Functions of IECs/IRBs

Ethical Review

IECs and IRBs evaluate the risk- benefit ratio, participant protection, and the fairness Of the informed consent process, along with other Ethical considerations in clinical studies

Procedure for approval: Before a clinical studies can begin, the IEC/IRB must review and approve the study protocol, which covers the study design, methods, and participant recruitment strategies.

The informed consent process: is supervised by IECs and IRBs, who Make certain that the informed consent forms for the trial are clear, accurate, and provide participants with sufficient information to allow them to make educated judgments.

Continuous Monitoring: After the experiment begins, IECs and IRBs monitor its progress and any unforeseen problems or negative results To guarantee that it remains within ethical constraints. They may also have to reschedule the experiment or make adjustments if safety concerns are brought up.

Composition of IECs/IRBs: The makeup of IECs and IRBs should be diverse, comprising experts with backgrounds in medicine, research, law, and ethics, To confirm a thorough Review of the trial's conduct. People with Patient care experience advocacy or participants in the local community Should be incorporated into the composition to represent The needs of participants.

Contract For clinical studies The Clinical studies Agreement)is a legally binding contract that is signed On behalf of the sponsor, usually a pharmaceutical company or research organization, and the participating clinical trial site, like a hospital or research institution. Both parties outline their respective roles, responsibilities, and financial arrangements in the CTA.

Important Rules and Instructions

EU Regulation No. 536/2014 on Clinical studies

The Clinical Studies Regulation (EU) No 536/2014 (CTR) is followed by the European Union (EU) and the European Economic Zone when performing clinical research. The legislation's goals are to ensure high levels of transparency in clinical studies across the EU, boost Participant well-being, and streamline the approval process. The clinical research directive (2001/20/EC) was replaced by the CTR, which allows for more central and efficient submission and approval of clinical studies.

Characteristics of the Clinical studies Law

CTIS or the Single Submission Portal

- Several European Union and EEA nations allow Sponsors are required to submit a single application for clinical studies permission through the Clinical studies Information System. This speeds up the approval process and reduces the administrative burden.
- The system will evaluate the trial application By the ethical review bodies and National Competent Authorities (NCAs) in the related countries.
- To better safety monitoring standards, the CTR requires sponsors to promptly report suspected unexpected severe adverse reactions (SUSARs) and severe adverse events (SAEs) to regulatory authorities.
- To safeguard trial participants, the regulation additionally enforces stricter standards for adverse event reporting and safety evaluations.

Transparency: By mandating that clinical studies data, including protocols, results, and safety information, Be made accessible to the public, the CTR seeks to improve transparency. This helps to foster confidence in clinical studies and

ensures that study data is publicly available. Clinical Trial Registers Supply details about ongoing or completed clinical studies inside the EU/EEA to the public.

Ethical Monitoring

The CTR's top priority remains ethical concerns. ECs review and approve the trial's ethics, including participant protection and the informed consent procedure.

Simplified Approval Process: The law speeds up the trial authorization process by allowing a comprehensive review of the application across many countries and by establishing a precise timetable for decision-making. Trial applications must be processed within the CTR's specified deadlines, which for global trials are typically 60 days.

Compliance with Good Clinical Practice (GCP)

A well-recognized set of guidelines for conducting Clinical studies with human participants is known as "good clinical practice" (GCP). GCP aims to verify the security, reliability, and correctness of clinical studies data while also protecting The protection and safety of trial participants GCP must be followed in all clinical studies conducted in the EU or EEA.

Principles of GCP

Ethics: Clinical trials must always adhere to ethical norms outlined in the Declaration of Helsinki, which guarantees the participants' rights, protection , and welfare. Participants must voluntarily and knowingly Approval of the experiment after being fully Made aware of all potential risks and rewards.

Observance of regulations and scientific principles

For studies to be regarded as scientifically sound, a well-documented study protocol That outlines the objectives, design, and methodology and statistical factors of the investigation is necessary. It is essential to maintain compliance with all regulatory requirements during the trial, including those outlined in Regulation (EU) No 536/2014.

Keeping an eye on and reporting: To ensure compliance with the procedure, ethical standards, and regulatory requirements, GCP requires ongoing clinical trial monitoring. Serious adverse events (SAEs), suspected unexpected severe adverse reactions (SUSARs), and any other significant findings that might compromise the conduct or safety of the studies must be reported by the sponsors and investigators.

Assurance of quality: To improve that every aspect of the clinical studies conforms with regulatory and GCP requirements, sponsors must implement quality assurance procedures. This entails conducting internal audits and inspections.

Guidelines for Pharmacovigilance

Pharmacovigilance is the study and application of recognizing, assessing, understanding, and averting adverse medication reactions. In clinical studies, pharmacovigilance helps detect any potential safety problems or an unforeseen risk associated with the drug and ensures the ongoing safety of investigational medical products (IMPs) during the trial.

Clinical trials must adhere to pharmacovigilance requirements

Techniques for Reporting Adverse Events (AEs): The sponsors of the clinical study must establish protocols for identifying, assessing, and disclosing adverse events (AEs), including serious adverse events (SAEs). The earliest possible identification of an adverse event (AE) is necessary. Before being reported to the relevant regulatory agencies, Unanticipated severe adverse reactions must occur within a specific period of time (for instance, within 7 days for SAEs and 15 days for SUSARs).

Methods of Lowering Risk: Risk reduction strategies must be devised in clinical studies to reduce participant exposure to known or potential dangers. To protect vulnerable groups, some examples of these include changing the dosage, implementing monitoring procedures, or adding exclusion criteria.

Reporting Schedules for Pharmacovigilance: To guarantee that regulatory bodies are immediately notified of any serious safety issues during the trial, the Clinicalstudies Regulation (EU) No. 536/2014 specifies precise reporting deadlines for adverse events and SUSARs.

Reports on Development Safety Updates (DSURs): Every year, sponsors are required to present safety reports that give a summary of the IMP's safety profile.

Monitoring Safety and Assessing Data: Pharmacovigilance ongoing safety observation is essential. Sponsors must continuously assess safety data during the study and, if new safety information is made available, modify the research design or take remedial action.

Documentation and Record-Keeping: Every pharmacovigilance activity, including as safety assessments, adverse event reports, and regulatory correspondence, must be meticulously documented. This material should be accessible for regulatory reviews and inspections.

Cooperation with Regulatory Bodies: In order to improve the effective exchange and evaluation of safety data from clinical studies, sponsors must preserve effective communication and collaboration with the European Medicines Agency (EMA) and National Competent Authorities (NCAs) with regard to pharmacovigilance initiatives.

Conditions for Investigational Medicinal Products (IMPs)

An experimental medicinal product (IMP) is a pharmaceutical product not yet approved for general use but being tested or used in a clinical studies. Since clinical trials are used to assess the safety, effectiveness, and pharmacological features of humans, these items are crucial. The IMP guidelines address a number of issues, including manufacturing, licensing, labeling, and good manufacturing practice (GMP). Meeting these objectives is necessary to improve the trial's scientific integrity, regulatory compliance, and patient safety.

Production and Permits

Production of Investigational Medicinal Products (IMPs)

To improve the medication's quality, safety, and effectiveness, manufacturing and IMP requires following strict regulatory requirements. The production process includes the following steps

Growth and Scale-Up: For preclinical research, the IMP is initially manufactured on a small scale. Once the product shows promising results in clinical testing, the production process must be scaled up.

Compliance with laws: Manufacturers operating in the European Union (EU) must comply with strict laws that ensure their production process complies with the Good Manufacturing Practice (GMP) standards, which will be discussed later.

IMP Licensing: Before being used in clinical studies within the European Union, an IMP must be authorized for clinical use by the European Medicines Agency (EMA) or the relevant National Competent Authorities (NCAs).

Form for Clinical studies Authorization: When a sponsor files a CTA with the regulatory agencies, they must provide all related information about the IMP, including

- The content and description of the product
- Production processes and infrastructure Guidelines for safety and quality assurance; data on preclinical and clinical development

GMP (Good Manufacturing Practice) adherence: Manufacturers must demonstrate that they are following GMP criteria, which ensure that IMPs are manufactured reliably and with the required efficacy, safety, and quality.

Distribution and Importation: IMPs may be imported from third countries, but only when that location's manufacture complies with EU GMP standards. For clinical studies, the import license is required to improve that the product meets the legal requirements.

GMP stands for Good Manufacturing Practices.

To improve that pharmaceutical products, including investigational medical products, or IMPs, are consistently created with high quality and meet safety criteria, manufacturers must follow a collection of guidelines known as "good manufacturing practice" (GMP). GMP is required to lessen the risks associated with IMP production, including contamination, errors, deviations, and mix-ups.

Aspects of GMP for IMPs

Quality Control Systems: In order to improve that the IMP meets all relevant requirements, including potency, stability, and homogeneity, and purity, extensive quality control (QC) systems must be established in manufacturing facilities. For these standards to be fulfilled, routine audits and inspections are required.

Training and Staff: Only capable people who have had enough instruction in GMP standards should oversee the manufacturing of IMPs. This includes training on legal compliance, hygiene, and risk management. **Facilities** must be properly designed, equipped, and maintained in accordance with GMP regulations to prevent contamination and ensure product safety. This include keeping industrial facilities clean and regulating temperatures, humidity, and air quality throughout manufacturing.

Records and Traceability: During the manufacturing process, comprehensive paperwork must be kept in order for the IMP to be traceable from production to final distribution. This includes certificates of analysis and batch information for each IMP batch. Logs documenting the cleaning, maintenance, and calibration process for the equipment are also available. Records of every raw material and supplier used during the production process

Registration and Reporting

Trials in to ensure accountability, openness, and the public and scientific community's Availability of the findings, clinical trial Must be enrolled and reported. Clinical Trials Regulation (EU) No 536/2014 and international guidelines, such as those published by the World Health Organization (WHO) and the International Committee of Medical Journal Editors (ICMJE), govern these criteria.

The Clinical studies Registry

Clinical trial outcomes need to be entered into a public database so that the general public, healthcare providers and government agencies can view them effectively. The public distribution of studies results, whether positive or negative, is ensured by registration, which also helps to prevent selective reporting.

System for Clinical studies Information, or CTIS

In the European Union, the Clinical studies Information System (CTIS) is the primary platform for registering and submitting clinical studies applications, as per Regulation (EU) No. 536/2014. The CTIS must be registered before any clinical study conducted in the EU or EEA can begin. When registering, you must enter important trial information like: A comprehensive description of the study's objectives, design, and methods is called the study protocol. "Investigational medicinal products" (IMPs) are details on the experimental drugs or therapies being tested.

Obligations for Reporting

Trials in Clinical studies sponsors and investigators are required to provide detailed reports on the conduct, progress, and outcomes of clinical studies For the purpose of preserve regulatory adherence and transparency. These rules for reporting also protect the public's trust in clinical research.

Conditions for Essential Reporting: The Development Safety Update Report (DSUR) is the name of the yearly safety report. It is mandatory to submit Development Safety Update Reports (DSURs) to the relevant National Competent Authorities (NCAs) annually. The safety profile of the investigational medical product (IMP) is compiled in these studies, which comprise

- Since the last report, additional safety-related information has emerged.
- The state of clinical studies today and new safety issues. Suspected unexpected significant adverse responses (SUSARs) and adverse events are included in the summary of the trial's safety data.

Trial Final Reporting

After a clinical studies is finished, end-of-trial reports must be reported to regulatory agencies. The report includes detailed information on safety, the trial's outcome, and any issues that arose. Disclosure of Results Clinical studies results must be disclosed to maintain the scientific integrity and openness of clinical research. To avoid publication bias and to allow for independent data analysis, both positive and negative results must be made available.

Requirements for Results Reporting

The final trial's findings: After a clinical studies is finished, the final results must be released to the public within a certain amount of time. It includes

Results of effectiveness: Information about the accomplishment of the experiment's primary and secondary goals.

Findings for safety: A summary of adverse events, encompassing both expected and unexpected reactions. Analysis of statistical data from trials, including studies design and methodology.

Clinical studies Registration: In compliance with Clinical studies Regulation (EU) No 536/2014, the Clinical Trials Information System (CTIS) must receive and make publicly available the final results of clinical studies conducted inside the EU. This will guarantee openness and enable the public and authorities to evaluate the study's findings.

Results Reporting in Public Databases: Results of clinical studies should also be made available via other public trial registries and databases, such as the following: For studies conducted within the Use the EU Clinical Trials Register: EU or EEA. International standards for results reporting are the goal of the WHO International Clinical Trials Registry Platform (ICTRP).

One of the most widely used trial databases globally is ClinicalTrials.gov.

Results must normally Be submitted within a year after the experiment's conclusion, or within six months in the event of child interventional studies. This ensures prompt reporting. Delays in reporting results could have regulatory consequences, like the suspension or rejection of future clinical study applications.

Publications subject to peer review: It is advised that trial sponsors send their results to publications that are subject to peer review. The scientific community can then critically assess the results, which leads to a more thorough understanding and application of the findings.

Documentation of Adverse Results: It should be mentioned that trials that fail to meet their main objectives or yield other unfavorable or ambiguous results also Must be documented. The scientific understanding of a product's Security and efficacy is distorted by publication bias, which only discloses positive data. This must be avoided.

Openness and Public Access: The EU drug regulator and other Regulatory bodies possess mandated that results be rendered publicly understandable. To do this, succinct summaries of the findings must be presented.

Data Protection and Privacy

When conducting clinical studies, it is essential to have safeguards for sensitive personal data. Make sure that European data privacy regulation of the European Union is strictly followed in order to treat personal data in a safe and morally responsible manner. To safeguard the rights of participants and privacy, clinical trial organizers must put strong data management and controls in place.

GDPR, or EU Data Protection Regulation Conformity

The European Union has enacted EU Data Protection Regulation to protect privacy and personal information both inside the EU and the EU Economic Area. Organizations must follow legal requirements for the handling of personal data, including information gathered from clinical studies conducted in EU countries.

Under GDPR, consent

- GDPR mandates that consumers provide clear, Pre-consent agreement their personal information is processed for clinical studies. Consent must be provided voluntarily, clearly, and explicitly.
- **It is essential to inform individuals about:** the cause of the data collection. What type of data— demographic or health—is being collected?
- The duration of data retention;
- The right to revoke consent whenever necessary. Consent forms need to be clear and concise, and they should be written in an understandable language.
- **Vulnerable Groups and Children:** When participants are members of vulnerable groups (such individuals with dementia) or are minors, further care must be taken, such as obtaining legal counsel clearance.

Pseudonymization and Data Anonymization

To protect participant privacy during data processing and analysis, methods like Anonymization and pseudonymization represent used.

- The practice of removing all personally identifiable Data is processed so that it cannot be connected to a specific individual is known as anonymization.
- Pseudonymization involves replacing identifiable information with pseudonyms, or codes, so that the data can be re-identified if necessary (for instance, for follow-up purposes).
- Pseudonymization is allowed in clinical studies, but researchers must improve that the key to reidentify the data is kept separate and safe.

Safety precautions: Clinical studies data should be protected by putting in place appropriate organizational and technical measures to avoid data security breaches. They include: All confidential information must be encrypted both during transmission (such as over the internet) and while storage on servers or databases.

- To ensure that data is not lost due to technical failures, natural disasters, or cyber-attacks, backup solutions should be used often.

Cyber security Procedures: To avoid hacking, unauthorized access, and other malicious activities, trials need to have robust cyber security protocols.

Insurance and Financial Requirements

Clinical study planning and execution must take insurance and financial constraints into account. These guidelines promote transparency in financial transactions, define funding responsibilities, and ensure the Participant protection

in research. The two main components are explained as follows: Trial participants' insurance, financial transparency, and budget.

Protection of Trial Participants

Ensuring the safety of clinical trial participants is one of the fundamental ethical standards. Clinical studies can be dangerous, particularly when testing new drugs, treatments, or methods. To lower these risks, insurance is required by law for trial participants in several jurisdictions, including the European Union (EU). This guarantees that participants will get payment in the event that their injuries are related to the experiment.

Types of Insurance

Clinical Trial Insurance

This type of insurance shields trial participants from any health hazards related to the use of investigational drugs (IMPs) or study procedures. If involvement in the experiment results in harm, death, or injury, it offers monetary compensation.

- The insurance must cover the Timeframe of the clinical trial, including any follow-up periods after the experiment.

Coverage types

Compensation for harm or Death

Insurance must cover all medical costs and damages for personal harm or death resulting from clinical research. Among these are: Expenses related to treating injuries sustained during a study. Income loss in the case of that a participant Is unable to perform work because of injuries they received during the survey. Funeral costs in the case of a trial participant's death.

Liability

Not Including Negligence: In general, damages resulting from the negligence or misconduct of the trial sponsor or staff are not covered by insurance. Nevertheless, if the experiment's harm stems from a violation of regulatory or Good Clinical Practice (GCP) standards, the research sponsor may be held liable

Insurance Coverage Levels

a. The minimum requirements established by the relevant regulatory agencies (such as the Including organizations like the European Medicines Agency, or national Competent Authorities) must be met by the insurance policy. This usually means having adequate financial coverage that is commensurate with the complexity and potential risks of the experiment.
b. Clearly stated in the insurance policy should be the conditions for submitting claims as well as the scope of coverage accessible to participants.

Budget and Financial Transparency

Effective clinical trials require transparent budgeting and well-defined financial planning. Maintaining accountability, providing comprehensive details on the trial's financial components, and managing finances in accordance with legal and ethical requirements are all part of this.

Recommendations for Budget and Financial Transparency Regulation: The Clinical Trials Regulation (EU) No 536/2014 highlights the importance of financial accountability and transparency in clinical studies, in addition to participant protection and trial conduct.

- As part of the application process for clinical trials, National Competent Authorities (NCAs) may demand a financial strategy and detailed budget.

Safety Observation

In clinical studies, safety monitoring is necessary to safeguard the health and Protection of participants and to identify and control any Possible risks linked to the investigational medicinal product (IMP). Safety monitoring

entails reporting Severe adverse events (SAEs), creating a Risk Management Plan (RMP), and having a Data Monitoring for Safety (DSMB) oversee the process. These systems are essential to maintain patient safety and ensure regulatory compliance.

Safety-related reports (SAEs)

Reporting and managing Severe adverse reactions(SAEs) Is a crucial part of safety monitoring in a clinical investigation. The term SAEs refers to unintended Life-threatening medical incidents a life-threatening situation, hospitalization, significant disability, or other medically critical problems that require intervention or continuous treatment. To identify the risks to trial participants and ensure that regulatory bodies are informed of any safety concerns, SAEs must be recorded.

DSMB (Monitoring of Data Safety Board)

An objective panel of professionals called a Monitoring of Data Safety Board (DSMB) was set up to supervise clinical trial participants' safety. Because of safety concerns, the DSMB is responsible for monitoring the progress of the study, evaluating intermediate findings, and providing recommendations about whether to continue, modify, or end it.

A risk management strategy (RMP)

A clinical trial sponsor might use a comprehensive plan called a Risk Management Plan (RMP) to identify, assess, and mitigate possible risks. Particularly in studies including innovative therapeutic interventions or investigational medicines (IMPs), participant safety is heavily reliant on the RMP.

Observation and Inspections

To ensure their Integrity, security, and compliance, clinical trials need to be tracked and audited. These protocols improve that the conduct of clinical studies complies with ethical guidelines, regulatory requirements, and Good Clinical Practice (GCP) recommendations. A detailed summary of the requirements for sponsor audits, regulatory audits, and site monitoring—all crucial to maintaining the trial's security and integrity—is given below.

Requirements for Site Watch

Clinical trial sites are routinely observed to improve that the trial is conducted In accordance with the approved protocol, relevant regulations, and Good Clinical Practice (GCP). We call this procedure site monitoring. To ensure Safety of participants, data precision, and compliance with study protocols, clinical research associates (CRAs) and other qualified professionals conduct routine monitoring visits.

Regulatory Examinations

The European drug regulatory agency (EMA) and National Health Authoritys (NCAs) are examples of national or regional regulatory authorities that carry out regulatory audits to assess whether clinical trials comply with applicable legislation, GCP, and ethical standards. These audits ensure that clinical studies are performed in a way that safeguards participants and preserves data integrity.

Sponsor audits

Sponsor audits can be performed by the sponsor's hired internal or external auditors. These audits are intended to ascertain that the sponsor's clinical studies are being conducted As per the trial protocol, GCP, and applicable regulations. Monitoring sponsor responsibilities and improving appropriate conduct during the study are the goals of these audits. Sponsor audits are intended to ensure that the study is being conducted in as per with the rules and guidelines.

- Identify that the trial's data was collected and documented correctly.
- To assess compliance with sponsor protocols and industry standards by trial locations, clinical staff, and investigators.

- To identify any gaps in trial operations or non-compliance and ensure that corrective actions are taken.

Trial Closure and Trial End

A clinical trial's closure marks the formal completion of the experiment and the completion of data collection, making it an essential step in the clinical research process. In addition to improving that all documents are properly stored, it involves improving that all legal, moral, and regulatory requirements are met. To support transparency and compliance, the end-of-trial process also includes notifying the relevant authorities and interested parties. The conclusion of Trial Notification and Archiving of studies Documentation are two crucial steps in the studies closure process, and they are outlined here.

Notice of Trial End

In order to formally announce the completion of a clinical trial, regulatory organizations require the conclusion of Trial Notification. This notification marks the complete of the clinical investigation and informs the Ethics Committee and national or regional regulatory agencies Including bodies like the National Competent Authorities (NCAs) and the European Medicines Agency (EMA) Regulatory agencies can keep an eye on clinical study completion when the completion of a trial is announced, which also ensures compliance with legal and ethical criteria.

Preservation of Trial Records

Upon completion or termination of a clinical studies, all trial records must be kept for future use and regulatory observance. This improve that participant data, trial-related correspondence, and raw data are all stored in a secure and organized manner. When a studies is properly archived, it also facilitates future audits, inspections, or legal claims.

Regulatory requirements Checklist for performing clinical trials in Europe(Table: 1)

Obligations Following a Trial

Sponsors and study leaders must fulfill a number of post-trial obligations when a medical study is completed to ensure ongoing participant safety, the moral sharing of trial results, and adherence to regulations. These obligations help maintain public confidence in clinical research while Additionally supporting the body of scientific knowledge. The primary post-trial duties are as follows: Publication of Trial Findings and Ongoing Safety Inspection.

Continuous Safety Oversight

Safety monitoring of investigational medical products (IMPs) must continue after the trial has concluded, especially if they are authorized for wider use following the trial. Ongoing safety monitoring makes it easier to spot any unusual or persistent adverse effects that might have gone undetected during the clinical studies.

Trial Results Publication

The publication of clinical studies data following the experiment is essential to preserving transparency and population trust in the scientific approach Ethical research methods require that trial outcomes, both positive and negative, be disclosed to the population. Publication trial results promotes medical science, enhance clinical practice and decision-making, and facilitates critical peer review.

CONCLUSION

Regulatory agencies, Trial funders and researchers have a number of ongoing responsibilities even though the end of a clinical studies is a significant milestone in the research and development process. To preserve The credibility of clinical research and safeguard patient safety, adherence to legal and ethical requirements is crucial. . The following provides an outline of the compliance requirements Along with the significance of ongoing reporting and Surveillance during clinical studies.

Short-Term Requirements for Compliance

In order to maintain the utmost Data integrity, participant safety, and transparency during a clinical investigation, several ethical and regulatory requirements need to be fulfilled. In conclusion, compliance with these regulatory requirements is essential to preserving clinical studies integrity, protecting study participants, and promoting the development of safe and effective medications.

The Importance of Ongoing Reporting and Monitoring

After the final person completes their final session, clinical trials proceed. An essential part of post-trial duties is ongoing reporting and monitoring, which continues long after the trial is concluded.

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Table.1: Regulatory requirements Checklist for performing clinical trials in Europe

S.N.O	REQUIREMENTS	YES	NO
1	Clinical studies Application (CTA) submitted via CTIS		
2	Sponsor registration in EU Portal (CTIS)		
3	EudraCT Number (if applicable under transitional trials)	(	
4	Ethics Committee Approval		
5	National Competent Authority (NCA) Authorization		
6	Informed Consent Form (ICF) compliant with GDPR		
7	Trial registered on EU CTR public database (via CTIS)		
8	Clinical studies Protocol (ICH-GCP compliant)		
9	Investigator's Brochure (IB)		
10	Risk Assessment and Justification of studies Design		
11	GMP Certification for IMP Manufacturing		

12	Labeling of IMP as per Annex VI of EU CTR		
13	Insurance/Indemnity Coverage		
14	Financial Disclosure Statements		
15	Clinical Trial Agreement (CTA) with Sites		
16	Data Monitoring Committee (DMC) setup (if applicable)		
17	Annual Safety Reports (Development Safety Update Report -DSUR)		
18	Serious Adverse Event (SAE) and SUSAR Reporting		
19	Archiving and Data Retention Policy (25 years)		
20	Trial Summary Results Posting within 12 months of completion		
21	GDPR-Compliant Data Management and Privacy Notices		
22	GCP Training Certificates for Investigators and Site Staff		
23	Quality Management System (QMS) in place for Sponsor/CRO		
24	Electronic Systems Validated (e.g., eCRFs, EDC systems)		
25	Review and Examination Readiness Plan		

RESEARCH ARTICLE

An Analysis of Britannia's Retail Marketing Practice and Customer Engagement, Chennai

P. Srinivas[1]* and Usha[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Associate Professor, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
P. Srinivas
Second Year MBA Student,
School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai,
Tamil Nadu, India.

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ABSTRACT

This study is essential because Britannia is one of India's leading FMCG (Fast-Moving Consumer Goods) brands, and its retail marketing strategies significantly attract customers. With growing competition in the food and beverage industry, it is essential to understand how Britannia promotes its products in retail stores and connects with its customers. This analysis can help identify what is working well and what can be improved to increase customer satisfaction and sales. Britannia has a strong presence in retail stores and is well-known among customers for its popular products. However, while the brand is familiar, it still has room to build a deeper connection with its customers. Currently, most of its customer engagement at the retail level is focused on selling products rather than creating a memorable or enjoyable shopping experience. The company can benefit by making its marketing more personalized and using technology to connect better with store shoppers. Also, combining its online marketing with in-store promotions can improve the overall customer experience. As customer preferences are changing quickly, especially after COVID, and with growing interest in health,Britannia needs to study the market and update its retail strategies regularly. By doing this, the company can stay competitive, strengthen customer loyalty, and improve its retail marketing practices.

Keywords: Customer Satisfaction, Health, Retail Marketing, Shopping Experience, Technology.

INTRODUCTION

The Indian biscuit industry was flourished in the baking industry since the second half of 20th century. The urban dweller has indicated that they want to eat cheaply. In early times biscuits were considered a sick person's food. Nevertheless, it has is becoming one of the many popular fast-food items out there after that attracts consumers of every age group. Biscuits are not only tasty but cheap and low in cholesterol. Biscuit industry is more developed in West Bengal and Maharashtra. Doesn't the Indian biscuit industry found to be generating Rs. 3000 cr small profit look like the largest one in food industry, The Indian subcontinent is the second largest manufacturer of biscuits after the United States. Into two classes the industry is divided, the official and the unofficial. At present biscuits are dearer and more genteel than bread. India is now the 3rd largest biscuit producer after China and the United States. Increasing consumer disposable income, shift to premium biscuit, establishment of new manufacturing units, growing health awareness, biscuit innovation and attractive packaging are the key drivers in the Indian biscuit market. The proposed biscuit industry accounts for more than 70% of the aggregate value of the Indian biscuit market, according to the new report, India Biscuit Market Overview, 2017-2023. Share Rs 400 billion market value by 2023: The industry is expected to record sales of over INR 400 billion by 2023. Culinary market consists of all household biscuit firms dealing in both rural and urban areas. Some players have a broad distribution network into the countryside: Parlé and Britain, for example. In villages any general shop has the packet of Parlé biscuits for five rupees,it is relatively easy in this case. In the organised category, cream, cookies, sugar, Marie, crackers, milk and other-products ascribable to biscuit are sold. Some of the clients here are Surya Food and Agro Limited (PNB), Britannia, Parlé, ETCE among others. make all four of the biscuits. Biscuits supplied in rural areas have improved in recent years. In addition to Indian major players, multiple regional companies produce cookies. The plays also have the same production facilities as the famous ones. Poor people can afford their biscuits there too because they are affordable, packaged in nice bags and just healthy enough. In response to slumping sales and reduced product values, firms have introduced an up-market biscuit in an under-size packaging. Urban Areas: Hypermarkets, supermarkets, general stores and "e-retailing" based distribution channels are prevalent in the urban locales. Electronic marketing provides discounts and gives consumers a choice during the months of holidays. It's easy to buy them online from the convenience of your living room. Some of India's top electronics retailers like Flipkart, Amazon, and Snapdeal have stocked biscuits of both Indian and International brands.

Statement of the Problem

In today's competitive market, it is essential for companies like Britannia to not only sell quality products but also to market them and build strong relationships with customers effectively. However, it is unclear whether Britannia's current retail marketing strategies are fully effective in attracting and retaining customers. It is also necessary to understand how well the company engages with its customers at the retail level and whether these efforts lead to customer satisfaction and loyalty. This study aims to identify gaps in Britannia's retail marketing practices and customer engagement and suggest ways to improve them for better business outcomes.

Need for the Study

This study is essential because Britannia is one of India's leading FMCG (Fast-Moving Consumer Goods) brands, and its retail marketing strategies significantly attract customers. With growing competition in the food and beverage industry, it is essential to understand how Britannia promotes its products in retail stores and connects with its customers. This analysis can help identify what is working well and what can be improved to increase customer satisfaction and sales.

Objectives of the Study

Primary Objective

To analyse Britannia's retail marketing strategies and customer engagement practices.

Secondary Objectives

- ❖ To understand Britannia's positioning and branding efforts.
- ❖ To examine customer perception and behaviour toward Britannia products.
- ❖ To evaluate the effectiveness of Britannia's in-store promotions and visibility.
- ❖ To suggest improvements in marketing and engagement.

Scope of the Study

- ★ The study focuses on Britannia's retail marketing practices, such as in-store promotions, product placement, packaging, pricing strategies, and visual displays.
- ★ It also covers customer engagement efforts like feedback systems, loyalty programs, and digital promotions.
- ★ The study mainly looks at the Indian market, focusing on urban retail settings like supermarkets, grocery stores, and retail chains.
- ★ It includes feedback from both customers and retailers to get a balanced view.

Limitations of the Study

- The study is limited to selected retail outlets and may not reflect the practices across all regions or stores.
- Time and budget constraints may limit the number of customer and retailer interviews or surveys.
- Rapid changes in market trends or consumer behaviour may affect the findings.
- The study relies on available data and responses, which may sometimes be biased or incomplete.

RESEARCH METHODOLOGY

Research design is a systematic approach for collecting, interpreting and analyzing information to help and frame the research problem. You might consider it the science of investigating the approach of science itself applying to research. We explore the variety of paths a researcher generally follows when exploring their research problem, and why, in this section. Researchers should know the methodology and research methods.

Domain of the Study

This study falls under the domain of Marketing Management, with a specific focus on Retail Marketing and Customer Relationship Management (CRM). It aims to explore how Britannia Industries Ltd. strategizes and implements its retail marketing practices to attract and retain customers, enhance customer satisfaction, and build brand loyalty. The study also delves into the company's customer engagement techniques across various retail platforms, including traditional outlets and modern trade channels.

Research Design

The research design serves as the descriptive framework to guide the actual research.
It is the framework within which data are collected, measured and analyzed. The design incorporates a description of the gamified researcher's work, from the writing of the hypothesis and its operational consequences to the last.
In brief, the research design should include, at a minimum, an explication of the research problem, Procedures, and data gathering techniques. The study sample and methods of treatment and analysis.

Population

A research population is generally an extensive collection of individuals or objects with similar characteristics, which is the primary focus of a scientific query. Research is done for the benefit of the population.

Sample Size

Sample size is the number of items chosen from the entire universe to make up a sample. Neither an excessively large nor a small sample size is ideal. It must be at its best. The sample size ought to reflect the population it was taken

from. The cost of data collection and the requirement for adequate statistical power are the two main factors that determine the sample size used in a study. The sample size is one hundred people.

Sampling Technique

Researchers employ sampling techniques as a means of conducting statistical sampling. After studying a sample of the same population, researchers use this procedure to draw conclusions about the entire population. Simple Random Sampling is the sampling strategy employed in this research. With simple random sampling, every member of the population has an equal chance of being selected for the sample, making it the purest and most straightforward probability sampling technique. By using simple random sampling, the selection process is free of bias.

Methods of Data Collection

After a clear research problem and a research design or plan are established, the data collection task starts. The researcher should consider two categories of data when choosing the study's data collection strategy, i.e. E. both primary and secondary information.

Primary Data

The researchers gather the data and finish the study process without consulting any secondary sources, so it is first-hand information. Large businesses with strong funding prospects are able to conduct primary research through surveys, in-person interviews, social media polls, feedback, and analysis of customer feedback, as well as by receiving responses through email. First-hand information and primary data served as the study's main sources of information. A random sampling technique was used to choose the participants. A sample of 100 Chennai-based respondents provided the data.

Mode of Collection of Primary Data

A structured questionnaire was used to collect data, comprising 15 questions. The gathered data was then transferred to a master table to facilitate an easy analysis of the study.

Secondary Data

It is data that has previously been gathered, prepared, and examined by someone. The findings are usable and may be useful to other researchers in the future who need to consult the data for their research. Government consensus, annual reports from public agencies, magazines, newspapers, journals, online databases, etc. are a few instances of secondary research. Based on the research topic and web searches, secondary sources such as newspaper articles, magazines, and the internet have gathered the information and research papers that are required.

Questionnaire Design

A questionnaire containing 15 questions was constructed to study an analysis of Britannia's retail marketing practices and customer engagement. The questionnaire comprisesclosed-ended questions and a Likert rating scale.

Data analysis and interpretation

ANOVA

One-Way ANOVA – Satisfaction with Britannia by Age Group

- F-value: 2.327
- Significance (p-value): 0.043
- Interpretation: Table 4.12 shows that since the p-value is less than 0.05, it shows a statistically significant difference in satisfaction levels among different age groups.
- This means age has a meaningful impact on how satisfied consumers are with Britannia products

FINDINGS

In the ANOVA table, Table 1 shows that since the p-value is less than 0.05, there is a statistically significant difference in satisfaction levels among different age groups. This means age directly impacts how satisfied consumers are with Britannia products.

Suggestions for Britannia to Improve Customer Engagement

Personalized Digital Campaigns

What to Do: Leverage AI and data analytics to run targeted digital marketing campaigns on platforms like Instagram, YouTube, and Google.

Why: Younger audiences spend more time online and expect personalized content and product recommendations.

Example: Customized ads for health-focused biscuits like NutriChoice for fitness enthusiasts and Treat for kids/teenagers.

Interactive Social Media Engagement

What to Do: Run weekly challenges, polls, user-generated content (UGC) contests, and story-based polls.

Why: Engages users and increases brand visibility through shareable content.

Example: MyGoodDayMoment contest, where customers post pictures of their special moments with Good Day biscuits.

Loyalty Program or Britannia Club App

What to Do: Launch a mobile app or loyalty card offering points for purchases, referrals, feedback, and reviews.

Why: Encourages repeat purchases and builds a direct communication channel with customers.

Example: Redeem points for exclusive Britannia merchandise or coupons.

Co-Creation Campaigns

What to Do: Allow customers to vote on or suggest new biscuit flavors, packaging designs, or slogans.

Why: Builds emotional ownership and involvement in product development.

Example: "Design the Next Good Day Pack" competition for design students and artists.

Influencer & Micro-Influencer Collaborations

What to Do: Work with food bloggers, fitness influencers, and parenting content creators to promote products authentically.

Why: Influencer trust can drive organic and authentic engagement, especially in niche segments.

Example: NutriChoice promoted by a popular dietitian or mommy-blogger.

In-Store Augmented Reality (AR) Experiences

What to Do: Introduce AR-based interactive games or product stories in retail stores via mobile scanning.

Why: Creates memorable brand interactions and draws footfall.

Example: Scan a pack of Treats to unlock a mini-game for kids.

Local Festival & Regional Campaign Tie-ins

What to Do: Run campaigns tied to local festivals (Pongal, Onam, Diwali, Baisakhi) with regionally flavored biscuits.

Why: Builds cultural connection and enhances regional brand loyalty.

Example: Launch a "Diwali Gifting Pack" of assorted Britannia biscuits with a special box design.

CSR-Driven Engagement (Emotional Branding)

What to Do: Involve customers in CSR by linking purchases to charity/donation initiatives (e.g., feeding children).

Why: Modern consumers value socially responsible brands and are likelier to engage.

Example: "For every pack you buy, we donate one to a needy child."

Gamification on Website/App
What to Do: Add games, quizzes, and activities on the Britannia app or website that reward users.
Why: Encourages repeat visits and builds a fun and loyal user base.
Example: "Spin the Wheel" to win coupons, or "Biscuit Trivia" games.

Feedback Loop and Community Forums
What to Do: Create a community platform or feedback hub where customers can share opinions, recipes, and ideas.
Why: Creates a sense of belonging and provides real-time market feedback.
Example: Britannia Recipe Club – fans share recipes using Marie Gold or NutriChoice biscuits.

CONCLUSION

Britannia has a strong presence in retail stores and is well-known among customers for its popular products. However, while the brand is familiar, it still has room to build a deeper connection with its customers. Currently, most of its customer engagement at the retail level is focused on selling products rather than creating a memorable or enjoyable shopping experience. The company can benefit by making its marketing more personalized and using technology to connect better with store shoppers. Also, combining its online marketing with in-store promotions can improve the overall customer experience. As customer preferences are changing quickly, especially after COVID, and with growing interest in health,Britannia needs to study the market and update its retail strategies regularly. By doing this, the company can stay competitive, strengthen customer loyalty, and improve its retail marketing practices.

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Table 1: Gender Wise Respondents

		Sum of Squares	df	Mean Square	F	Sig.
	Between Groups	1.436	1	1.436	1.197	.277
Q1	Within Groups	117.554	98	1.200		
	Total	118.990	99			
	Between Groups	.918	1	.918	.410	.524
Q2	Within Groups	219.672	98	2.242		
	Total	220.590	99			
	Between Groups	.689	1	.689	.536	.466
Q3	Within Groups	126.061	98	1.286		
	Total	126.750	99			
	Between Groups	.821	1	.821	.379	.539
Q4	Within Groups	212.089	98	2.164		
	Total	212.910	99			
	Between Groups	.387	1	.387	.221	.639
Q6	Within Groups	171.403	98	1.749		
	Total	171.790	99			
	Between Groups	4.842	1	4.842	2.591	.111
Q7	Within Groups	183.158	98	1.869		
	Total	188.000	99			
	Between Groups	3.184	1	3.184	1.676	.199
Q10	Within Groups	186.176	98	1.900		
	Total	189.360	99			
	Between Groups	.244	1	.244	.116	.734
Q11	Within Groups	206.666	98	2.109		
	Total	206.910	99			

REVIEW ARTICLE

Documentation of Various *Varmam* Injuries (*Odivu Murivugal)* Mentioned in Siddha *Varmam* Literatures and Correlation with Modern Science - A Literature Review

M. Fouziya Thasim[1]*, N.J. Muthukumar[2] and P. Samundeswari[3]

[1]PG Scholar, Department of Varma Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University), Chennai, Tamil Nadu, India.
[2]Professor and Director General, Central Council for Research in Siddha, Chennai, (Ministry of Ayush, Government of India), Tamil Nadu, India.
[3]Assitant Professor, Department of Varma Maruthuvam, National Institute of Siddha, Tambaram Sanatoruim, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University Guindy), Chennai, Tamil Nadu, India.

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***Address for Correspondence**
M. Fouziya Thasim
PG Scholar, Department of Varma Maruthuvam,
National Institute of Siddha, Tambaram Sanatoruim,
(Affiliated to The Tamil Nadu Dr. M.G.R. Medical University),
Chennai, Tamil Nadu, India.
E.Mail: fouziyathasim@gmail.com

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ABSTRACT

Siddha system is one of the traditional system of medicine which provides holistic health. *Varmam* is one of the unique speciality in the *siddha* system of medicine. It is the vital energy circulating inside the body, which can be regulated by pressure manipulation over a prescribed *Varmam* points with a particular force for the specified time. *Varmam* injury occurs when a particular force implied on a particular *Varma* point (*Varma thalam*). The medicine which deals with the *Varmam* injuries is known as *Varma Odivu Murivu Maruthuvam* (Fracture injury). It deals with the management of fractures, dislocations, subluxations and injuries of nerves, vessels, ligaments and tendons. Many available *Varmam* literatures deals with different school of thoughts in terms of name, location, various types of *Varmam* injuries (*Odivu Murivugal*) yet it was difficult to understand clearly about what they were described and that was also not in practical application. Hence this study will focus on compilation of various *Varmam* injuries (*Odivu Murivugal*) by highlighting the different types of injuries related to *Varmam* mentioned in the *Varmam* literatures and few of the *Varmam* injuries were correlated with modern science for better understanding. In future, this literature review may used in clinical purpose for the diagnosis of that condition in practical application and also for the academic purpose.

Keywords: *Odivu Murivugal,* Fractures, *Varmam* injuries, *Vilagal*, Dislocations.

INTRODUCTION

Siddha system is one of the traditional system of medicine which provides holistic health. *Varmam* is one of the unique speciality in the siddha system of medicine. *Varmam* is the state in which the vital energy (flow of *vaasi* or *pranan*) circulates or spreads throughout the body. Sometimes this flow of energy can be blocked by attack of external factors in certain parts of the body. This can be regulated by pressure manipulation over a prescribed *Varmam* points with a particular force for the specified time which is known as *Varmam* therapy. There are numerous vital points in the human body that are located in the junction of nerves, joints, bones, muscles, ligaments, internal organs. These are interconnected by channels in definite way and governing on various vital activities of human body. Injury occurs when a force hits on the body and it affects organs like skin, muscle, tendons, membranes, joints, bones, nerves. *Varmam* injury occurs when a particular force implied on a particular *Varma* point (*Varma thalam)*. There are different types of *Varmam* injuries in *Varma* texts namely *odivu murivugal, vilagal, nazhuval, savvu kayangal, thasai naan kayangal* etc.. The medicine which deals with the *Varmam* injuries is known as *Varma Odivu Murivu Maruthuvam* (Fracture injury). It deals with the management of fractures, dislocations, subluxations and injuries of nerves, vessels, ligaments and tendons. The different types of *Varmam* injuries given in various *Varmam* texts which is difficult to locate or find the particular type of injury. In this literature review, the *Varmam* injuries were documented and compiled based on the regions they located and based on the type of injury. And also this review deals with the modern correlation of *Varmam* injuries to facilitate better understanding.

MATERIALS AND METHODS

STUDY TYPE: Literature review
STUDY PERIOD: 6 months
STUDY PLACE: National Institute of siddha, Tambaram sanatorium
VISITED LIBRARIES: • Roja muthiah research library, Taramani • Government siddha medical college and hospital, Arumbakkam • Siddha central research library, Arumabakkam • Library of National Institute of Siddha, Tambaram sanatorium.

RESULTS

List of *Varmam* literatures reviewed regarding *Varma* injuries: Table 1
Types of *Varmam* injuries and its modern correlation: Table 2 to table 9
The above table 1 outlines the list of ten *Varmam* literatures which have foot prints of *Varmam* injuries.
The above **2** table presents a comprehensive correlation between *Varmam* injuries n the upper limb and modern medical terminology. It categorizes 14 types of injuries, including fracture of clavicle, scapula, humerus, forearm and wrist bones, metacarpals and phalanges. The above table **3** presents a comprehensive correlation between *Varmam* injuries in the lower limbs and modern medical terminology. The table categorizes 11 types of *Varmam* injuries, including fracture of long bones in thigh and leg along with metatarsals and phalanges in foot. The above table **4** presents a list of *Varmam* injuries in the head, neck and face and its correlation with modern medical terminology. The table categorizes 8 types of varmam injuries, including fracture of Skull, orbital bone, nasal bone, mandible and also fracture of frontonasal suture, parietal and occipital bone. The above table **5** presents a list of *Varmam* injuries in the axial skeleton and its correlation with modern medical terminology. It categorizes 7 types of injuries especially fracture to the sternum and its parts like manubrirum, body and xiphoid process, fracture to ribs and hyoid bone. The above table 6 lists out the vilagal (dislocations) in varmam literatures and its correlation with modern medical terminology.
The table categorizes dislocations in both upper and lower limbs. Including
• Dislocations of acromioclaviclular joint, shoulder joint, elbow and wrist joints in the upper limb
• Dislocations of hip joint, knee joint, talus and imterphalangeal joint in lower limb

The above table 7 list outs the 5 types of *Nazhuval* (Subluxations) in *Varmam* literatures and correlated with modern medical terminology. It facilitates the understanding of varmam concepts and its integration with modern practices. This table 8 represents the list of *Savvu kayangal* (ligament injuries) and its modern correlation and also it categorizes the various types of injuries, those affecting the shoulder, wrist, knee, and ankle. This table 9 represents the list of *Murindhu vilagal* (fracture dislocations) and its correlation with modern terminology. The table also categorizes the various types of fracture dislocations those affecting the cervical spine, thoracic spine, lumbar spine, coccyx, ribs, costal cartilages and sternum.

SOME EXAMPLES ALONG WITH VERSES IN EACH TYPES OF *VARMAM* INJURIES

Injuries in upper limb

Kaarai elumbin nadupaguthi murivu / thirai nadu murivu – Mid clavicular fracture

- *Nalamaga kaarai nadumaiyathil thane*
- *Naadiyea murindhalo nadukamaagi*
  - *Varma odivu murivu saari* 1500
- *Thamaraga kazhuthinadi kaarai ellu*
- *Idikondu naduvathilo murindhittalo*
  - *Odivu murivu kattu soothiram* 60

***Muzhankai maiyya/thandupaguthi murivu/kai mannai murivu* – Fracture of shaft of forearm bones**

- *Pathiyana karamathilea kendaithanni*
- *Panbudanea maiyamdhu odiyumanaal*
- *Varma odivu murivu saari* 1500
- *Kelappa muzhankaiyin mathipathil*
- *Kettiyaga murindhadhendral*
  - *Varma nithanam* 500
- *Asaivana kaimannai elumburendum*
- *Appanea naduvodindhal paazhathagum*
  - *Odivu murivu kattu soothiram* 60
- *Kandidavea muzhankaithaan mathipathil*
- *Kadithaga odiyumagil kutramaagum*
  - *Odivu murivu kattu murai saari 110*

***Muzhankai mootu vilagal* – Dislocation of elbow joint**

- *Naadiyea muzhankaiyil balamuraithu*

Nandraga muttadhilea visaithaaneri
Saadimuzhankai elumbu muttaivittu
Thaanaga saadivisai konduthendral

- *Varma odivu murivu saari 1500,*

- *Idhamana muzhankaithan baarayeettaal*

Isangidum muttadhuvum idarinindral

- *Odivu murivu kattu murai saari*

Injuries of lower limb

***Thodai enbu thandu murivu* – Fracture of shaft of femur**

- *Kelappa thodainaduvin mathipathil*

Kettiyana varmamadhu murindhathaanal

- *-Varma Nithanam* 500

- *Thappana thudaimaiyam thanodindhu*

Thalladi savvatru purameasadil

○ *Odivu murivu kattu murai saari* 110

***Padankaal enbu murivu* – Fracture of metatarsals**

• *Paarappa padangalin naduvuthannil*
Pathiyavea odinthathendral sollakelu
○ *Varma odivu murivu sara soothiram 1200*
• *Thaanana nadupadam murindhadhanaal*
○ *Odivu murivu kattumurai saari*
• *Theerumea nadupadankaal murinerukki*
○ *Odivu murivu kattu soothiram*

Injuries in vertebral column

***Anjunirai mun elumbu murivu* – Cervical spine injury**

• *Anbaana kazhuthadiyin pinpakkathil*
Arulana anjunirai munnelumbil
Thunbamutru murindhu iyanginindru kondaal
Sootchamoru moondramnaal maranamaavaan
Narambana kazhuthuthaan odiyumaanal
○ *-Odivu murivu kattumurai saari* 110

***Puthelumbu murivu* –Fracture of Transverse process of vertebrae**

• *Nilaiyana narambunilai saripattegum*
Nindrmadhil puthelumbu murindha dhaanaal
○ *Varma odivu murivu saari* 1500

Injury to ribs

***Vaari elumbu kayangal* – Injury to rib bones**

• *Paarthidunee vaariellu murindhu thazhnthaal*
○ *Varma odivu murivu sara soothiram* 1200
• *Kooravea pakkamathil vaariellu*
Koodiyea murindhudanea ullaethaznthaa
○ *odivu murivu kattumurai saari* 110

Injury of skull

***Kan keezh mel ulla ellu murivu* - Blowout fracture of orbital bone**

Naayaganea nayanamadhil melumkeezhum
Nandrenavea valaimthirukum ellurendu
Vaayagamai modhivizhunthu odiyumaagil
○ *Odivu Murivu Kattu Murai Saari*

DISCUSSION

In this Literature Review, ten *Varmam* literatures are cited for the compilation of *Varmam* injuries which were reviewed by visiting various Siddha libraries. There are nearly 240 *Varmam* injuries were mentioned in ten different *Varmam* texts. The injuries are enumerated and classified into eight categories namely fractures, dislocations, subluxations, ligament injuries and fracture dislocations, nerve injuries, soft tissue injuries and *Varmam* injuries. They are arranged according to the regions—head and neck, lower limb, and axial skeleton—where the injury takes place. Subsequently, they are correlated with modern science. By comparing traditional descriptions with current medical knowledge, researchers can gain insights into the diagnosis and treatment of these conditions in a traditional manner.

Additionally, this correlation can help bridge the gap between traditional and modern medicine, leading to improved patient care and outcomes. The most common types of injuries discovered include dislocations, *narambu kaayngal* (nerve injuries), and *Murivugal / Odivugal* (fractures). Very few additional injuries, including *Savvu kayangal*, *Murindhu vilagal, Menthisu kaayangal* and *Nazhuval* were observed. All literature recite the majority of common injuries, such as *Thol puja netti thazhal, Gundumani vilagal*, *Muzhankai,* and *Muzhankaal mathi murivu*. The specific injuries like *kankeezh mel ulla ellu murivu* (fracture of orbital bone), *Alavelumbu murivu* (fracture of mandible) were described only in the text *Odivu Murivu Kattu Murai Saari*. Only the texts *Varma Odivu Murivu Saari Soothiram* 1500 and *Varma Odivu Murivu Sara Soothiram* 1200 discuss the injuries related to the skull, ribs,vertebrae,nerves and soft tissues. Not only fractures and dislocations, but ligament injuries *(savvu kayangal*) like *manikattu savvu kayam* (wrist ligament injury), *mootu savvu kayam* (knee ligament injury) and ankle sprain with their symptoms and line of treatment were also mentioned in that reviewed literatures. This literature review and analysis indicates that major types of injuries are mentioned in the texts such as *Varma odivu murivu saari* 1500, *Varma odivu murivu sara soothiram* 1200, *Odivu murivu kattu murai saari* 110, and 60. It is also noted that the same injury was mentioned under different names in the texts, indicating variations in terminology across different sources. Besides the types of injuries, there symptoms and management of injuries were also mentioned in that literatures. Some of the injuries like *kuthiraimuga mathi murivu*, *kuthi naramabu kayam* were considered as dangerous type of injury and also they were difficult to treat. In the management of injuries, specific types of management or treatment methods were mentioned for each type of injuries. The treatment methods includes internal medicines like *murivuku chooranam, elathy nei* and external medicines like *murivuennai, oorennai, vasavu ennai* etc. Along with internal and external medicines, external therapies like varmam manipulation techniques, ottradam (fomentation), pattru (poultice), pattikattal or kattu muraigal (bandaging techniques) also were mentioned Additionally, the detailed descriptions of these injuries suggest a deep understanding of anatomy and medical knowledge during that time period. Furthermore, the texts also provide information on the treatment methods for these injuries. Overall, this review highlights the advanced medical practices and knowledge present in ancient Indian literature related to musculoskeletal injuries. Compiling all this information can provide valuable insights into the medical practices of ancient India and help bridge the gap between traditional and modern medicine.

CONCLUSION

The literature review includes the compilation of different types of *Varmam* injuries and also they are correlated with modern science. This literature review may used in academic purpose for the better understanding of that condition in future. It may also helpful in clinical purpose for diagnosis of that injury and also for research and documentation.

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Table 1: List of *Varmam* literatures which has footprints of *Varma* injuries

S.NO:	*VARMAM* LITERATURES
1	*Varma odivu murivu saari soothiram 1500*
2	*Varma odivu murivu maruthuvam*
3	*Varma odivu murivu gnanam*
4	*Odivu murivu kattu soothiram 60*
5	*Odivu murivu kattu saari 110*
6	*Agathiyar odivu murivu saari soothiram*
7	*Varma odivu murivu saari 1200*
8	*Sathuramani soothiram*
10	*Varma nithanam* 500

Table 2: Types of *Varmam* injuries in upper limb and its modern correlation

S NO	*MURIVUGAL* IN UPPER LIMBS	MODERN CORRELATION
1.	*Kaarai enbu murivu*	Fracture of clavicle
2	*Kaarai elumbin nadupaguthi murivu/thirai nadu murivu*	Mid clavicular Fracture
3	*Sippi enbu murivu*	Fracture of scapula
4	*Puja enbin mel munai murivu/ gundumani norungal*	Fracture of proximal humeral head
5	*Puja elumbin udal kooru kazhuthu paguthi murivu*	Fracture of anatomical neck of humerus
6	*Puja elumbin aruvai kazhuthu paguthi murivu*	Fracture of surgical neck of humerus
7	*Puja elumbin thandu paguthi murivu/ mundakai enbu murivu/ thivalai enbu murivu*	Fracture of shaft of humerus
8	*Mundakai keezhpaguthi murivu*	Fracture of lower end of humerus
9	*Muzhankai elumbugalin melmunai murivu*	Fracture of proximal end of forearm bones
10	*Muzhankai elumbugalin keezh paguthi murivu*	Fracture of distal end of forearm bones
11	*Kai mannai murivu/ muzhankai maiyya (thandu) paguthi murivu/ kai mathi murivu*	Fracture of shaft of forearm bones
12	*Manipantham murivu*	Fracture of wrist bones
13	*Padankai odivu/ karaba enbu murivu/ullankai murivu*	Fracture of metacarpals
14	*Viral murivu*	Fracture of phalanx

Table 3: Types of *Varmam* injuries in lower limb and its modern correlation

S NO	*MURIVUGAL* IN LOWER LIMBS	MODERN CORRELATION
1	*Thodai enbu thalai paguthi murivu*	Fracture of head of femur
2	*Thodai nadu paguthi murivu/ thodai maiyya murivu/ nadu thodai enbu odivu*	Fracture of shaft of femur
3	*Muttu murivu*	Fracture of knee
4	*Kuthirai muga mathi murivu(thirantha murivu)/ muzhangal mathi murivu (maiyya enbu) murivu*	Fracture of shaft of tibia and fibula
5	*Kanukaal enbu murivu*	Fracture of ankle

6	*Thattai varma paguthi murivu/ Kaal kulachu enbu kayam*	Fracture of talus
7	*Kudhi vellai murivu/ uppu kutri kaalam murivu*	Fracture of calcaneum
8	*Kaal kulachu padankaal poruthu kayam*	Injury to tarsometatarsal joint
9	*Padankaal nadu murivu*	Fracture of metatarsal bones
10	*Padankaaal – viral poruthu murivu*	Fracture of metatarsophalangeal joint
11	*Peruviral kannu murivu*	Fracture of great toe
12	*Poovelumbu murivu*	Fracture of sesamoid bone

Table 4: Types of *Varmam* injuries in head,neck and face and its modern correlation

S NO	MURIVUGAL IN HEAD, NECK and FACE	MODERN COMPARISON
1	*Kabala murivu*	Skull fracture
2	*Mandai ucchi murivu*	Fracture of parietal bone
3	*Pidari enbu murivu*	Fracture of occipital bone
4	*Kan keezh mellulla ellu murivu*	Fracture of orbital bone
5	*Thilartha kaala poruthu murivu*	Fracture of frontonasal suture
6	*Naasi nadu murivu*	Fracture of nasal bone
7	*Aakiranabala kurunthu murivu*	Fracture of nasal bridge
8	*Alavelumbu murivu*	Fracture of mandible

Table 5: Types of *Varmam* injuries *murivugal* in axial skeleton and its modern correlation

S no	***Murivugal* in axial skeleton**	**Modern correlation**
1	*Thudi enbu murivu*	Fracture of manubrium
2	*Pushpathi nadu murivu*	Fracture of body of sternum
3	*Nadumathi elumbinadi murivu*	Fracture of xiphoid process
4	*Nenjelumbu norungal*	Injury of sternum
5	*Thanu elumbu/ sanguthiri enbu murivu*	Fracture of hyoid bone
6	*Vaari ellu murivu*	Fracture of rib bones
7	*Moonara kayam*	Injury of sacrum

Table 6: Types of *Varmam* injuries *Vilagal* in upper and lower limb and its modern correlation

S NO	***Vilagal* (dislocations)/ *Thazhal* in upper limb**	**Modern correlation**
1	*Karai enbu vilagal*	Dislocation of clavicle
2	*Karai enbu munai vilagal*	Dislocation of lateral end of clavicle
3	*Kaarai enbu munpakka vilagal*	Anterior dislocation of clavicle
4	*Kai puja sippi thazhal/ thol poruthu vagu enbu thazhal*	Dislocation of acromio humeral joint
5	*Urulai perunsippi nagandal/ Thol puja netti thazahal*	Dislocation of shoulder joint
6	*Kai kuzhi gundu isangal*	Dislocation of head of humerus
7	*Kai muttu vilagal*	Dislocation of elbow joint
8	*Izhupatta muzhankai mootu*	Pulled elbow
9	*Manipantham vilagal*	Dislocation of wrist
10	*Nadu viral poruthu vilagal*	Dislocation of interphalangeal joint of middle finger
S NO	***Vilagal* in lower limb**	**Modern correlation**
11	*Gundumani vilagal/ Urulai pootu vittal/ Iduppu poruthu vilagal/ gundu vilagal*	Dislocation of hip joint

12	*Muzhankaal muttu vilagal*	Dislocation of knee
13	*Kuthikaal gundu vilagal/Nilai gundu vilagal / Kaaladi gundu isangal*	Dislocation of talus
14	*Viralidai poruthu vilagal*	Dislocation of interpahalangeal joint of phalanges

Table 7: Types of *Varmam* injuries *Nazhuval* (subluxations) and its modern correlation

S NO	*NAZHUVAL*	*MODERN CORRELATION*
1	*Manikattu gundu nazhuval*	Subluxation of wrist joint
2	*Pujam nazhuval*	Subluxation of humerus
3	*Muttu nazhuval*	Subluxation of knee
4	*Naangana poruthu nazhuval*	Subluxation of sacroiliac joint
5	*Muchadhura palagai nazhuval*	Subluxation of sacro coccygeal joint

Table 8: Types of *Varmam* injuries *Savvu kayangal* (ligament injuries) and its modern correlation

S NO	*SAVVU KAYANGAL* (LIGAMENT INJURIES)	MODERN CORRELATION
1	*Thol poruthu savvu kayam*	Injury to shoulder ligament
2	*Manikattu savvu kayam*	Injury to ligaments of wrist
3	*Muttu savvatral/ muzhankaal muttu savvu murivu/ muttu savvu murivu*	Knee ligament injury
4	*Kanukaal suluku*	Ankle sprain

Table 9: Types of *Varmam* injuries *murindhu vilagal* (fracture dislocations) and its modern correlation

S NO	MURINDHU VILAGAL (FRACTURE DISLOCATIONS)	MODERN CORRELATION
1	*Kazhuthu natellu murivu/ anju nirai elumbu*	Fracture of cervical spine
2	*Maarbu and iduppu natelumbu*	Fracture of thoracic and lumbar spine
3	*Mudhugu natellu murivu*	Fracture of lumbar spine
4	*Mookai enbu murivu*	Fracture of spinous process of vertebrae
5	*Puthelumbu murivu*	Fracture of transverse process of vertebrae
7	*Kathir elumbu murivu/ monai ellu murivu*	Fracture of coccyx
8	*Koogai ellu murindhu vilagal*	Fracture dislocation of thoracic spine
9	*Thilai enbu murindu vilagal*	Fracture dislocation of upper thoracic spine
10	*Poonellumbin keezh elumbu murindhu vilagal*	Fracture dislocation of lower thoracic costovertebral joints
11	*Poonellu murindhu thazhal*	Fracture dislocation of costal cartilage
12	*Vaari ellu murindhu thazhal*	Fracture dislocation of ribs
13	*Nadumathi elumbin adi murinthu vilagal*	Fracture dislocation of manubrium of sternum
14	*Pirai ellu murindhu agam or puram vilagal*	Fracture dislocation of sternocostal joint of first rib

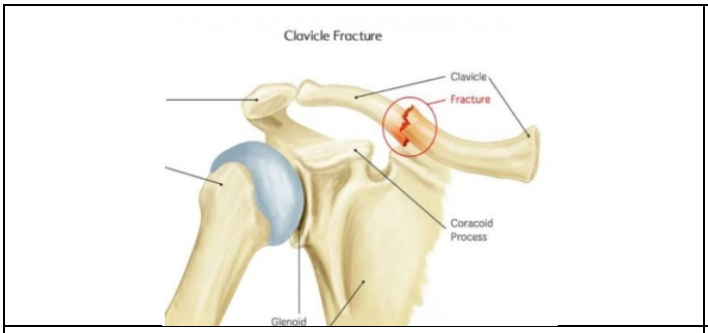


Image 1: Fracture of clavicle

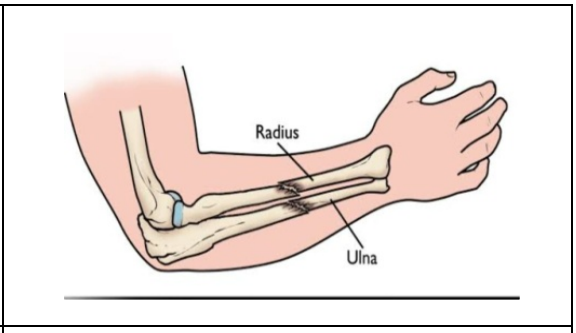
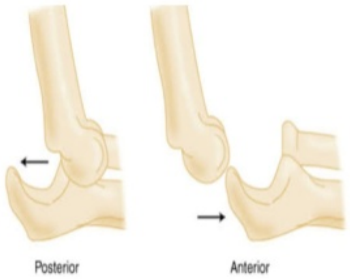


Image 2: Fracture of shaft of radius and ulna



3: Dislocation of elbow

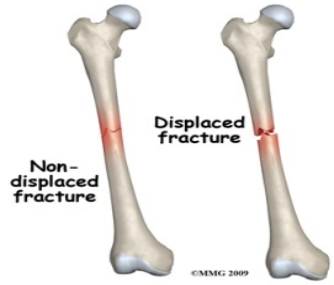


Image 4: Fracture of shaft of femur

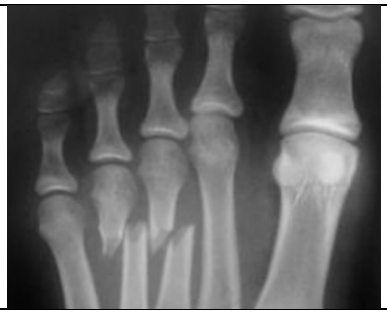


Image 5: Fracture of Metatarsal bones

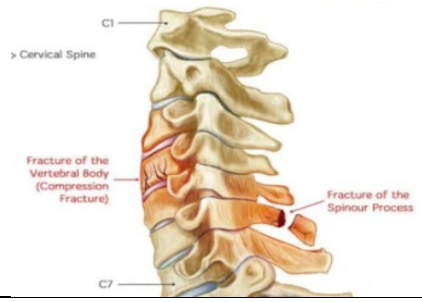


Image 6: Fracture of body of cervical vertebrae

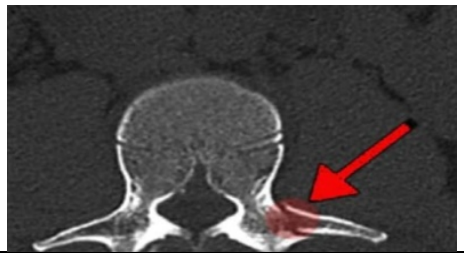


Image 7: Fracture of transverse process of vertebrae

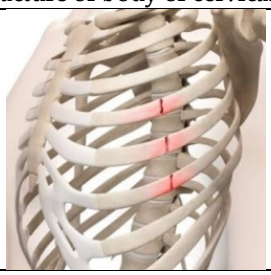


Image 8: Fracture of rib bones

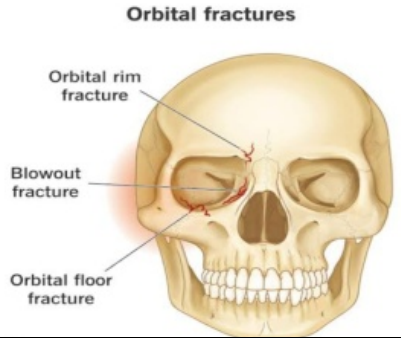


Image 9: Fracture of orbital bone

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REVIEW ARTICLE

Role of Plant Growth Promoting Rhizobacteria (PGPR) acting as Biofertilizers and their Biological Approach towards the Salinity Stress: A Comprehensive Review

Zeeshan Ali[1], Rekha Sirohi[2], Mohammad Rehan[1] and Umesh Kumar[3]*

[1]Ph.D Scholar, Department of Biochemistry, Sir Syad Faculty of Science, Mohammad Ali Jauhar University, Rampur, Uttar Pradesh, India.
[2]Assistant Professor, Department of Biochemistry, Sir Syad Faculty of Science, Mohammad Ali Jauhar University, Rampur, Uttar Pradesh, India.
[3]Associate Professor, College of Allied Health Care (Affiliated to Swami Vivekanand Subharti University), Meerut, Uttar Pradesh, India.

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***Address for Correspondence**
Umesh Kumar
Associate Professor,
College of Allied Health Care
(Affiliated to Swami Vivekanand Subharti University),
Meerut, Uttar Pradesh, India.
E.Mail: umesh.biochem@gmail.com

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ABSTRACT

Salinity stress is a major environmental factor that negatively affects plant growth and agricultural productivity. PGPR, which are beneficial bacteria residing in the rhizosphere, have been recognized for their potential to mitigate the adverse effects of salt stress on plants by promoting growth, enhancing nutrient uptake, and improving stress tolerance. In this study, PGPR strains were isolated from the rhizosphere of *Pisum sativum* grown in saline soil environments. These bacterial isolates were screened for biofilm formation ability as biofilm-producing PGPR strains are known to offer enhanced protection and colonization ability under stressful conditions. The isolated bacteria were characterized through phenotypic and molecular approaches, including 16S rRNA gene sequencing, to identify the bacterial species and assess their biofilm-forming capacity using crystal violet staining. The results showed that several isolates exhibited significant biofilm formation and were identified as strains belonging to genera such as *Pseudomonas*, *Bacillus*, and *Stenotrophomonas*. In vitro tests also demonstrated that these isolates could significantly enhance pea plant growth, improve root development, and reduce the detrimental effects of salinity stress. These findings suggest that biofilm-forming PGPR can serve as a promising biological agent for alleviating the negative impact of salinity stress on *Pisum sativum*, opening new avenues for sustainable agricultural practices under saline conditions.

Keywords: PGPR, Biofilm Formation, Salinity Stress, *Pisum sativum*, Molecular Characterization, Salt Tolerance.

INTRODUCTION

Salinity is one of the most significant abiotic stresses affecting global agriculture, limiting crop productivity, and reducing food security. The increasing salinization of arable lands due to factors such as irrigation practices, climatic changes, and poor soil management has led to a growing concern about its impact on plant growth and crop yields. *Pisum sativum* (pea), a vital legume crop, is highly sensitive to salt stress, which hampers its germination, growth, nutrient uptake, and overall productivity. Therefore, finding sustainable and cost-effective methods to mitigate salinity-induced stress is crucial for improving agricultural output in saline environments. One promising approach to addressing salinity stress is the use of Plant Growth-Promoting Rhizobacteria (PGPR), a group of beneficial bacteria that colonize the plant rhizosphere and stimulate plant growth through various mechanisms. PGPR enhance plant growth by improving nutrient availability, producing phytohormones, fixing nitrogen, and protecting plants against pathogens. More importantly, certain PGPR have the ability to form biofilms—dense, organized communities of bacteria embedded in a self-produced matrix. Biofilm formation enhances bacterial survival and colonization under stressful conditions, including salinity, by providing protection against environmental stresses such as desiccation, UV radiation, and the presence of toxic compounds. Salinity stress is known to induce osmotic and ionic imbalances in plants, which can be alleviated by the application of biofilm-forming PGPR. These bacteria help in mitigating the negative effects of salinity by improving the plant's ability to absorb nutrients, producing exopolysaccharides that protect plant roots from salt-induced dehydration, and stimulating the production of stress-related hormones such as abscisic acid and cytokinin. Moreover, biofilm formation enhances the stability and persistence of these beneficial bacteria in the rhizosphere, ensuring prolonged plant growth promotion under saline conditions. In recent years, molecular tools and techniques, including 16S rRNA gene sequencing, have enabled the identification and characterization of PGPR at the genetic level, offering valuable insights into their functional properties, biofilm formation potential, and mechanisms of salt tolerance. Understanding the molecular basis of biofilm formation and salt tolerance in PGPR is crucial for harnessing their full potential as natural bio-inoculants in agriculture. This study aims to isolate and molecularly characterize biofilm-forming PGPR strains from the rhizosphere of *Pisum sativum* grown under saline conditions. The research will explore the ability of these PGPR to form biofilms, their salt tolerance mechanisms, and their effects on plant growth, providing a foundation for developing PGPR-based biotechnological solutions to combat salinity stress in crops.

Salinity Stress and its Impact on *Pisum sativum* (Pea)

Soil salinity is a major environmental challenge for agriculture, significantly impairing plant growth, productivity, and overall crop yield. In legumes like *Pisum sativum* (pea), salinity stress affects key physiological processes, such as water absorption, ion homeostasis, photosynthesis, and nitrogen fixation. Salt stress induces osmotic and ionic imbalances in plants, leading to dehydration, reduced nutrient uptake, and oxidative stress. Specifically, it leads to the accumulation of harmful ions, like sodium (Na^+) and chloride (Cl^-), which disrupt cellular processes and damage plant tissues (Munns and Tester, 2008). In legumes, such as *Pisum sativum*, the effect of salinity is even more pronounced due to their dependency on nitrogen-fixing bacteria, which are sensitive to high salt concentrations. This leads to a reduction in nitrogen fixation, thus impairing plant nutrition (Zahran, 1999). Consequently, the need for effective solutions to mitigate salt-induced stress is urgent for improving the productivity of legumes under saline conditions.

Role of Plant Growth-Promoting Rhizobacteria (PGPR) in Alleviating Salinity Stress

Plant Growth-Promoting Rhizobacteria (PGPR) are a diverse group of microorganisms that live in the rhizosphere and provide benefits to plants through various mechanisms, including nutrient enhancement, pathogen suppression, and stress tolerance (Glick, 2012). Under saline conditions, PGPR have been shown to enhance plant growth by improving root development, facilitating nutrient uptake, and increasing the plant's resistance to salt-induced damage (Zaidi *et al*., 2006). PGPR can produce growth hormones, such as auxins, cytokinins, and gibberellins, which help in enhancing root elongation and branching, allowing the plant to better access nutrients and water even under saline stress (Sood *et al*., 2018). Additionally, PGPR can produce exopolysaccharides (EPS), which help in creating a

protective biofilm around the plant roots. These biofilms improve bacterial colonization and create a physical barrier against the toxic effects of high salinity, thus improving the overall stress resilience of the plant (Madhaiyan *et al*., 2009).

Biofilm Formation by PGPR and its Role in Stress Tolerance

Biofilm formation is a key feature of many PGPR, which involves the aggregation of bacteria in a self-produced matrix of polysaccharides, proteins, and DNA (Flemming and Wingender, 2010). Biofilm-forming PGPR are particularly beneficial in saline environments due to their enhanced stability, protection from desiccation, and resistance to various environmental stresses. Biofilms protect bacteria from harmful environmental factors, such as high salt concentrations, by creating a microenvironment with reduced osmotic stress (O'Toole *et al*., 2000). The ability of PGPR to form biofilms under saline stress is crucial for their survival and plant growth promotion. Studies have shown that biofilm-forming PGPR can improve plant growth under salt stress by enhancing the uptake of essential nutrients like nitrogen, phosphorus, and potassium (Vivas *et al*., 2010). Furthermore, biofilm formation in PGPR has been linked to the production of specific enzymes and metabolites that enhance stress tolerance in plants, such as exopolysaccharides, which help maintain osmotic balance and mitigate salt-induced damage (Xu *et al*., 2007).

Molecular Characterization of PGPR under Salinity Stress

Recent advances in molecular biology have enabled the identification of PGPR strains and the exploration of their mechanisms of action under salinity stress. Gene sequencing, particularly 16S rRNA gene analysis, has been widely used for the molecular identification of PGPR strains. Moreover, molecular studies have highlighted key genes involved in salt tolerance, such as *ompC* (a porin protein that helps regulate the entry of salt into the bacterial cell) and *glnA* (a gene involved in nitrogen assimilation), which are commonly found in salt-tolerant PGPR (Verma *et al*., 2010). The molecular basis of biofilm formation in PGPR has also been elucidated, with studies identifying the role of genes involved in the synthesis of exopolysaccharides (EPS) and other extracellular components that help in biofilm development. For example, the *pel* and *psl* genes, which are responsible for EPS production in *Pseudomonas* spp., have been shown to be involved in biofilm formation under saline conditions (Flemming and Wingender, 2010).

PGPR-Based Solutions for Salinity Stress Mitigation in *Pisum sativum*

Several studies have demonstrated the beneficial effects of PGPR on alleviating salt stress in different crops, including legumes like *Pisum sativum*. PGPR can significantly improve plant growth parameters such as root length, shoot biomass, and chlorophyll content under saline conditions (Zaidi *et al*., 2009). For example, *Pseudomonas* spp., which are well-known biofilm-forming PGPR, have been shown to improve the growth of *Pisum sativum* under salt stress by enhancing nutrient uptake and mitigating oxidative damage (Chakraborty *et al*., 2011). In a study conducted by Vivas *et al*. (2010), biofilm-forming PGPR strains isolated from saline soils were found to significantly increase pea plant biomass and root length under salt stress. The study attributed this effect to the bacteria's ability to produce EPS and other compounds that alleviated osmotic stress and promoted root development. Furthermore, molecular characterization of these PGPR strains revealed the presence of salt tolerance genes, such as *glnA* and *ompC*, which were linked to their ability to survive and function in saline enviroment.

CONCLUSION

This study demonstrates the potential of biofilm-forming Plant Growth-Promoting Rhizobacteria (PGPR) in mitigating salinity stress in *Pisum sativum* (pea) by enhancing plant growth and promoting stress tolerance. The isolation and molecular characterization of PGPR from the rhizosphere of *Pisum sativum* grown in saline environments revealed that several bacterial strains exhibited strong biofilm-forming capabilities, which is a crucial trait for bacterial survival under saline conditions. The ability of these PGPR strains to form biofilms not only protected them from environmental stressors like salinity but also facilitated their sustained interaction with plant roots, resulting in improved plant growth, enhanced root development, and better nutrient uptake. Molecular identification using 16S rRNA gene sequencing allowed the identification of beneficial strains, including those

belonging to genera such as *Pseudomonas* and *Bacillus*, which are well known for their salt tolerance and biofilm-forming abilities. Furthermore, the salt tolerance experiments confirmed that the isolated PGPR strains could thrive in media containing up to 8% NaCl, highlighting their potential for application in saline soils. Under controlled greenhouse conditions, the inoculation of *Pisum sativum* with these biofilm-forming PGPR strains resulted in significant improvements in plant growth parameters, such as root length, shoot height, fresh weight, and chlorophyll content, even under saline stress. This suggests that PGPR can enhance plant resilience to salinity by improving physiological functions and reducing salt-induced damage. Overall, the findings of this study underscore the promising role of biofilm-forming PGPR as a sustainable, environmentally friendly solution for alleviating salinity stress in crops like *Pisum sativum*. The results support the idea that these bacteria can be used as effective bio-inoculants in saline agriculture to promote crop productivity and improve soil health. Further studies on the molecular mechanisms involved in biofilm formation and salt tolerance in PGPR will provide a deeper understanding of their functionality and pave the way for the development of targeted biotechnological applications in saline environment.

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RESEARCH ARTICLE

Soft Multi Filters

V. Inthumathi[1], A. Gnanasoundari[2]* and M. Maheswari[2]

[1]Associate Professor and Head (Rtd.), Department of Mathematics, Nallamuthu Gounder Mahalingam College, Pollachi, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, Nallamuthu Gounder Mahalingam College, Pollachi, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
A. Gnanasoundari
Assistant Professor,
Department of Mathematics,
Nallamuthu Gounder Mahalingam College, Pollachi,
(Affiliated to Bharathiar University), Coimbatore,
Tamil Nadu, India.
E.Mail: gnana.ngm@gmail.com

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ABSTRACT

This paper's primary goal is to present soft multi filters and describe its fundamental aspects. Additionally, we provide ideas about soft multi filter base, soft multi ultrafilter and convergence of soft multi filter and look into their basic characteristics.

Keywords: Soft multiset, soft multi topology, generalized semi closed soft multiset, generalized semi soft multi connectedness and generalized semi soft multi compactness.
2020 AMS subject classifications: 03E70, 54A05, 54B05, 54A10.

INTRODUCTION

Multiset theory was introduced by Cerf *et al*. [4] in 1971. Then Yager [17] and Blizard [3] initiated further contributions to it. Continuing this study, multiset relation and multiset function were introduced by Girish and John [7,8]. In addition, these authors [9,10] using multiset relations gave multiset topology. In 1999, Molodstov [14] introduced the concept of soft set theory as a mathematical tool for dealing with uncertainties. In 2013, Babitha and John [2] acquainted the thought of soft multi sets as an amalgamation of soft sets and multi sets that can be used to solve real life problems in many fields. Muhammad Riaz *et al*. [15] gave properties of soft multiset topology in 2020. In 2022, Inthumathi *et al*. refined the concepts soft multi points and soft multi neighbourhood in [11,12]. On these bases, they were described the soft multi function. The filter's study is conducted in a very natural manner to characterize convergence in general topology. In 2014, SaziyeYuksel *et al*.[16] proposed the concept of soft filter. Moreover, in 2018 Alireza Bagheri Salec[1]investigated the concept of soft ultrafilter and some of the related

structures. In this paper we acquaint the conception of soft multi filters and also acquaint the notion of soft multi filter base and soft multi ultrafilter and look into their basic characteristic.

Preliminaries

Throughout this paper SMTS denotes the soft multi topological spaces, smset denotes soft multi set.

Definition 2.1. [5] Let M be an universal mset, L be a set of parameters and $I \subseteq L$. Then, an ordered pair (S, I) is called a smset where S is mapping given by $S: I \to P^*(M)$; $P^*(M)$ is the power set of a mset$M$.
For all $l \in I$, $S(l)$ mset represents by count function $C_{S(l)}: M^* \to N$ where N represents the set of non-negative integers and M^* represents the support set of M.

Definition 2.2. [5] Let $\tau \subseteq SMS(M)_L$, then τ is said to be a smtopology on M if the following conditions hold:
1. $\tilde{\phi}$, $\tilde{M}$ belong to τ,
2. The union of any number of smsets in τ belongs to τ,
3. The intersection of any two smsets in τ belongs to τ.
τ is called a smtopology over M and the triple (M, τ, L) is called a SMTS over M. Also, the members of τ are said to be open smsets in M. A soft mset(S, L) in $SMS(M)_L$ is said to be a closed smset in M, if its complement $(S, L)^c$ belongs to τ.

Definition 2.3. [11] Let f_L be a smset over M_L. f_L is called a smpoint over M, if there exists $l \in L$ and $n/v \in M$, $1 \leq n \leq m$ such that
$f(\epsilon) = \begin{cases} \{n/v\} & if\ \epsilon = l, 1 \leq n \leq m \\ \phi & if\ \epsilon \in L - \{l\} \end{cases}$
We denote f_L by $[(n/v)_l]_L$. The family of all smpoints over M is denoted by $P(M, L)$ or P.
i.e. $P(M, L) = \{[(n/v_i)_{l_j}]_L : v_i \in M, l_j \in L, 1 \leq n \leq m\}$.

Definition 2.4. [15] Let $\tilde{\tau}$ be a SMTS and $\tilde{B} \subseteq \tilde{\tau}$. If every element of $\tilde{\tau}$ can be written as a union of members of $\tilde{B}$, then $\tilde{B}$ is called a smbasis for SMTS $\tilde{\tau}$.

Soft multi filters

Definition 3.1. Let M be an universal mset and L be a parameter set. The non-empty family Γ of sub smsets of $\tilde{M}$ is called a soft multi filter (in summary sm-f) if Γ satisfies the following properties.
1. $\tilde{\phi} \notin \Gamma$,
2. If $S_L \in \Gamma$ and $T_L \in \Gamma$, then $S_L \cap T_L \in \Gamma$,
3. If $S_L \in \Gamma$ and $C_{S(l)}(v) \leq C_{T(l)}(v)$ then $T_L \in \Gamma$.

Example 3.2. Let $M = \{2/v_1, 2/v_2\}$, $L = \{l_1, l_2\}$ and $\Gamma = \{\tilde{M}, (S_L)_1, (S_L)_2, (S_L)_3, (S_L)_4, (S_L)_5, (S_L)_6, (S_L)_7, (S_L)_8\}$, where $S_1(l_1) = \{2/v_1\}, S_1(l_2) = \{2/v_1\}, S_2(l_1) = \{2/v_1\}, S_2(l_2) = \{2/v_1, 1/v_2\}, S_3(l_1) = \{2/v_1\}, S_3(l_2) = \{M\}, S_4(l_1) = \{2/v_1, 1/v_2\}, S_4(l_2) = \{2/v_1\}, S_5(l_1) = \{2/v_1, 1/v_2\}, S_5(l_2) = \{2/v_1, 1/v_2\}, S_6(l_1) = \{2/v_1, 1/v_2\}, S_6(l_2) = \{M\}, S_7(l_1) = \{M\}, S_7(l_2) = \{2/v_1\}, S_8(l_1) = \{M\}, S_8(l_2) = \{2/v_1, 1/v_2\}$. Then Γ is sm-f on $\tilde{M}$.

Proposition 3.3. Sm-f is not sm-f on $\tilde{\phi}$.

Proof. Presume that Γ is sm-f on $\tilde{\phi}$, then $\tilde{\phi} \notin \Gamma$ which is contrary. As a consequence Γ is not sm-f on $\tilde{\phi}$.

Proposition 3.4. Each sm-f incudes $\tilde{M}$.

Proof. Make Γ a non-empty sm-f. There is a minimum of one smsetS_L in it and $C_{S(l)}(v) \leq C_{M(l)}(v)$, so $\tilde{M} \in \Gamma$.

Proposition 3.5. Sm-f has finite intersection property.

Proof. $(S_L)_1, (S_L)_2, \ldots (S_L)_n$ belong to a sm-f called Γ. $(S_L)_1 \cap (S_L)_2 \cap \ldots \cap (S_L)_n$ is then a member of Γ as well and since none of $\Gamma's$ member are empty. As a result, $C_{S_1 \cap S_2 \cap \ldots \cap S_n(l)}(v) \leq C_{\phi(l)}(v)$.

Remark 3.6. 1. $P^*(M)$, power smset is not a sm-f on $\widetilde{M}$.

2. In general, the intersection of a set and its counterpart is empty but it may not be under SMTSs. As a result, sm-f may contain a smset and its counterpart as shown from the following example.

Example 3.7. Let $M = \{2/v_1, 3/v_2\}$, $L = \{l_1, l_2\}$ and let$\Gamma = \{\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{1/v_1, 2/v_2\})\}$,$\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\{(l_1, \{1/v_1, 1/v_2\}), (l_2, \{M\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{1/v_1, 2/v_2\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\{(l_1, \{1/v_1, 2/v_2\}), (l_2, \{M\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, \{1/v_1, 2/v_2\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\{(l_1, \{1/v_1, 3/v_2\}), (l_2, M\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{1/v_1, 2/v_2\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\{(l_1, \{2/v_1, 1/v_2\}), (l_2, \{M\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{1/v_1, 2/v_2\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\{(l_1, \{2/v_1, 2/v_2\}), (l_2, \{M\})\}$, $\{(l_1, \{M\}), (l_2, \{1/v_1, 1/v_2\})\}$, $\{(l_1, \{M\}), (l_2, \{1/v_1, 2/v_2\})\}$,$\{(l_1, \{M\}), (l_2, \{1/v_1, 3/v_2\})\}$, $\{(l_1, \{M\}), (l_2, \{2/v_1, 1/v_2\})\}$, $\{(l_1, \{M\}), (l_2, \{2/v_1, 2/v_2\})\}$, $\widetilde{M}\}$.

Proposition 3.8. Let (M, τ, L) be a SMTS and $[(n/v)_l]_L \in \widetilde{M}$. Then the collection $\widetilde{N}_{[(n/v)_l]_L}$ of all sm-nbds of $[(n/v)_l]_L$ is a sm-f on $\widetilde{M}$.

Proof. Since $\widetilde{M}$ is a sm-nbd of $[(n/v)_l]_L$, $\widetilde{M} \in \widetilde{N}_{[(n/v)_l]_L}$ and therefore $\widetilde{N}_{[(n/v)_l]_L} \neq \phi$. By proposal, $\widetilde{N}_{[(n/v)_l]_L}$ must contain $[(n/v)_l]_L$. Therefore no member of $\widetilde{N}_{[(n/v)_l]_L}$ is empty. If $S_L \in \widetilde{N}_{[(n/v)_l]_L}$ and $C_{S(l)}(v) \leq C_{H(l)}(v)$, then H_L is a sm-nbd of $[(n/v)_l]_L$. Therefore $H_L \in \widetilde{N}_{[(n/v)_l]_L}$. If $S_L, H_L \in \widetilde{N}_{[(n/v)_l]_L}$, then $S_L \cap H_L$ is a sm-nbds of $[(n/v)_l]_L$. Wherefore $S_L \cap H_L$ is a member of $\widetilde{N}_{[(n/v)_l]_L}$. Hence $\widetilde{N}_{[(n/v)_l]_L}$ is a sm-f on $\widetilde{M}$.

Proposition 3.9. Let Γ be a sm-f on $\widetilde{M}$. Then the collection $\aleph = \{S(l): S_L \in \Gamma\}$ for each $l \in L$ does not define a multi filter over M.

Proof. If $S(l) = \phi$ for any $l \in L$, then $\phi \in \aleph$. Hence $\aleph$ is not a multi filter over M in mset topology.

Proposition 3.10. Let Γ_i, $i \in I$ be a family of sm-f's over $\widetilde{M}$. Then $\cap_{i \in I} \Gamma_i$ is a sm-f over $\widetilde{M}$.

Proof. GivenΓ_i, $i \in I$ be a family of sm-f's over $\widetilde{M}$. Then $\tilde{\phi} \notin \cap_{i \in I} \Gamma_i$. Let $S_L \in \cap_{i \in I} \Gamma_i$ and let $C_{S(l)}(v) \leq C_{H(l)}(v)$. Then $S_L \in \Gamma_i$. Since each Γ_i is sm-f implies that $H_L \in \Gamma_i$, $i \in I$. Thereupon $H_L \in \cap_{i \in I} \Gamma_i$, $i \in I$. Let $(S_L)_1, (S_L)_2 \in \cap_{i \in I} \Gamma_i$. Then $(S_L)_1, (S_L)_2 \in \Gamma_i$. Since each $\Gamma_i$sm-f, $(S_L)_1 \cap (S_L)_2 \in \Gamma_i$. Therefore $(S_L)_1 \cap (S_L)_2 \in \cap_{i \in I} \Gamma_i$, $i \in I$ and so $\cap_{i \in I} \Gamma_i$ is a sm-f over $\widetilde{M}$.

Remark 3.11. (i) The intersection of all sm-f's on $\widetilde{M}$ is $\{\widetilde{M}\}$ which is coarsest of all sm-f's on $\widetilde{M}$.
(ii) The union of two sm-f's need not be sm-f on $\widetilde{M}$ as shown from the following example.

Example 3.12. Let $M = \{2/v_1, 2/v_2\}$, $L = \{l_1, l_2\}$ and $\Gamma_1 = \{\widetilde{M}, (S_L)_1, (S_L)_2\}$ where $S_1(l_1) = \{2/v_1\}, S_1(l_2) = \{2/v_1\}, S_2(l_1) = \{2/v_1, 1/v_2\}, S_1(l_2) = \{2/v_1, 1/v_2\}$ and $\Gamma_2 = \{\widetilde{M}, (T_L)_1, (T_L)_2\}$ where $T_1(l_1) = \{2/v_2\}, T_1(l_2) = \{2/v_2\}, T_2(l_1) = \{1/v_1, 2/v_2\}, T_2(l_2) = \{1/v_1, 2/v_2\}$ be two sm-f's on $\widetilde{M}$. Then their union $\Gamma_1 \cup \Gamma_2$ is not sm-f on $\widetilde{M}$.

Soft multi filter base

Definition 4.1. Let (M,τ,L) be a SMTS. Let $[(n/v)_l]_L \in \widetilde{M}$. Let $\widetilde{N}_{[(n/v)_l]_L}$ be the family of sm-nbd of $[(n/v)_l]_L$. A family $\mathcal{B}_{[(n/v)_l]_L}$ of $\widetilde{N}_{[(n/v)_l]_L}$ is called a soft multi local base (in summary sm-lb) at $[(n/v)_l]_L$, if corresponding to each $N_L \in \widetilde{N}_{[(n/v)_l]_L}$, there exists a $B_L \in \mathcal{B}_{[(n/v)_l]_L}$ such that $[(n/v)_l]_L \leq C_{B(l)}(v) \leq C_{N(l)}(v)$.

Example 4.2. Let $M=\{2/v_1, 2/v_2\}$, $L=\{l_1,l_2\}$ and $\tau=\{\tilde{\phi},\widetilde{M},F_L\}$ where $F(l_1)=\{2/v_1\}, F(l_2)=\{2/v_1\}$. Then $\mathcal{B}_{[(2/v_1)_{l_1}]_L}=\{(e_1,\{2/v_1\}),(e_2,\{2/v_1\})$ is a sm-lb at $[(2/v_1)_{l_1}]_L$.

Remark 4.3. In [15], Muhammad Riaz *et al*. defined soft multi basis. With the help of this definition, we have given the characterization of a soft multi basis.

Theorem 4.4. A sub family $\mathcal{B}$ of SMTS (M,τ,L) is a sm-b for (M,τ,L) if and only if for each open smset G_L and each $[(n/v)_l]_L \in G_L$ there exists a member B_L in $\mathcal{B}$ such that $[(n/v)_l]_L \leq C_{B(l)}(v) \leq C_{G(l)}(v)$.

Proof. Let $\mathcal{B}$ be a sub family of (M,τ,L) which is a sm-b. Then, every open smset is the union of members of $\mathcal{B}$. Let G_L be an arbitrary open smset. Then by assumption, G_L is the union of members of $\mathcal{B}$. If $[(n/v)_l]_L \in G_L$, then G_L being union of members of $\mathcal{B}$, there exists a B_L such that $[(n/v)_l]_L \leq C_{B(l)}(v) \leq C_{G(l)}(v)$.
Conversely, let G_L be any open smset, there exists a B_L in $\mathcal{B}$ such that $[(n/v)_l]_L \leq C_{B(l)}(v) \leq C_{G(l)}(v)$. Thus $G_L = \cup\{B_L : [(n/v)_l]_L \in G_L$ and each $B_L \in \mathcal{B}\}$. Hence every open smset is the union of members of $\mathcal{B}$ and so $\mathcal{B}$ is a sm-b of (M,τ,L).

Definition 4.5. Let M be the non-empty mset and L be a parameter set. A soft multi filter base (in summary sm-fb) on $\widetilde{M}$ is a non-empty family $\varrho$of sub smsets of $\widetilde{M}$ such that
(i) $\tilde{\phi} \notin \varrho$
(ii) If $(S_L)_1, (S_L)_2 \in \varrho$ there exists a smset $S_L \in \varrho$ with the property that $C_{S(l)}(v) \leq C_{(S_1 \cap S_2)(l)}(v)$.

Example 4.6. Let $M=\{2/v_1, 2/v_2\}$, $L=\{l_1,l_2\}$ and $\varrho=\{(S_L)_1,(S_L)_2\}$ where $S_1(l_1)=\{2/v_1\}, S_1(l_2)=\{2/v_1\}, S_2(l_1)=\{M\}, S_2(l_2)=\{M\}$ is a sm-fb on $\widetilde{M}$.

Remark 4.7. If ϱ is a sm-fb on $\widetilde{M}$, then it follows by finite induction that the intersection of a finite number of members of ϱ contains a member of ϱ, which is non-empty. So ϱ has the finite intersection property.

Proposition 4.8. Every non-empty smset is a sm-fb.

Proof. Let $\tilde{\phi} \neq S_L \in \widetilde{M}$. Then the family $\varrho=\{S_L\}$ is a sm-fb on $\widetilde{M}$. Since $\tilde{\phi} \neq \varrho$ and $C_{S(l)}(v) \leq C_{S(l)}(v)$.

Proposition 4.9. Every sm-f is a sm-fb.

Proof. Let Γ be a sm-f on $\widetilde{M}$. Then $\tilde{\phi} \notin \Gamma$. Further $(S_L)_1, (S_L)_2 \in \Gamma$ implies $(S_L)_1 \cap (S_L)_2 \in \Gamma$ such that $C_{(S_1 \cap S_2)(l)}(v) \leq C_{(S_1 \cap S_2)(l)}(v)$. This implies that the intersection of any two members of Γ contains a member of Γ. Wherefore Γ is a sm-fb.

Proposition 4.10. Let (M,τ,L) be a SMTS. Then the intersection of two sm-nbds of $[(n/v)_l]_L \in \widetilde{M}$, is also a sm-nbd of $[(n/v)_l]_L$.

Proof. Let $(N_L)_1$ and $(N_L)_2$ be two sm-nbd of $[(n/v)_l]_L$. Then there exists open smsets$(G_L)_1$ and $(G_L)_2$ such that $[(n/v)_l]_L \leq C_{G_1(l)}(v) \leq C_{N_1(l)}(v)$ and $[(n/v)_l]_L \leq C_{G_2(l)}(v) \leq C_{N_2(l)}(v)$. Therefore $[(n/v)_l]_L \leq C_{G_1 \cap G_2(l)}(v) \leq C_{N_1 \cap N_2(l)}(v)$. Thus $(G_L)_1 \cap (G_L)_2$ is an open smset containing $[(n/v)_l]_L$ and contained in $(N_L)_1 \cap (N_L)_2$. Hence $(N_L)_1 \cap (N_L)_2$ is also sm-nbd of $[(n/v)_l]_L$.

Proposition 4.11. Let (M, τ, L) be a SMTS and $[(n/v)_l]_L \in \widetilde{M}$. Let $\mathscr{B}$ be a sm-lb at $[(n/v)_l]_L$. Then $\mathscr{B}$ is a sm-fb.

Proof. Given $\mathscr{B}$ be a sm-lb at $[(n/v)_l]_L$ then by Theorem 4.4. each member of $\mathcal{B}$ contains $[(n/v)_l]_L$. So, $\tilde{\phi} \notin \mathcal{B}$. Next if $(S_L)_1, (S_L)_2 \in \mathcal{B}$, then by definition of sm-lb and above proposition, then $C_{S(l)}(v) \leq C_{(S_1 \cap S_2)(l)}(v)$. Hence $\mathcal{B}$ is a sm-fb on $\widetilde{M}$.

Proposition 4.12. Let ϱ be a sm-fb on $\widetilde{M}$. Then the family Γ consisting of all those sub smsets of $\widetilde{M}$ which contain a member of ϱ is a sm-f on $\widetilde{M}$.

Proof. As, every member of ϱ is also a member of Γ, $\varrho \subseteq \Gamma$.
(i) Since ϱ is non-empty, Γ is also non-empty. Since $\tilde{\phi} \notin \varrho$, $\tilde{\phi} \notin \Gamma$. (ii) Let $S_L \in \Gamma$ and $C_{S(l)}(v) \leq C_{T(l)}(v)$. Then there exists $R_L \in \varrho$ with the property that $C_{R(l)}(v) \leq C_{S(l)}(v) \leq C_{T(l)}(v)$. Hence $T_L \in \Gamma$. (iii) Let $(S_L)_1, (S_L)_2 \in \Gamma$. Then there exists $(T_L)_1, (T_L)_2 \in \varrho$ such that $C_{T_1(l)}(v) \leq C_{S_1(l)}(v)$ and $C_{T_2(l)}(v) \leq C_{S_2(l)}(v)$. Wherefore $C_{T_1 \cap T_2(l)}(v) \leq C_{S_1 \cap S_2(l)}(v)$, since ϱ is a sm-fb and so $(S_L)_1 \cap (S_L)_2 \in \Gamma$. Hence $\Gamma$is a sm-f on $\widetilde{M}$.

Remark 4.13. 1. The above sm-f is called a sm-f generated by the sm-fb.
2. Two sm-fb's are said to be equivalent if and only if they generate the same sm-f.

Theorem 4.14. Two sm-fb's ϱ and ϱ^* on $\widetilde{M}$ are equivalent if and only if every member of ϱ contains a member of ϱ^* and every member of ϱ^* contains a member of ϱ.

Proof. Suppose that sm-fb ϱ and ϱ^* are equivalent and suppose that each sm-fb generates the same sm-f Γ. Then ϱ and ϱ^* are sub family of Γ. Let $T_L \in \varrho$. Then $T_L \in \Gamma$. Also ϱ^* being a sm-fb which generates Γ and $T_L \in \Gamma$there exists $T_L^* \in \varrho^*$ such that $C_{T^*(l)}(v) \leq C_{T(l)}(v)$. Thus every member of ϱ contains a member of ϱ^*.
Similarly every member of ϱ^* contains a member of ϱ.
Conversely, let Γ and Γ^* be the sm-f's generated by sm-fb ϱ and ϱ^* respectively. Let $S_L \in \Gamma$. Then there exists $T_L \in \varrho$with the property that $C_{T(l)}(v) \leq C_{S(l)}(v)$. By proposal, corresponding to $T_L \in \varrho$ there exists $T_L^* \in \varrho^*$such that $C_{T^*(l)}(v) \leq C_{T(l)}(v)$. Thus S_L contains a member of ϱ^* and so $S_L \in \Gamma^*$. Thus $\Gamma \subseteq \Gamma^*$. Similarly $\Gamma^* \subseteq \Gamma$. Hence $\Gamma = \Gamma^*$. i.e. ϱ and ϱ^* generate same sm-f.

Definition 4.15. Let Γ be a sm-f on $\widetilde{M}$. Then a sub family ϱ of Γ is called a soft multi base (in summary sm-b) of Γ if and only if every member of Γ contains a member of ϱ.

Proposition 4.16. $\mathcal{B}_{[(n/v)_l]_L}$ is a sm-b of a sm-f.

Proof. Let (M, τ, L) be a SMTS and let $[(n/v)_l]_L \in \widetilde{M}$. Then the family $\widetilde{N}_{[(n/v)_l]_L}$ of all sm-nbd of $[(n/v)_l]_L$ is a sm-f on $\widetilde{M}$. Now $\mathcal{B}_{[(n/v)_l]_L}$ is a sm-lb at $[(n/v)_l]_L$, then clearly $\mathcal{B}_{[(n/v)_l]_L}$ is a sub family of $\widetilde{N}_{[(n/v)_l]_L}$ and every member of $\widetilde{N}_{[(n/v)_l]_L}$ contains a member of $\mathcal{B}_{[(n/v)_l]_L}$. Hence $\mathcal{B}_{[(n/v)_l]_L}$ is a sm-b of a sm-f $\widetilde{N}_{[(n/v)_l]_L}$.

Theorem 4.17. A sm-f Γ with sm-b ϱ is coarser than a sm-f Γ^* with sm-b ϱ^* if and only if every member of ϱ contains a member of ϱ^*.

Proof. Let Γ be coarser than Γ^* and let $B_L \in \varrho$. Then $B_L \in \Gamma$ and so $B_L \in \Gamma^*$. Since ϱ^* is sm-b for Γ^*, there exists a smset$B_L^* \in \varrho^*$ like that $C_{B^*(l)}(v) \leq C_{B(l)}(v)$. Wherefore every member of ϱ contains a member of ϱ^*.
Conversely, let $F_L \in \Gamma$. Since ϱ is a sm-b of Γ, there exists a $B_L \in \varrho$ like that $C_{B(l)}(v) \leq C_{F(l)}(v)$ and by proposal, there exists $B_L^* \in \varrho^*$ like that $C_{B^*(l)}(v) \leq C_{B(l)}(v) \leq C_{F(l)}(v)$. Thus F_L contains a member of ϱ^*. Since ϱ^* is a sm-b of Γ^*, $F_L \in \Gamma^*$. Therefore $\Gamma \subseteq \Gamma^*$ and so Γ is coarser than Γ^*.

Theorem 4.18. Let ϱ^* be any non-empty collection of sub smsets of $\tilde{M}$ having the finite intersection property. Then there subsists a sm-f on $\tilde{M}$ containing ϱ^*.

Proof. Let ϱ be the family obtained by taking all finite intersections of members of ϱ^*. Since ϱ^* has finite intersection property, no member of ϱ^* is empty. So, $\tilde{\phi} \notin \varrho^*$. Let Γ be the family of those sub smsets of $\tilde{M}$ which contain a member of ϱ. Then ϱ is a sub family of Γ. Since $\tilde{\phi} \notin \varrho$ and each member of Γ contains a member of ϱ, so$\tilde{\phi} \notin \Gamma$. Let $S_L \in \Gamma$ and let $C_{S(l)}(v) \leq C_{T(l)}(v)$. Then there subsists a $B_L \in \varrho$ like that $C_{B(l)}(v) \leq C_{S(l)}(v) \leq C_{T(l)}(v)$. Thus T_L contains a member of ϱ and $T_L \in \Gamma$. Let $(S_L)_1, (S_L)_2 \in \Gamma$. Then there exist $(B_L)_1, (B_L)_2 \in \varrho$ such that $C_{B_1(l)}(v) \leq C_{S_1(l)}(v)$ and $C_{B_2(l)}(v) \leq C_{S_2(l)}(v)$ and therefore $C_{(B_1 \cap B_2)(l)}(v) \leq C_{(S_1 \cap S_2)(l)}(v)$. Now, $(C_L)_1, (C_L)_2 \in \varrho$, so each one of them is the intersection of finite number of members of ϱ^* and so is $(C_L)_1 \cap (C_L)_2$. Consequently, $(C_L)_1 \cap (C_L)_2 \in \varrho$ and therefore $(S_L)_1 \cap (S_L)_2 \in \Gamma$. Hence Γ is a sm-f on $\tilde{M}$.

Remark 4.19. Let Γ be asm-f on $\tilde{M}$. Then a subfamily ϱ^* of Γ is called smsub base of Γ if and only if the family of all finite intersections of members of ϱ^* is a sm-b for sm-f Γ. Clearly Γ is the smallest sm-f on $\tilde{M}$ containing ϱ^*.

Theorem 4.20. Let Γ be a sm-f on a non-empty smset$\tilde{M}$ and $C_{S(l)}(v) \leq C_{M(l)}(v)$. Then there exists a sm-f Γ^* on $\tilde{M}$ containing the smset S_L such that Γ^* is finer than Γ if and only if every member of Γ intersects S_L.

Proof. Suppose that every member of Γ intersects S_L. Then $C_{(S \cap T)(l)}(v) \neq C_{\phi(l)}(v) \forall T_L \in \Gamma$. Let $\varrho^* = \{S_L \cap T_L, \forall\, T_L \in \Gamma\}$. Now, if $\{S_L \cap (T_L)_i; i = 1,2,3, \ldots n\}$ be a collection of finite number of members of ϱ^*, then $C_{\cap_{i=1}^n (S \cap T_i)(l)}(v) = C_{S \cap (\cap_{i=1}^n T_i)(l)}(v) \neq C_{\phi(l)}(v)$, since $\cap_{i=1}^n (T_L)_i \in \Gamma$. Thus ϱ^* is a family of non-empty sub smsets of $\tilde{M}$ having finite intersections property. So, there exists a sm-f Γ^* on $\tilde{M}$ generated by ϱ^*. Clearly Γ^* contains S_L. Now let $T_L \in \Gamma$. Then $S_L \cap T_L \in \varrho^*$ which is contained in Γ^* and thereupon $S_L \cap T_L \in \Gamma^*$. Since Γ^* is a sm-f on $\tilde{M} S_L \cap T_L \in \Gamma^* C_{(S \cap T)(l)}(v) \leq C_{T(l)}(v)$, $T_L \in \Gamma^*$. Thus, $T_L \in \Gamma$ implies that $T_L \in \Gamma^*$. Therefore Γ^* is finer than Γ.
Conversely, let Γ^* be a sm-f on $\tilde{M}$ containing S_L and let $\Gamma \subseteq \Gamma^*$. Let $T_L \in \Gamma$, it follows that $T_L \in \Gamma^*$ and by proposal $S_L \in \Gamma^*$. Since Γ^* is sm-f, $S_L \cap T_L \in \Gamma^*$ this implies $C_{(S \cap T)(l)}(v) \neq C_{\phi(l)}(v)$. Hence every member of Γ intersects S_L.

Definition 4.21. A sm-f on $\tilde{M}$ is allegatedly soft multi ultra filter (in summary sm-uf) if and only if Γ is not properly contained in any other sm-f on $\tilde{M}$.

Example 4.22. Let $M = \{2/v_1, 2/v_2\}$, $L = \{l_1, l_2\}$. Consider the following sm-f's on $\tilde{M}$. $\Gamma_1 = \{\tilde{M}, (S_L)_1\}$ where $S_1(l_1) = \{M\}, S_1(l_2) = \{2/v_1, 1/v_2\}\Gamma_2 = \{\tilde{M}, (T_L)_1, (T_L)_2, (T_L)_3, (T_L)_4, (T_L)_5, (T_L)_6, (T_L)_7, (T_L)_8\}$ where $T_1(l_1) = \{2/v_1\}, T_1(l_2) = \{2/v_1\}, T_2(l_1) = \{2/v_1\}, T_2(l_2) = \{2/v_1, 1/v_2\}, T_3(l_1) = \{2/v_1\}, T_3(l_2) = \{M\}, T_4(l_1) = \{2/v_1, 1/v_2\}, T_4(l_2) = \{2/v_1\}, T_5(l_1) = \{2/v_1, 1/v_2\}, T_5(l_2) = \{2/v_1, 1/v_2\}, T_6(l_1) = \{2/v_1, 1/v_2\}, T_6(l_2) = \{M\}, T_7(l_1) = \{M\}, T_7(l_2) = \{2/v_1\}, T_8(l_1) = \{M\}, T_8(l_2) = \{2/v_1, 1/v_2\}$ and $\Gamma_3 = \{\tilde{M}, (W_L)_1, (W_L)_2\}$ where $W_1(l_1) = \{2/v_2\}, W_1(l_2) = \{M\}$, $W_2(l_1) = \{1/v_1, 2/v_2\}, W_2(l_2) = \{M\}$. Γ_1 is not a sm-uf on $\tilde{M}$. Since Γ_2 properly contains Γ_1. But there is no sm-f on $\tilde{M}$ which is strictly finer than any of the sm-f's Γ_2, Γ_3. Accordingly each one of the sm-f's Γ_2, Γ_3 is a sm-uf on $\tilde{M}$.

Theorem 4.23. A sm-f Γ on $\tilde{M}$ is sm-uf if and only if Γ contains all those sub smsets of $\tilde{M}$ which intersects every member of Γ.

Proof. Let Γ be a sm-f on $\tilde{M}$ which contains all those sub smsets of $\tilde{M}$ which intersects every member of Γ. If possible, Γ^* be a sm-f on $\tilde{M}$ strictly finer than Γ. Let $S_L \in \Gamma^*$. Then S_L intersects every member of $\Gamma^*$and since $\Gamma \subseteq \Gamma^*$, it follows that S_L intersects every member of Γ. Consequently, $S_L \in \Gamma$. Therefore $\Gamma^* \subseteq \Gamma$, contradiction the assumption. Hence there isno sm-f on $\tilde{M}$which strictly finer than Γ. Accordingly, Γ on $\tilde{M}$ is sm-uf.
Conversely, let Γ be a sm-f on $\tilde{M}$ and let $C_{T(l)}(v) \leq C_{M(l)}(v)$ like that T_L intersects every member of Γ. Let $\Gamma^* = \{S_L^* : C_{S^*(l)}(v) \geq C_{T \cap S(l)}(v)$ for some $S_L \in \Gamma\}$. Clearly Γ^* contains Γ. Since $C_{(T \cap S)(l)}(v)) \neq C_{\phi(l)}(v) \forall S_L \in \Gamma$ and every member of Γ^* is super smset of $T_L \cap S_L$ for some $S_L \in \Gamma$, so $\tilde{\phi} \notin \Gamma^*$. Let $S_L^* \in \Gamma^*$ and $C_{H^*(l)}(v) \geq C_{S^*(l)}(v)$. Then $C_{H^*(l)}(v) \geq C_{S^*(l)}(v) \geq C_{T \cap S(l)}(v)$ for some $S_L \in \Gamma$. Therefore $H_L^* \in \Gamma^*$. Let $(S_L)_1^*, (S_L)_2^* \in \Gamma^*$. Then $C_{S_1^*(l)}(v) \geq C_{T \cap S_1(l)}(v)$

and $C_{S_2^*(l)}(v) \geq C_{T\cap S_2(l)}(v)$ for some $(S_L)_1^*, (S_L)_2^* \in \Gamma$. Therefore $C_{S_1^*\cap S_2^*(l)}(v) \geq C_{(T\cap S_1)\cap(T\cap S_2)(l)}(v) = C_{(T\cap(S_1\cap S_2))(l)}(v)$, where $(S_L)_1^* \cap (S_L)_2^* \in \Gamma$. This shows that $(S_L)_1^*, (S_L)_2^* \in \Gamma^*$. Thus Γ^* is a sm-f on $\widetilde{M}$ and contains Γ. But Γ is a sm-uf on $\widetilde{M}$, it follows that $\Gamma = \Gamma^*$. Therefore $\widetilde{M} \in \Gamma$ implies $S_L \cap \widetilde{M} \in \Gamma^* = \Gamma$ implies $S_L \in \Gamma$. Hence Γ contains all those sub smsets of $\widetilde{M}$ which intersects every member of Γ.

Theorem 4.24. A sm-f Γ on $\widetilde{M}$ is a sm-uf then for any sub smsetS_L of $\widetilde{M}$ either $S_L \in \Gamma$ or $S_L^c \in \Gamma$.

Proof. Let Γ be a sm-uf and let $C_{S(l)}(v) \leq C_{M(l)}(v)$. If there exists $T_L \in \Gamma$ like that $C_{(T\cap S)(l)}(v)) = C_{\phi(l)}(v)$, then $C_{T(l)}(v) \leq C_{S^c(l)}(v)$. Thus $S_L^c \in \Gamma$. Otherwise $C_{(T\cap S)(l)}(v)) \neq C_{\phi(l)}(v)$. By using above theorem implies that $S_L \in \Gamma$.

Theorem 4.25. A sm-f Γ on $\widetilde{M}$ is a sm-uf on $\widetilde{M}$. Then for each two sub smsets $(N_L)_1, (N_L)_2$ of $\widetilde{M}$ such that $(N_L)_1 \cup (N_L)_2 \in \Gamma$, either $(N_L)_1 \in \Gamma$ or $(N_L)_2 \in \Gamma$.

Proof. Let Γ be a sm-uf on $\widetilde{M}$ and let $(N_L)_1, (N_L)_2$ be the sub smsets of $\widetilde{M}$ such that $(N_L)_1 \cup (N_L)_2 \in \Gamma$. If possible, let $(N_L)_1 \notin \Gamma$ or $(N_L)_2 \notin \Gamma$. Since $(N_L)_1 \cup (N_L)_2 \in \Gamma$, both of $(N_L)_1$ and $(N_L)_2$ cannot be empty. So let $C_{N_1(l)}(v)) \neq C_{\phi(l)}(v)$. Consider the family $\Gamma^* = \left\{R_L : C_{R(l)}(v)\right) \neq C_{\phi(l)}(v), C_{R(l)}(v) \leq C_{M(l)}(v)$ and $R_L \cup (N_L)_2 \in \Gamma\}$. Since $C_{N_1(l)}(v)) \neq C_{\phi(l)}(v)$ and $(N_L)_1 \cup (N_L)_2 \in \Gamma$, it follows that$(N_L)_1 \in \Gamma^*$. So, $\tilde{\phi} \neq \Gamma^*$. Since $\tilde{\phi} \cup (N_L)_2 = (N_L)_2 \in \Gamma$, it follows that $\tilde{\phi} \notin \Gamma^*$. Let $R_L \in \Gamma^*$, $C_{R(l)}(v) \leq C_{H(l)}(v)$. Then $C_{R\cup N_2(l)}(v) \leq C_{H\cup N_2(l)}(v)$. Since Γ is a sm-f $H_L \cup (N_L)_2 \in \Gamma$and therefore $H_L \in \Gamma^*$. Let $(R_L)_1, (R_L)_2 \in \Gamma^*$. Then $(R_L)_1 \cup (N_L)_2 \in \Gamma$ and $(R_L)_2 \cup (N_L)_2 \in \Gamma$. Therefore $(R_L)_1 \cup (N_L)_2 \cap (R_L)_2 \cup (N_L)_2 \in \Gamma$ implies that $((R_L)_1 \cap (R_L)_2) \cup (N_L)_2 \in \Gamma$. Consequently $((R_L)_1 \cap (R_L)_2) \in \Gamma^*$. So, Γ^* is a sm-f on $\widetilde{M}$. Now, $S_L \in \Gamma$ implies $(S_L) \cup (N_L)_2 \supseteq (S_L) \in \Gamma$ implies that $(S_L) \cup (N_L)_2 \in \Gamma$ implies $S_L \in \Gamma^*$. That is each member of Γ is in Γ^* and so $\Gamma \subseteq \Gamma^*$. This is contradiction. Hence $(N_L)_1 \cup (N_L)_2 \in \Gamma$ implies either $(N_L)_1 \in \Gamma$ or $(N_L)_2 \in \Gamma$.

Convergence of soft multi filters

Definition 5.1. Let Γ be a sm-f on $\widetilde{M}$ and let $C_{S(l)}(v) \leq C_{M(l)}(v)$. A sm-f is referred as smeventually in S_L if and only if $S_L \in \Gamma$.

Definition 5.2. A sm-f Γ is allegatedly as smfrequently in S_L if and only if $C_{(S\cap T)(l)}(v)) \neq C_{\phi(l)}(v)$, $\forall T_L \in \Gamma$.

Proposition 5.3. If Γ is issmeventually in S_L, then Γ is smfrequently in S_L.

Proof. Given $S_L \in \Gamma$, then $C_{(S\cap T)(l)}(v)) \neq C_{\phi(l)}(v)$, $\forall T_L \in \Gamma$, since $\tilde{\phi} \notin \Gamma$.

Remark 5.4. Converse of the above proposition do not necessarily true as evidenced by the following.

Example 5.5. Let $M = \{2/v_1, 2/v_2\}$, $L = \{l_1, l_2\}$ and $\tau = \{\widetilde{M}, S_L\}$ where $S(l_1) = \{1/v_1, 2/v_2\}$,$S(l_2) = \{M\}$. Let T_L be sub smset of $\widetilde{M}$ such that $T(l_1) = \{2/v_2\}$,$T(l_2) = \{2/v_2\}$. Then Γis smfrequently in T_L. Since T_L intersects every member of Γ. But $T_L \notin \Gamma$, so, Γ is not smeventually in T_L.

Definition 5.6. Let (M, τ, L) be a SMTS. Then a sm-f Γ on $\widetilde{M}$ is supposedly as smconverge to $[(n/v)_l]_L \in \widetilde{M}$ if and only if Γ is smeventually in every smnbd of $[(n/v)_l]_L$.

Example 5.7. 1. Every sm-nbd of $[(n/v)_l]_L$ is smconverges to $[(n/v)_l]_L$.

2. In an Indiscrete SMTS, every sm-f Γ on $\widetilde{M}$ smconverges to every smpoint of $\widetilde{M}$. Since the only sm-nbd of $[(n/v)_l]_L$ is $\widetilde{M}$ which is in Γ.

Proposition 5.8. Let (M, τ, L) be a SMTS and let Γ be a sm-f on $\widetilde{M}$ which smconverges to smpoint$[(n/v)_l]_L \in \widetilde{M}$. Then every sm-f on $\widetilde{M}$, finer than Γ also smconverges to $[(n/v)_l]_L$.

Proof. Let Γ be a sm-f on $\widetilde{M}$ which smconverges to smpoint $[(n/v)_l]_L \in \widetilde{M}$ and let Γ* be a sm-f on $\widetilde{M}$, finer than Γ. Then Γ is smeventually in every sm-nbd of $[(n/v)_l]_L$, by proposal. This implies that every sm-nbd of $[(n/v)_l]_L$ is contained in Γ implies every sm-nbd of $[(n/v)_l]_L$ is contained in Γ* implies Γ* is smeventually in every sm-nbd of $[(n/v)_l]_L$ implies that Γ* is smconverges to $[(n/v)_l]_L$.

Proposition 5.9. Let τ and τ^* be two topologies on a smset$\widetilde{M}$ such that τ is finer than τ^* and let Γ be a sm-f on $\widetilde{M}$ which τ-converges to smpoint$[(n/v)_l]_L \in \widetilde{M}$. Then Γ also τ^*-converges to $[(n/v)_l]_L$.

Proof. Since τ is finer than τ^*, every τ^*-nbd of $[(n/v)_l]_L$ is a τ-nbd of $[(n/v)_l]_L$. Now Γ smconverges to $[(n/v)_l]_L$ with respect to τ. This implies that Γ is smeventually in every smnbd of $[(n/v)_l]_L$ implies every τ-nbd of $[(n/v)_l]_L$ is contained in Γ implies that τ^*-nbd of $[(n/v)_l]_L$ is contained in Γ which implies that Γ is smeventually in every τ^*-nbd of $[(n/v)_l]_L$ implies that Γ smconverges to $[(n/v)_l]_L$ with respect to τ^*.

Proposition 5.10. Let (M, τ, L) be a SMTS. Then a sm-f Γ on $\widetilde{M}$ smconverges to smpoint $[(n/v)_l]_L \in \widetilde{M}$ if and only if every sm-uf on $\widetilde{M}$ containing Γ smconverges to $[(n/v)_l]_L$.

Proof. Let Γ smconverges to $[(n/v)_l]_L$ and let Γ* be any sm-uf containing Γ. Then Γ smconverges to $[(n/v)_l]_L$ implies Γ is smeventually in every τ-nbd of $[(n/v)_l]_L$which implies every τ-nbd of $[(n/v)_l]_L$ is contained in Γ implies that every τ-nbd of $[(n/v)_l]_L$ is contained in Γ* which implies that Γ* is smeventually in every τ-nbd of $[(n/v)_l]_L$ implies Γ* smconverges to $[(n/v)_l]_L$.
Conversely, let every sm-ufon $\widetilde{M}$ containing Γ smconverges to $[(n/v)_l]_L$. Then every sm-nbd of $[(n/v)_l]_L$ is contained in every sm-uf on $\widetilde{M}$ which contains Γ. Consequently, every sm-nbd of $[(n/v)_l]_L$ is contained in all sm-uf's containing Γ. But Γ is the intersection of all sm-uf's containing Γ. So, every sm-nbd of $[(n/v)_l]_L$ is in Γ. Hence Γ smconverges to $[(n/v)_l]_L$.

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RESEARCH ARTICLE

Comparative Study of Organic Acids for Inhibiting Enzymatic Browning in Fresh-cut Banana, Guava and Apple

Archana Gautam[1]*, Anjana Sagar[2], Neelam Anand[3], Manpreet Kaur[4] and Naveen Dogra[4]

[1]Assistant Professor, Department of Chemistry, Sri Guru Tegh Bahadur Khalsa College, (Affiliated to University of Delhi), Delhi, India.
[2]Associate Professor, Department of Botany, Sri Guru Tegh Bahadur Khalsa College, (Affiliated to University of Delhi), Delhi, India.
[3]Associate Professor, Department of Chemistry, Sri Guru Tegh Bahadur Khalsa College, (Affiliated to University of Delhi), Delhi, India.
[4]Student, Department of Chemistry, Sri Guru Tegh Bahadur Khalsa College, (Affiliated to University of Delhi), Delhi, India.

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***Address for Correspondence**
Archana Gautam
Assistant Professor,
Department of Chemistry,
Sri Guru Tegh Bahadur Khalsa College,
(Affiliated to University of Delhi), Delhi, India.
E.Mail: gautamarchana98@gmail.com

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ABSTRACT

Enzymatic browning is a natural process in fruits and vegetables, posing a critical issue as it impacts their appearance and degrade the quality. In the present study, we explore the inhibitory effect of various organic acids viz. oxalicacid, ascorbicacid, citricacid and tartaric acid, on the enzymatic browning of some fruits namely bananas, apples, and guava. Polyphenol oxidase (PPO) is the primary enzyme responsible for enzymatic browning and is present in most of the fruits and vegetables. PPO catalyses the oxidation of phenolic compounds into brown component (Melanin). In this paper, 5% and 8% stock solutions of above mentioned acids were prepared and applied on the fruitsfor inhibiting enzymatic browning. One batch of fruits were dipped in the acid solutions for 10 minutes and in another batch, acids were applied on fruit surface. Observations were recorded for one hour period. The acids used have shown different level of inhibitory effect on various fruits and inhibition was done though mechanism such as metal ion chelation and pH reduction. Out of all acids, Oxalic acid emerged as most effective inhibitor due to its strong chelating process and lowering PPO activity. Ascorbic acid has shown its antioxidative and reverse quinone formation property. While citric and tartaric acids were moderately effective with their property

of chelation and pH reduction. This study emphasizes the capability of organic acid in preserving the colour, appearance, taste and quality of fruits by pacifying enzymatic browning.

Keywords: Enzymatic browning, Inhibition, chelation, Antioxidative and Polyphenol oxidase.

INTRODUCTION

Browning is a chemical process that causes food to turn brown due to reactions occurring within it. This process is a type of oxidation commonly studied in food science, with significant implications for health, food quality, and nutritional technology. Browning reactions in food products are generally classified into two main categories: enzymatic browning and non-enzymatic browning (NEB), each driven by different mechanisms[1]. Enzymatic browning occurs when enzymes like polyphenol oxidase react with oxygen, leading to brown pigments in fruits and vegetables. Non-enzymatic browning involves chemical reactions like the Maillard reaction and caramelization, which develop flavours and colours incooked foods without enzymes. The Browning reaction takes place in fruits and vegetables when they are bruised and subsequently exposed to air [2]. The technique employed to mitigate unwanted browning in fruits and vegetables relies on the inhibition of polyphenol oxidase (PPO), a process that can be effectively achieved through heat treatment. One prevalent method for treating fresh-cut fruits and vegetables is blanching, which involves immersing the cut produce in hot water or exposing it to steam at temperatures ranging from 96 to 99 °C for a duration of 1 to 2 minutes, followed by rapid cooling to 7 to 10 °C. But this approach often leads to an undesirable softening of the product[3]. Edible coatings, consist of a thin layer of consumable material, represents a promising approach to prolong the shelf-life of fresh-cut products. This technique functions by forming a barrier on the surface, thereby reducing water loss and lowering the respiration rate during their storage[4]. In addition, the utilization of edible coatings on fresh-cut fruits has been shown to mitigate colour alterations and delay the ripening process. Many research revealed that these coatings can also serve as vehicles for various beneficial compounds, including antioxidants, antimicrobial agents, texture enhancers and precursors for flavours and volatiles [5,6].This research investigates the effectiveness of various organic acids in inhibiting enzymatic browning in three commonly consumed fresh-cut fruits: banana, guava, and apple. Our findings provide valuable insights into potential natural alternatives for preserving the quality and appearance of fresh-cut produce, which could have significant implications for the food industry and consumer health.

Role of Polyphenol Oxidase (PPO) in Enzymatic Browning

Enzymatic browning represents a significant oxidative process that can rapidly alter the visual and gustatory qualities of fresh fruits and vegetables, transforming their vibrant hues to brown within a short time frame. This phenomenon is primarily mediated by two oxidoreductase enzymes: polyphenol oxidase (PPO) and peroxidase (POD). The action of PPO involves two distinct reactions as shown in Fig.1, initially it facilitates the hydroxylation of monophenols into diphenols, a relatively slow process that yields colourless compounds. Subsequently, it catalyses the swift oxidation of diphenols into quinones, resulting in the formation of coloured products [7,8]. Polyphenol oxidase (PPO), classified under EC1.10.3.1, is a copper-dependent enzyme that is part of the oxidoreductase family. The Enzyme Commission categorizes oxidoreductases into various subclasses, including EC1.14.18.1, which encompasses monophenol monooxygenase, cresoltase and tyrosinase, as well as EC1.10.3.1, which includes diphenol oxidase, catechol oxidase, and o-diphenol oxygen oxidoreductase, based on their substrate specificity. Typically, the term PPO is used to denote the enzyme classified under EC1.10.3.1. This enzyme is found in the tissues of both plants and animals, with a notable presence in various fruits such as apples, guava, bananas, apricots, berries, mangoes, avocados, and grapes, as well as in vegetables like potatoes, lettuce, burdock, guava, melons, eggplants, and mushrooms [9].The significance of PPO activity in regulating enzymatic browning, is essential to consider the factors that influence this activity, including the type and concentration of endogenous phenolic compounds, the availability of oxygen, and the pH level, in order to mitigate browning reactions [10]. The application of thermal treatment is recognized as the most effective and economically viable strategy for the inactivation of polyphenol oxidase (PPO)

and the control of enzymatic browning in canned or frozen fruits and vegetables. However, this technique is not appropriate for fresh products, particularly fresh-cut fruits and vegetables. As a result, there is increasing interest in alternative nonthermal preservation methods, including the use of organic acids, to mitigate enzymatic browning [11].

Compounds Responsible for Enzymatic Browning in Fruits

Chlorogenic acid, a prominent polyphenolic compound found in apples, is integral to the enzymatic browning that occurs when the fruit is sliced or damaged. When exposed to polyphenol oxidase (PPO) and oxygen, chlorogenic acid undergoes transformation into quinone. This quinone then engages in a reaction with catechins, another polyphenol present in apples, leading to the formation of brown pigments. Such interactions expedite the browning process, thereby influencing both the visual appeal and overall quality of the apple [12]. Furthermore, tyrosine, an amino acid, undergoes oxidation by tyrosinase, another type of PPO, leading to the production of dopaquinone, which promotes melanin formation. These compounds together are vital to the browning that occurs in cut or damaged guava and banana blossoms experience enzymatic browning when their tissues are severed or injured, resulting in an undesirable brown hue that detracts from both the aesthetic appeal and nutritional quality of the fruits. This browning phenomenon is primarily attributed to the activity of essential enzymes, including polyphenol oxidase (PPO), peroxidase (POD), and phenylalanine ammonia lyase (PAL). In both types of fruit, PPO facilitates the oxidation of phenolic compounds, leading to the production of brown pigments. Additionally, PAL is activated by tissue damage, which elevates the levels of phenolic compounds and exacerbates the browning process. While the specific roles of these enzymes may differ among fruit species, their collective influence is a significant factor in the browning observed in guava and banana blossoms, posing a common challenge for preserving the quality and marketability of these fruits [13].

Organic Acids Exhibiting Ant Browning Properties

As a highly effective chelating agent, oxalic acid binds to essential metal ions, including copper, which are crucial for the activity of the polyphenol oxidase (PPO) enzyme involved in the enzymatic browning of fruits. The chelation of these metal ions by oxalic acid leads to a marked reduction in PPO activity, thereby significantly impeding the browning process. Furthermore, oxalic acid lowers the pH, creating an acidic environment that further diminishes the activity of PPO enzymes. This dual mechanism of action renders oxalic acid particularly effective in curtailing browning. In comparative studies, oxalic acid has consistently demonstrated greater effectiveness in preventing enzymatic browning than other acids, underscoring its potential applications in food preservation and quality assurance. Ascorbic acid or Vitamin C, is recognized for its efficacy as a reducing agent and antioxidant, playing a crucial role in mitigating enzymatic browning in fruits. It accomplishes this by converting quinones, the agents responsible for browning, back into their original phenolic forms, thus reversing the browning reaction. Moreover, ascorbic acid reduces the pH, establishing an acidic medium that hinders the function of polyphenol oxidase (PPO) enzymes, which facilitate the browning process. Beyond its influence on pH levels, ascorbic acid can also interact directly with PPO enzymes, further diminishing their activity and preventing the formation of the undesirable brown pigment. The inhibitory effects of citric acid and tartaric acid on enzymatic browning can be attributed to their ability to create acidic environments that reduce the activity of polyphenol oxidase (PPO) enzymes. By lowering the pH to levels that are less than optimal for PPO, which typically operates best in neutral to slightly acidic conditions, these acids effectively inhibit the enzyme's action. In addition, citric and tartaric acids act as chelators, binding to essential metal ions like copper that are necessary for PPO activity. This reduction in the availability of metal ions further restricts the enzymatic browning process. Moreover, citric acid is known to work in conjunction with other inhibitors, such as ascorbic acid, to further bolster the prevention of browning [1,14,15].

EXPERIMENTAL

All the weighing was carried out using Sartorius quintix analytical balance. Apparatus were washed and cleaned properly. Stainless steel knife was used for cutting fruits.

Synthesis of Stock Solutions

5% and 8% Stock solutions of organic acids namely, oxalic acid, tartaric acid, citric acid and ascorbic acid were used. 5% stock solution of oxalic acid was prepared by dissolving 0.7875g in 250mL distilled water in a standard flask. Similarly, 0.9381g, 0.8050g and 2.2015g of tartaric acid, citric acid and ascorbic acid were respectively weighed and dissolved in three different standard flask in 250mL distilled water to obtain their 5% stock solution. 8% Stock solution of ascorbic acid and citric acid were prepared by dissolving 3.5224g and 1.2880g respectively in 250mL distilled water.

METHODOLOGY

A set of four, 100mL beakers were taken and30mL of 5% stock solution of every acid was added to every single beaker using 50mL measuring cylinder and a fresh-cut slice of banana was dipped in the stock solution of each acid for 10 minutes. Then these slice were taken out using forceps and placed on watch glass. A timer of one hour was started to note the observations. These steps were then followed to record the observations for guava and apple. Same procedure was repeated with 8% stock solution of each acid to record the observations for banana, guava and apple one by one (Table 1,2,3). Another set of observations were recorded for on surface application method. For this, a fresh-cut slice of banana was placed in four separate watch glasses and 5-6 drops of 5% stock solution of every acid was poured to them. A timer of one hour was started and observations were recorded. These steps were then followed to record the observations for guava and apple. Same procedure was repeated with 8% stock solution of each acid to record the observations for banana, guava and apple one by one as reported in (Table 1,2,3).

SUMMARY OF OBSERVATIONS AND RESULT

In banana, oxalic acid was more effective when dipped as compared to surface application with 5% acid solution. Citric acid was moderately effective on the surface, while tartaric acid was the least effective. With 8% acid solution, ascorbic acid and citric acid showed moderate effectiveness(Table 1). In guava, with 5% acid solution, oxalic acid was effective on surface and nearly one hour when dipped. Tartaric acid was least effective when applied on the surface and took more than one hour when dipped. With 8% acid solution, ascorbic acid was highly effective for both surface and dipped conditions, while citric acid was less effective(Table 2). In apple, with 5% acid solution, oxalic acid was found to be the most effective acid in delaying browning, both on the surface and when dipped in acid solution. Ascorbic acid, tartaric acid and citric acid were less effective, with browning times ranging from 20 to 38 minutes. In 8% acid solution, ascorbic acid showed moderate effectiveness, while citric acid showed a faster browning time(Table 3).

CONCLUSION

This research investigates the effectiveness of various organic acids in inhibiting enzymatic browning in three commonly consumed fresh-cut fruits: banana, guava, and apple. Our findings provide valuable insights into potential natural alternatives for preserving the quality and appearance of fresh-cut produce, which could have significant implications for the food industry and consumer health. Oxalic acid consistently showed the highest effectiveness in inhibiting enzymatic browning in apple, banana and guava, predominantly when used in a 5% solution and applied by dipping. Ascorbic acid also demonstrated good effectiveness, particularly at higher concentrations (8%), while the effectiveness of tartaric acid and citric acid varied with type of fruit and also with the method of application. These results indicate that oxalic acid is a potent inhibitor of enzymatic browning, ascorbic acid as a secondary choice depending on the application conditions and type of fruit.

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Table 1. Browining Inhibition Time for Banana with the Organic Acids

FRUIT	STRENGTH OF ACIDS USED (%)	PARAMETERS	NAME OF ACIDS	BROWNING TIME(OBSERVED FOR 1 HOUR)
BANANA	5	ON SURFACE	OXALIC ACID	54 MINUTES
			ASCORBIC ACID	23 MINUTES
			TARTARIC ACID	< 1 HOUR
			CITRIC ACID	19 MINUTES
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	OXALIC ACID	< 1 HOUR
			ASCORBIC ACID	28 MINUTES
			TARTARIC ACID	< 1 HOUR
			CITRIC ACID	28 MINUTES
	8	ON SURFACE	ASCORBIC ACID	45 MINUTES
			CITRIC ACID	35 MINUTES
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	ASCORBIC ACID	56 MINUTES
			CITRIC ACID	39 MINUTES

Table 2. Browining Inhibition Time for Guava with the Organic Acids

FRUIT	STRENGTH OF ACIDS USED (%)	PARAMETERS	NAME OF ACIDS	BROWNING TIME (OBSERVED FOR 1 HOUR)
GUAVA	**5**	**ON SURFACE**	**OXALIC ACID**	**29 MINUTES**
			ASCORBIC ACID	**44 MINUTES**

			TARTARIC ACID	**25 MINUTES**
			CITRIC ACID	**29 MINUTES**
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	**OXALIC ACID**	**56 MINUTES**
			ASCORBIC ACID	**< 1 HOUR**
			TARTARIC ACID	**< 1 HOUR**
			CITRIC ACID	**< 1 HOUR**
	8	**ON SURFACE**	**ASCORBIC ACID**	**< 1 HOUR**
			CITRIC ACID	**39 MINUTES**
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	**ASCORBIC ACID**	**< 1 HOUR**
			CITRIC ACID	**< 1 HOUR**

Table 3. Browining Inhibition time for guava with the organic acids

FRUIT	STRENGTH OF ACIDS USED (%)	PARAMETERS	NAME OF ACIDS	BROWNING TIME (OBSERVED FOR 1 HOUR)
APPLE	**5**	**ON SURFACE**	**OXALIC ACID**	**55 MINUTES**
			ASCORBIC ACID	**20 MINUTES**
			TARTARIC ACID	**30 MINUTES**
			CITRIC ACID	**20 MINUTES**
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	**OXALIC ACID**	**< 1 HOUR**
			ASCORBIC ACID	**27 MINUTES**
			TARTARIC ACID	**38 MINUTES**
			CITRIC ACID	**22 MINUTES**
	8	**ON SURFACE**	**ASCORBIC ACID**	**50 MINUTES**
			CITRIC ACID	**21 MINUTES**
		DIPPED IN ACID SOLUTION FOR 10 MINUTES	**ASCORBIC ACID**	**53 MINUTES**
			CITRIC ACID	**18 MINUTES**

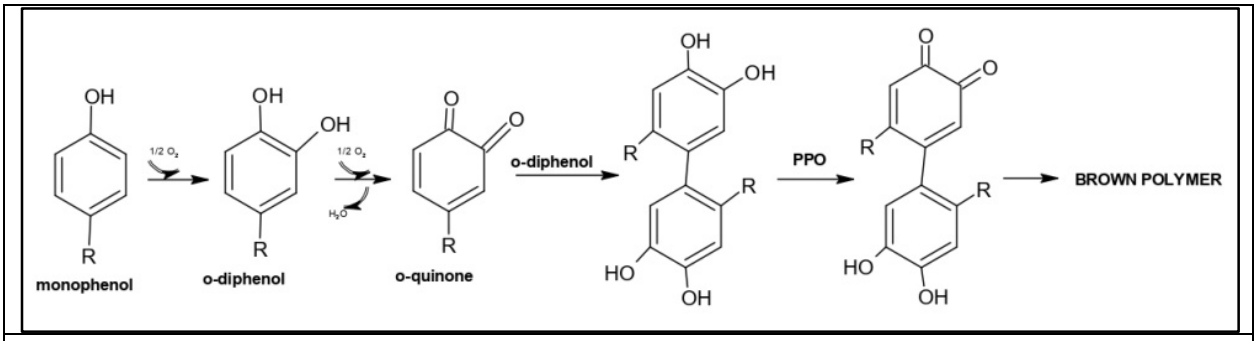


Figure 1. Mechanism of PPOin enzymatic browning

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RESEARCH ARTICLE

Phytochemical Profiling and Antibacterial Activity of *Elettaria Cardamomum* and *Cuminum Cyminum* Extracts

Lavanya S[1] and Dhivya R[2]*

[1]Post Graduate Student, Department of Zoology, Nirmala College for Women, Red Fields, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Zoology, Nirmala College for Women, Red Fields, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
Dhivya R
Assistant Professor,
Department of Zoology,
Nirmala College for Women, Red Fields,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: dhivya.adu@gmail.com

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ABSTRACT

The study investigated the phytochemical screening and antibacterial activity of aqueous extracts of spices viz., *Elettaria cardamomum and Cuminum cyminum.* The phytochemical screening revealed the presence of flavonoids, anthraquinones and carbohydrates in *E.cardamomum*, while *C.cuminum* showed flavonoids, anthraquinones, proteins, carbohydrates, glycosides and coumarins. Using the agar well diffusion method, antibacterial activity against Escherichia coli and Staphylococcus aureus was assessed.The aqueous extract of *E.cardamomum* exhibited maximum inhibition 20mm at 50µl against both bacteria, while *C.cyminum* showed greater efficacy with inhibition zonesof 25mm against *S.aureus* and 20mm against *E.coli* at the same concentration.

Keywords: Phytochemical Analysis, Antibacterial Activity, Spices, *Elettaria cardamomum*, *Cuminum cyminum*

INTRODUCTION

Natural products from plants and its significant parts have been proved to have lesser side effects in comparison to chemotherapeutics, hence they possess potential in being used as an “alternative remedy [1]. According to the most pertinent definition, "kitchen spices" are "vegetative items" or mixtures devoid of unnecessary ingredients that are used to flavor or add aroma to food. These spices are usually derivatives from different parts of plants like barks, leaves, fruits, buds, rhizomes and resins [2]. Most of the Asian countries have rich diversity of wide range of spices

used for various ailments [3]. Ayurveda, spices contribute significantly for the treatment of key disorders of human body [4]. Spices and herbs are typically regarded as safe and have been shown to be successful in treating a variety of illnesses. It is only in recent years that modern science has started paying attention to the properties of spices [5]. The presence of several substances referred to as phytochemicals or secondary metabolites gives the spices their distinct flavor and scent [6,7]. Secondary metabolites or phytochemicals, naturally occurring in plants are biologically active and play important role in defence system of plant [8]. Numerous classes of phytochemicals including isoflavonoids, anthocyanins and flavonoids are found associated with the spices [9]. The phytochemicals involuntarily protect humans in contrast to pathogens employing anti-microbial medicines. Some phytochemicals act as antioxidants that inhibit carcinogens and provide nutrition for normal health and repairs of the cell. These are also recognised to have therapeutic and prophylactic properties [10]. The most essential components of a human diet are spices and herbs. Herbs and spices are recognized for their medicinal and preservation properties in addition to their ability to enhance flavor [11,12]. Antimicrobial activity of spices and herbs has been known and described and used for medicinal purposes for several centuries [13,14]. The antimicrobial activity of some spices was studied against pathogenic yeasts and bacteria. In order to determine whether extracts can be used as a substitute for or in addition to well-established chemotherapeutic drugs, a comparison of the antibacterial activity of spices and various antibiotics has also been conducted. Chemotherapy's effectiveness is attributed to the ongoing hunt for novel medications to combat the threat posed by resistant strains. The investigation of certain indigenous plants for their antimicrobial properties may yields useful results. A large number of plants are used to combat different diseases [15,16] and possess antimicrobial activities [17]. In this regard, the objective of the present study was to compare phytochemical profiles and antibacterial activities of selected spices *Elettaria cardamomum* and *Cuminum cyminum.*

MATERIALS AND METHODS

The spice seeds, namely *Elettaria cardamomum and Cuminum cyminum* were collected from Madhampatti, Coimbatore, Tamil Nadu, India.

Preparation of Spice Powders and Extracts

The spices, namely *Elettaria cardamomum* and *Cuminum cyminum* were collected and dried to a moisture-free state. Dried spices were powdered using an electric pulverizer. 10g of each spice seed powder was weighed using electric balance. In a conical flask, 50 milliliters of aqueous solution were combined with 10 grams of powdered spice seeds. The mixture was placed an orbital shaker at room temperature for 72 hours. Extract was filtered using a Whatsman No. 1 filter paper. For later usage, the aqueous extract was kept in the refrigerator in firmly sealed vials.

Phytochemical Analysis of Selected Spices

Qualitative analysis

Preliminary phytochemical screening of aqueous extract of selected spice seeds was carried out using the standard procedures.

Test of Alkaloids

Mayer's test: A drop or two of Mayer's test reagent was added to a portion of the extract along the test tube's sidewalls, and the precipitate's formation—either white or cream in color was monitored.

Wagner's test: A fraction of extract was treated with Wagner's reagent along the sides of the test tube and observed for the formation of reddish-brown colour precipitate.

Hager's test: One or two milliliters of Hager's reagent were added to a few milliliters of extract, and the production

Test for Tannins

Ferric chloride test: After mixing roughly 0.5 g of extract with 10 ml of distilled water, the mixture was filtered. Two milliliters of the filtrate were mixed with a few drops of 1% ferric chloride solution, and the blue-black, green, or blue-green precipitate was then watched for.

Test for Phenols

Ferric chloride test: After dissolving a small amount of the extract in water and filtering it, two milliliters of 10% aqueous sodium hydroxide were added to create a yellow tint. The presence of flavonoids was shown by a color shift from yellow to colorless upon addition to diluted hydrochloric acid.

Lead acetate test: A small amount of lead acetate solution was added to 50 mg of test extract in a test tube, and the mixture was watched for the formation of a yellow precipitate.

Test for Flavonoids

NaOH test: After dissolving a small amount of the extract in water and filtering it, two milliliters of 10% aqueous sodium hydroxide were added to create a yellow tint. The presence of flavonoids was shown by a color shift from yellow to colorless upon addition to diluted hydrochloric acid.

Lead acetate test:A small amount of lead acetate solution was added to 50 mg of test extract in a test tube, and the mixture was watched for the formation of a yellow precipitate.

Test for Steroids

Liebermann-Burchard test: Two milliliters of acetic anhydride are used to dissolve the extract (50 mg). To this one or two drop of Conc. H_2SO_4 is added along the side of the test tube and observed for an array of colour changes.

Test for Glycosides

Keller Kilianin test: Five milliliters of each extract were combined with two milliliters of glacial acetic acid, and then a few drops of ferric chloride solution and one milliliter of strong sulfuric acid were added. The development of a brown ring at the contact indicates the presence of glycosides.

Test for Terpenoids

Liebermann- Burchard test: 50 grams of the extract were dissolved in ethanol. After adding one milliliter of acetic anhydride, concentrated H_2SO_4 was added. Terpenoids were indicated by a color shift from pink to violet.

Test for Saponins

Foam test: The extract (50 gm) or dry powder was diluted with distilled water and made up to 20 ml. After 15 minutes of vigorous shaking in a graduated cylinder, the suspension is checked for the development of a 2cmthick layer of foam.

Antibacterial Activity of Selected Spices

Test Microorganism

The bacterial strains used were *Staphylococcus aureus* and *Escherichia coli.*

Procedure for Antibacterial Activity

The activity of aqueous solvent extract of spice seeds, namely *Elettaria cardamomum* and *Cuminum cyminum* on selected bacterial strains was assayed by agar well diffusion method (Kirby-Bauer method). From an agar plate culture, at least three to five distinct colonies with the same morphology are chosen. After touching the top of each colony with a loop, the growth is put into a tube with 4–5 milliliters of an appropriate broth medium, like Muller-Hinton broth. The broth culture is incubated at 35ºC until it achieves or exceeds the turbidity. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity. This results in a suspension containing approximately 1 to 2 x 108 CFU/ml for *S. aureus and E.coli.* It is ideal to dip a sterile cotton swab into the modified suspension of inoculum within 15 minutes of changing the solution's turbidity. Rotate the swab multiple times and press it firmly against the tube's inner wall above the fluid level. This will rid the swab of extra inoculum. The dried surface of the Muller-Hinton agar plate is inoculated by streaking two more times. To guarantee that the inoculum is distributed evenly, rotate the plate by about 60 degrees each time. The agar's rim is swabbed as a last step. Before applying the drug-impregnated disks, the lid may be left open for three to five minutes, but no more than fifteen minutes, to allow for the absorption of any surplus surface moisture. The media

was punctured by making a well of 6mm in diameter and filled with 10-50µl of a sample. Further the petriplate were placed inversely for complete diffusion and inhibition zones were examined by measuring the diameter (mm) formed around the well after 24 hours incubation at 37ºC. The zones were measured by using standard (Hi-Media) scale.

RESULTS AND DISCUSSION

Phytochemical Analysis of Selected Spices

Phytochemical analysis was carried out in the aqueous extracts for spice seeds namely *Elettaria cardamomum* and *Cuminum cyminum.* The results of phytochemical analysis of *E. cardamomum* and *C. cyminum* spice seed extract is presented in Table 1. The aqueous extracts of *E. cardamomum* and *C. cyminum* showed presence of various bioactive compounds.

Phytochemical Analysis of Aqueous Extract of *Elettaria cardamomum*

The present study's phytochemical analysis of the E. cardamomum aqueous extract showed the presence of carbohydrates, anthraquinones, and flavonoids. In a study conducted by Bano *et al*., [18], the methanol extract of cardamom seeds showed the presence of flavonoids, terpenoids and glycosides while phenolic, alkaloids, tannins and saponins were not deducted. Likewise, in the present study of the phytochemical analysis of the aqueous extract of *E. cardamomum* revealed the presence of flavonoids, anthraquinones, and carbohydrates. Umesh *et al*.,[19] screened the phytochemicals in the oils of clove and cardamom. According to the findings of a phytochemical investigation, cloves contain phenolic compounds, alkaloids, glycosides, steroids, carbohydrates, terpenoids, and tannins. And the phytochemical analysis of cardamom extracts showed the presence of alkaloids, glycoside, steroids, protein, carbohydrates, terpenoids, tannins and phenolic compound.

Phytochemical Analysis of Aqueous Extract of *Cuminum cyminum*

In the present study of phytochemical analysis of *C. cyminum* contains flavonoids, anthraquinones, protein, carbohydrates, glycosides and coumarins. In concordance with the results of the present study, Rai *et al*., [20] carried out phytochemical screening of *C. cyminum* extracts. The results of phytochemical analysis revealed the presence anthraquinone, protein, alkaloid, glycoside, steroid, coumarin, saponin, flavonoid, Resin, and tannin in *C. cyminum*. Ashvin *et al.,* [21] reported that the aqueous extract of *C. cyminum* L. showed presence of the secondary metabolites like alkaloids, saponin, tannin, proteins, flavonoids, diterpenes, phenol and cardiac glycosides. It was also reported that its efficiency was more than the ethanol extract, as the ethanol extract showed the presence of alkaloids, saponin, coumarin, protein, flavonoids and cardiac glycosides.

Antibacterial Activity of Selected Spices

Antibacterial activity was carried out in the aqueous extracts for spice seeds namely *Elettaria cardamomum* and *Cuminum cyminum.* The results of antibacterial activity of *E. cardamomum* and *C. cyminum* spice seed extract is presented in Table 2 and 3 and Plate 1.

Antibacterial activity Aqueous Extract of *Elettaria cardamomum*

The result of the antibacterial activity of *E.cardamomum*in aqueous extract against *Staphylococcus aureus* and *E. coli* is presented in Table 2 and Plate 1. The antibiotic, Gentamycin, was used as a positive control in this study. The zone of inhibition for gentamycin measured to be 26 mm against *S. aureus* and 23 mm against *E. coli* (Plate 1 A and B). In this study, at 50µl the aqueous extract exhibited maximum antibacterial activity with a zone of inhibition of 20 mm against both *S. aureus* and *E.coli* (Plate 1 C and D).At the concentration of 10 to 40 µl the zone of inhibition was measured to be as 0 mm as there was no zone of inhibition observed. No inhibitory activity was detected at concentrations ranging from 10 to 40 µl. Inhibition zone diameter measured in millimetre (mm)

Antibacterial activity Aqueous Extract of *Cuminum cyminum*

The result of the antibacterial activity of *C. cyminum* in aqueous extract against *S. aureus* and *E.coli*is presented in Table 3 and Plate 1. The antibiotic, Gentamycin, was used as a positive control in this study. The zone of inhibition for gentamycin was measured to be 26 mm against *S.aureus*and 23 mm against *E.coli* (Plate 1 A and B). The study revealed that, against *S. aureus*, the *C. cyminum*aqueous exhibited a maximum zone of inhibition of 25 mm and a maximum zone of inhibition of 20 mm against *E.coli*, at a concentration of 50 µl (Plate 1 E and F). At a concentration of 40 µl, the zone of inhibition values were 22 mm against *S. aureus* and 19 mm against *E.coli*. As the concentration decreased the antibacterial activity was found to decrease. Therefore, at the concentration of 30 µl a zone of inhibition was only measured as 17mm against *E.coli*. No inhibition was observed against *S. aureus* at concentrations of 10, 20 and 30 µl and against *E.coli*at a concentrations of 10 and 20 µl. The antibacterial activity was observed to be dose dependent in the present study. Inhibition zone diameter measured in millimetre (mm) In the present study, at 50µl the aqueous extract of *E.cardamomum* exhibited maximum antibacterial activity with a zone of inhibition of 20 mm against both *S. aureus* and *E. coli*.In comparison to the present study Hamza *et al*., [22] carried out a study to test the antibacterial activity of cardamom essential oil against both gram positive and gram-negative bacteria viz., *Bacillus subtilus* and *Staphylococcus aureus*. The result revealed that the essential oil was active against gram positive and the gram negative bacteria except *E.coli.* The essential oil showed the highest inhibition towards the gram positive bacteria *Staphylococcus aureus* 10 mm and *Bacillus subtilis* 5 mm, *Pseudomonas aureus* 1mm, while *E.coli* didn't show any inhibition zone. In the present investigation the aqueous extract of *C. cyminum* at the concentration of 30 µl showed a zone of inhibition of 17mm against *E.coli*. No inhibition was observed against *S. aureus* at concentrations of 10, 20 and 30 µl. In another study Md. Mahfuzul *et al.,*[23] has reported that, the essential oil of clove has antibacterial properties and has shown to inhibit all tested organisms. This extract was more active on *Staphylococcus aureus* than on *E. coli* according to his study. In the present study aqueous extract of both Cardamom and Cumin showed strong antibacterial activity against both the test bacteria viz., *S. aureus* and *E.coli*. Similarly Barera Rani *et al*., [24] tested the antibacterial activity of *T.ammi, C.verum, F.vulgare, C.longa and P.nigrum*against *S.aureus*. The results showed that the aqueous extracts of all the tested spices *P.nigrum* worked well against *S.aureus* and exhibited an inhibiting effect at high concentration.

CONCLUSION

*E.cardamomum*and *C.cyminum* has been used as a very famous traditional folk medicine, and has been subjected for extensive phytochemical and bioactive investigations. But, its chemical components and bioactivities have not been completely investigated yet. In our present study many phytochemicals have been identified from the both spices by phytochemical screening. The aqueous extracts of these spices showed very good antibacterial activity, which suggested the presence of potent antimicrobial agents. The results indicated that the gram negative bacteria were more sensitive to the extracts, especially to *C.cyminum*, indicating the availability of broad-spectrum antibacterial agents. These findings indicated that both spices, especially *C. cyminum* would be excellent sources for natural antibacterial agents. Hence, in future the molecular level of studies can be undertaken to understand further properties. Further, isolation of individual phytochemical compounds may lead to discovery of a novel therapeutic drug.

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Table 1: Phytochemical Analysis of *Elettaria cardamomum* and *Cuminum cyminum*

S. No.	Metabolite	Test performed	*Elettaria cardamomum*	*Cuminum cyminum*
1	Alkaloids	+ Mayer's reagent	-	-
		+ Dragendorff's reagent	-	-
2	Flavonoids	Alkaline test	-	+
		+ H2SO4	+	+
		+ Lead acetate	-	+
		Shinoda test	-	-
3	Sterols (Libermann's test)	+ CHCL3+Acetic anhydride+ Conc. H2SO4	-	-
4	Terpenoids (Libermann's test)	+ CHCL3+Acetic anhydride+ Conc. H2SO4	-	-
5	Anthraquinone (Borntrager's test)	+ FeCl3 + Conc. HCl +diethyl ether +Ammonia	+	+
6	Anthocyanin	HCl test	-	-
7	Proteins	+2% Ninhydrin reagent	-	-
		+2% CuSo4 + 95% Ethanol +KOH pellet	-	-
		+ Conc.HNO3	-	+
8	Phenolic compounds	+ 5% neutral FeCl3	-	-
		Gelatin test	-	-
		+ Ellagic acid test	-	-
9	Quinones	Conc. HCl	-	-
		Alcoholic KOH	-	-
10	Carbohydrates	Molisch's test	+	+
		Fehling's test	+	+
11	Tannin	Brayer's test	-	-
		+ Gelatin test	-	-
		10% NaOH test	-	-
12	Saponins	Shaken with water	-	-
13	Cardiac glycosides	+ Baljet reagent	-	-
		Bromine water test	-	-
		Keller-killani test	-	-
14	Glycoside's test	Borntrager's test	-	-
		Aq.NaOH test	-	+
15	Lignin	+ Gallic acid	-	-
16	Coumarins	+ 10% NaOH + CHCl3	-	+
17	Volatile oils	Fluorescence test	-	-

Table 2: Antibacterial activity of Aqueous Extract of *Elettaria cardamomum* against *Staphylococcus aureus* and *Escherichia coli*

Treatment	Zone of Inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
Control (Gentamycin)	26 mm	23 mm
10 µl	0 mm	0 mm
20 µl	0 mm	0 mm

30 µl	0 mm	0 mm
40 µl	0 mm	0 mm
50 µl	20 mm	20 mm

Table 3: Antibacterial activity of Aqueous Extract of *Cuminum cyminum* against *Staphylococcus aureus* and *Escherichia coli*

Treatment	Zone of Inhibition (mm)	
	Staphylococcus aureus	*Escherichia coli*
Control (Gentamycin)	26 mm	23 mm
10 µl	0 mm	0 mm
20 µl	0 mm	0 mm
30 µl	0 mm	17 mm
40 µl	22 mm	19 mm
50 µl	25 mm	20 mm

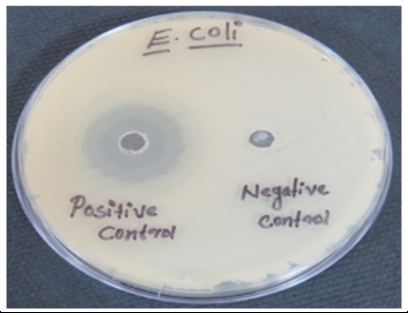


Plate A: Antibacterial Activity of Control Against *E.coli*

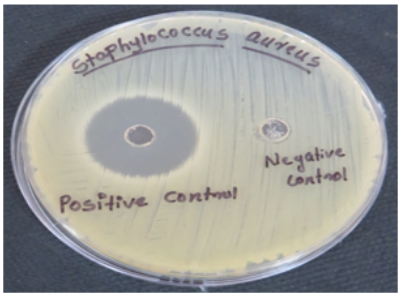


Plate B: Antibacterial Activity of Control Against *S.aureus*

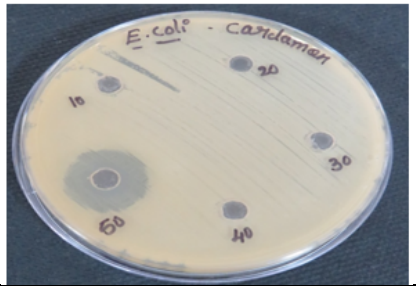


Plate C: Antibacterial Activity of *E.cardamomum* Against *E.coli*

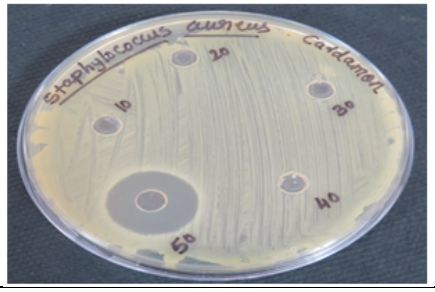


Plate D: Antibacterial Activity of *E.cardamomum* Against *S.aureus*

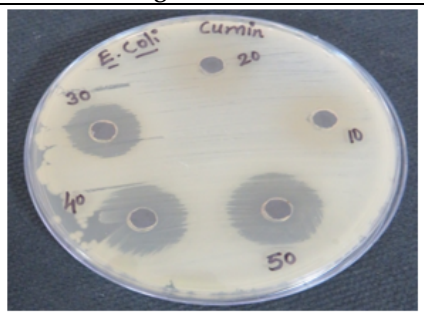


Plate E: Antibacterial Activity of *C.cyminum* Against *E.coli*

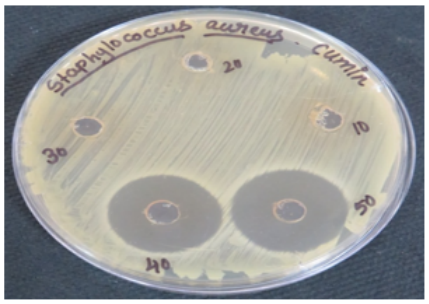


Plate F: Antibacterial Activity of *C.cyminum* Against *S.aureus*

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RESEARCH ARTICLE

A Study on Cash Flow Analysis at Femtosoft Technologies, Chennai

L. Clement Babu[1]* and C.Sathish Kumar[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Assistant Professor (SG), School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
L. Clement Babu
Second Year MBA Student,
School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai, Tamil Nadu, India.

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ABSTRACT

Technology is crucial in driving innovation and supporting digital transformation across industries. It encompasses software development, IT services, data analytics, cloud solutions, and other digital infrastructures contributing to business efficiency and growth. With increasing demand for automation, remote access, and AI integration, this sector continues to evolve rapidly and shape the global economy. However, the industry faces challenges such as fast-paced technological changes, rising competition, data security concerns, and the need for continuous upskilling. Technology companies must invest consistently in research and development, talent acquisition, and agile methodologies to stay ahead. Femto Soft Technologies is a growing player in the Indian IT and software services landscape, offering various solutions from enterprise software development to custom web and mobile applications. The company has gained recognition for delivering value-driven technological services and maintaining strong client relationships. This study focuses on the cash flow analysis at Femto Soft Technologies, examining how the company manages its inflows and outflows of cash over a specific period. A cash flow statement is a vital financial tool to assess liquidity, operational efficiency, and the company's ability to generate cash to meet obligations and invest in growth. The analysis helps identify the patterns of cash movement in operating, investing, and financing activities, offering insights into the company's financial health. Furthermore, it aims to guide better cash management strategies and long-term financial planning, which are essential for sustainable performance in the dynamic tech sector.

Keywords: AI Integration, Cloud Solutions, Data Analytics, IT Services, Technology.

INTRODUCTION

Femtosoft Technologies operates within the IT and software solutions sector—a dynamic, rapidly evolving industry marked by continuous innovation, high competition, and significant reliance on digital transformation. Companies in this space offer services from custom software development to cloud computing and IT consultancy, addressing diverse needs across various sectors such as finance, healthcare, manufacturing, and retail.

Need of the Study

Credit-based sales are common in the IT industry. Studying receivables helps assess how efficiently Femtosoft collects payments to maintain a healthy cash flow for operations. The study aims to identify gaps in credit evaluation and collection practices that could lead to delayed payments or defaults, thus helping to minimize bad debt losses. Efficient receivables management allows better use of available funds, supporting smoother operations and reducing reliance on external financing. As Femtosoft expands, managing receivables effectively ensures financial stability, vital for funding new projects, R&D, and technology upgrades. The study helps analyze existing credit terms and suggests improvements to balance customer satisfaction with financial discipline. Understanding current challenges in receivables can drive the adoption of automation tools and software that streamline invoicing, follow-ups, and collections.

Objectives of the Study

Primary objective:

- To analyse the accounts receivable management and its performance in Femtosoft Technologies

Secondary Objectives:

- To know how the accounts receivable were managed.
- To examine the average collection period and identify any delays in receivables.
- To evaluate the company's credit policy and its enforcement.
- To suggest improvements for optimizing the receivables management process.

Scope of the Study

- This study evaluates the impact of accounts receivable turnover on Femtosoft's financial performance by analyzing how quickly receivables are collected. The study aims to understand the correlation between turnover rates and the company's profitability.
- This study focuses on assessing the effectiveness of Femtosoft's collection strategies.It examines the current methods employed for debt collection to identify areas where efficiency can be enhanced, thereby reducing overdue accounts.
- This study explores the relationship between credit terms offered and customer payment behavior. Understanding how different credit terms influence timely payments can help formulate policies encouraging prompt settlements.
- This study focuses on identifying the role of staff training in accounts receivable management. It investigates whether ongoing training programs for the finance team contribute to more effective receivables management.
- This study focuses on analyzing the impact of economic fluctuations on receivables. The company can better anticipate and mitigate risks by studying how external economic factors affect customer payment capabilities.
- This study benchmarks Femtosoft's accounts receivable performance against industry peers.

Limitations of the Study

- The data is gathered mainly through secondary sources; no independent verification has been done.
- The time series analysis of ratios has been attempted with no effects being given to inflation.
- Detailed analysis could not be carried out for the project work because of the limited period

- Only five years' financial reports have been considered, i.e., from 2021 to 2025.
- The total statements are given as secondary sources, and they don't provide a means to undergo scrutiny of those statements.

RESEARCH METHODOLOGY

The methodology adopted for this study on Femto Soft Technologies involves an analytical and quantitative approach, using secondary data derived from the company's cash flow statements over five years. The study aims to assess the cash inflows and outflows through a detailed evaluation of operating, investing, and financing activities. Key financial tools such as ratio analysis, trend analysis, and percentage comparisons are used to interpret the data. This approach enables a clear understanding of the company's liquidity, financial health, and cash management practices, providing insights that can guide future financial decisions and strategies.

Sources of data collection

Primary:

In primary content, we will use a fresh form of records used for the first time, and indicate an institution's original documents.

This project's primary data was from the Tube Investment India Limited staff and the guide.

Information will be collected with the help of a firm auditor.

The observation method is used to understand various tools used in logistics management and their features.

Secondary:

The secondary contents will be mainly interpreted from various websites, publications, periodicals, etc.

Sampling Method

Since this study focuses on the cash flow analysis of a single company, Femto Soft Technologies, the purposive sampling method has been adopted. This non-probability sampling technique was chosen to analyze the financial data of Femto Soft Technologies over five financial years. The sample includes annual cash flow statements representing the company's operational and financial performance, allowing for meaningful analysis and interpretation relevant to the study's objectives.

Tools for Analysis

To effectively examine the cash flow patterns of Femto Soft Technologies, the following financial and statistical tools have been used:

- Cash Flow Ratio Analysis
- Cash Flow to Debt Ratio
- Trend Analysis
- Percentage Analysis
- Comparative Analysis

Data Analysis and Interpretation

Interpretation:

From the above table, it is understood that the firm's cash flow from operating activities is less in the year ending 31st March 2021, with a 33.29% cash flow of 664.77 cr and a total cash flow of 1996.38 cr. The cash flow from operating activities is higher for the year ending 31st March 2020. The cash flow is 527.72 Cr, and the total is 9 94.67 with 53.05%.

Interpretation:

The book table shows that the firm's cash flow from investing activities islessintheyearending31stMarch2028, with 16.48% and the Cash flow of 127.66 cr, and the total cash flow of774.18 cr. The cash flow from investing activities for the year ending 31st March 2020 is 1005.51 Cr; the total cash flow is 1996.38, with 50.36%.

Interpretation:
The table shows that the cash flow from financial activities was less in the year ending 31st March 2022, with 6.33%. The cash flow is 41.65 cr, and the total is657.87 cr. The cash flow from financial activities is more in the year ended 31st March 2018, with 33.28 cr, the cash flow is 290.81, and the totalcashflowis873.79cr.4.1.3financialactivities to total cash flow.

DISCUSSIONS AND INFERENCES

Cash Flow from Operating Activities to Total Cash Flow (Table 1)
The analysis shows a steady increase in cash flow from operating activities from 44.87% in 2018 to 53.05% in 2020, reflecting the company's improved ability to generate cash from its core operations. However, in 2021, despite a significant rise in total cash flow, the percentage of cash flow from operating activities sharply declined to 33.24%. This could be attributed to disruptions such as the impact of the COVID-19 pandemic, which likely affected operational efficiency or led to a greater reliance on non-operating income. The cash flow from operating activities improved in 2022, rising to 49.52%, but it remained below the peak seen in 2020, suggesting recovery without fully reaching pre-pandemic levels.

Cash Flow from Investing Activities to Total Cash Flow (Table 2)
Cash flow from investing activities remained relatively low, comprising 16-21% of total cash flow from 2018 to 2020. A sharp increase occurred in 2021, accounting for 50.36% of total cash flow. This surge indicates that the company made significant investments, possibly in growth initiatives, capital expenditures, or asset acquisitions. The proportion declined to 44.14% in 2022, which could reflect a reduction in investment activity or a strategic shift toward operational cash flow generation.

Cash Flow from Financial Activities to Total Cash Flow (Table 3)
The share of cash flow from financial activities was substantial in 2018, at 38.64%, suggesting a reliance on external financing such as loans or capital raises. Over the years, this percentage steadily declined, reaching just 6.33% in 2022. This shift reflects the company's decreasing need for external financing, potentially indicating improved financial stability and self-sufficiency, as it increasingly generated enough internal cash to fund operations and investments without relying on debt or equity funding.

General Inferences:
Impact of External Factors (COVID-19): The drastic fluctuations in cash flow percentages in 2021, especially in operating and investing activities, align with the disruptions caused by the COVID-19 pandemic. This suggests the company faced challenges maintaining its normal operations, resulting in greater reliance on non-operating income and external financing.

Operational Efficiency and Investment Strategy: The increase in cash flow from investing activities in 2021 suggests a strategic focus on growth, despite the drop in operational efficiency. This may indicate that the company invested heavily in future projects or expansion, potentially at the cost of immediate operational performance.

Declining Financial Dependence: The decreasing reliance on financial activities over the years highlights a positive trend in the company's financial health. The debt reduction and external financing indicate growing operational profitability, enabling the firm to fund itself internally without substantial borrowing or equity issuance.

Volatility and Recovery: The fluctuations in cash flows between 2020 and 2022 highlight the volatility caused by the pandemic and the firm's recovery efforts. While the company faced challenges in 2021, it appears to be regaining balance and operational strength, moving towards a more stable and sustainable financial position.

SUGGESTIONS

The company's financial position is perfect, but I found some areas during my analysis that sharply alarm the firm's effective cash management.

- To increase efficiency in the delivery of services and, in turn, increase profit, I advise the company to develop and implement an effective management strategy.
- For the increase in profit, the firm must make alternative plans.
- The company must diversify its plan into other areas, such as EVs.
- The firm should conduct market research at regular intervals, which could help increase the demand for its sales and services.
- It would be suggested that the firm replace the fixed assets with new, modern, and improved technology, which helps reduce idle capacity, increase overall stability, and help earn high returns.
- The firm should try to decrease its profit by paying back all the debts, which will result in high interest payments.
- The company should implement innovative advertising strategies to promote its sales and enhance its reach.
- The firm should form a research and development department to keep up with the recent trends in the competitive global market

CONCLUSION

The study on cash flow analysis at Femto Soft Technologies highlights the critical role of effective cash management in ensuring the company's financial stability and operational efficiency. A detailed examination of the company's cash flow statements over five years showed that consistent monitoring of operating, investing, and financing activities is essential for maintaining liquidity and supporting strategic decisions. The analysis revealed that the company's ability to generate sufficient cash from operations is vital in funding its investments and meeting financial obligations. Moreover, understanding the trends in cash inflows and outflows enabled the identification of potential areas for improvement, such as optimizing working capital, balancing investment decisions, and minimizing unnecessary financing costs. Overall, the study reinforces the importance of cash flow as a key indicator of financial health, especially for IT and service-based firms like Femto Soft Technologies. The company can enhance its resilience, sustain growth, and build long-term value for stakeholders by applying structured financial tools and maintaining a disciplined approach to cash flow management. The process of managing financial risk has been going on for a while. It is necessary to develop and improve new strategies to transform the market. Changes in consumer expectations, changes in the corporate environment, or potential changes in the global climate can all be considered as reflections of refinement.

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Table 1: Cash flow from operating activities to total cash flow

Year	Cash flow	Total cash flow	Percentage
2018	347.41	774.18	44.87
2019	411.18	800.4	51.37
2020	527.72	994.67	53.05
2021	664.77	1996.38	33.24
2022	325.82	657.87	49.52

Table 2: Cash flow from investing activities to total cash flow

Year	Cash flow	Total cash flow	Percentage
2018	127.66	774.18	16.48
2019	134.44	800.34	16.79
2020	217.54	994.67	21.87
2021	1005.51	1996.38	50.36
2022	290.40	657.87	44.14

Table 3: Financial activities to total cash flow

Year	Cash flow	Total cash flow	Percentage
2018	299.11	774.18	38.64
2019	254.72	800.34	31.82
2020	249.41	994.67	25.07
2021	326.10	1996.38	16.33
2022	41.65	657.87	6.33

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REVIEW ARTICLE

Clinical Significance of *Dosha Amshansha Kalpana*: A Comprehensive Approach to *Ayurvedic* Diagnosis and Treatment

Kesha Patel[1]* and Vijay Bhagat[2]

[1]PG Scholar, Department of Samhita Siddhant, Parul Institute of Ayurveda, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[2]Professor, Department of Samhita Siddhant, Parul Institute of Ayurveda, Parul University, P.O. Limda, Waghodia, Vadodara,Gujarat, India.

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***Address for Correspondence**
Kesha Patel
PG Scholar,
Department of Samhita Siddhant,
Parul Institute of Ayurveda,
Parul University, P.O. Limda,
Waghodia, Vadodara, Gujarat, India.
E.Mail: keshapatel04@gmail.com

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ABSTRACT

Ayurveda, an ancient Indian medical science, underscores the significance of health as foundational for achieving *Purushartha Chatushtya - Dharma, Artha, Kama*, and *Moksha*. The holistic health definition provided by *Acharya Sushruta* encompasses the equilibrium of *Doshas* (*Vata, Pitta, Kapha*), *Agni* (digestive fire), *Dhatus* (tissues), *Malas* (excretory products), and mental well-being. Diseases arise from vitiation of *Doshas* due to exposure to causative factors (*Nidana*), making the elimination of these factors (*Nidana Parivarjana*) the primary line of treatment. Charaka's Nidana Sthana introduces the concept of *Nidana Panchaka -Nidana, Purvarupa, Rupa, Upashaya, and Samprapti* as a diagnostic framework, with Samprapti being pivotal for understanding disease progression and pathogenesis. This study highlights the clinical relevance of *Dosha Amshansha Kalpana* and *Vikalpa Samprapti*, which involve fractional analysis of *dosha* attributes and their contribution to disease manifestation. The framework enables precise identification of predominant Dosha and Gunas, aiding in individualized treatment protocols. Personalized interventions focus on opposing the vitiated *Gunas* to restore homeostasis. Early identification of *Dosha* imbalances during prodromal stages(Purvarupa) allows for preventive measures, minimizing disease progression.
The study further emphasizes the practical applications of *Dosha Amshansha Kalpana* in assessing pathogenesis, determining *Dosha* predominance, and tailoring interventions for conditions like *Raktapitta*, *Gulma*, and *Prameha*. By providing a detailed understanding of disease evolution, this concept enhances diagnosis, prevention, and treatment strategies. Integrating *Amshansha Kalpana* into clinical practice

ensures precision, improves therapeutic outcomes, and aligns with Ayurveda's primary aim of health preservation.

Keywords: *Dosha Amshansha Kalpana, Vikalpa Samprapti, Nidana Sthana, Nidana Panchaka.*

INTRODUCTION

Ayurveda, the ancient Indian science of life, emphasizes the importance of health as a prerequisite for achieving *Purushartha Chatushtya -Dharma*, *Artha*, *Kama*, and *Moksha*. Considering the attainment of *Purushartha Chatushtaya*, health becomes a prerequisite, and any disease hindering this must be managed effectively to ultimately achieve the supreme goal of liberation (*Moksha*)[1]. The foundation of Ayurveda lies in maintaining health and preventing diseases, as emphasized in the classical texts. *Acharya Sushruta* defined health as a harmonious state of *Sama Dosha, Sama Agni, Sama Dhatu, Sama Mala, and Prasanna Atma, Indriya, and Mana.* Achieving this holistic health ensures the capability to fulfill life's goals effectively[2]. In the context of treatment, the two critical factors to be evaluated are *Roga* (disease) and *Rogi* (patient). This evaluation aids in developing appropriate treatment protocols. Before selecting therapeutic interventions (*Aushadha*), it is essential to thoroughly examine the disease, with the *Nidana Panchaka* (five diagnostic tools) serving as a vital framework. These include *Nidana* (etiology), *Purvarupa* (prodromal symptoms), *Rupa*(clinical manifestations), *Upashaya* (therapeutic tests), and *Samprapti* (pathogenesis)[3]. Doshas-Vata, Pitta, and Kapha are the physiological entities in Ayurveda responsible for maintaining body functions. When vitiated due to improper Nidana (causative factors), they lead to various diseases. *Charaka's Nidana Sthana* introduces the concept of *Nidana Panchaka- Nidana*(etiology), *Purvarupa* (prodromal symptoms), *Rupa*(clinical manifestations), *Upashaya* (therapeutic tests) and *Samprapti* (pathogenesis) - which serve as diagnostic tools. Among these, *Samprapti* provides a detailed understanding of disease progression, making it pivotal for diagnosis and treatment[4]. The *Charaka Samhita* elaborates on *Nidana Panchaka* in its *Nidana Sthana* along with the etiology and pathogenesis of eight specific diseases. The concept of *Nidana Sevana* (exposure to causative factors) illustrates the *Karya-Karana Bhava* (cause-effect relationship), emphasizing that treatment begins with the elimination of causative factors (*Nidana Parivarjana*)[5]. *Samprapti* further includes *Vikalpa Samprapti*, which focuses on the fractional analysis of *Dosha* attributes (Gunas) involved in disease manifestation. This concept, known as *Dosha Amshansha Kalpana*, plays a crucial role in identifying the predominant Dosha and its specific attributes responsible for the disease, aiding in personalized and targeted treatment approaches[6]. This study aims to explore the clinical relevance of *Dosha Amshansha Kalpana* in understanding and managing diseases.

MATERIALS AND METHODS

Source of Literature: Primary references were taken from *Charaka Samhita* (*Nidana Sthana*) and relevant commentaries by *Chakrapani*, *Gangadhara*, and others. Secondary references included contemporary studies on Dosha Gunas and their role in disease pathogenesis.

Methodology: A systematic review of classical Ayurvedic texts was conducted to extract information on *Dosha Amshansha Kalpana* and its application in diagnosing and treating diseases.

REVIEW OF LITERATURE

Samprapti in Ayurveda

Samprapti refers to the process of disease formation, beginning with *Nidana* exposure and progressing through six stages of *Kriyakala*: *Sanchaya* (accumulation), *Prakopa* (aggravation), *Prasara* (spread), *Sthana Samshraya*(localization),

Vyakti (manifestation), and *Bheda* (complications). This progression highlights the sequential interaction of vitiated *Doshas* with *Dushyas* (tissues)[7].

Vikalpa Samprapti and Amshansha Kalpana

- *Vikalpa Samprapti* identifies the varying fractions of *Doshas* contributing to disease. It is instrumental in determining the predominant *Dosha* or *Guna* responsible for specific symptoms.
- *Amshansha Kalpana* extends this analysis to the Gunas of *Doshas*, such as *Ruksha* in *Vata*, *Ushna* in *Pitta*, and *Snigdha* in *Kapha*, which are pivotal in understanding disease pathology.

Clinical Utility

By recognizing the Dosha predominance and their specific Gunas, treatments can be tailored to pacify the vitiated attributes using oppositional qualities. For instance, a disease caused by the *Sheeta Guna* of *Vata* may require *Ushna* therapies.

DISCUSSION

The concept of *Dosha Amshansha Kalpana* plays a transformative role in *Ayurvedic* diagnosis and treatment, ensuring precision and holistic management of diseases. It delves into the fractional analysis of *Doshas* and their specific *Gunas*, allowing for a deeper understanding of pathogenesis and therapeutic interventions. Below, the clinical implications of this concept are elaborated in detail

Identification of Nidana

Amshansha Kalpana facilitates the identification of causative factors through patient history and clinical examination. For instance, consumption of *Kalaya* (pea) aggravates *Vata* due to *Laghu*, *Sheeta*, and *Ruksha* qualities, while *Kandekshu* aggravates Vata due to *Sheeta* and *Ruksha* qualities. By understanding such correlations, physicians can recommend avoiding specific etiological factors to prevent disease progression and facilitate recovery.

Understanding Pathogenesis

This framework aids in evaluating the role of specific *Gunas* (qualities) of vitiated *Doshas* in disease pathogenesis. For example, excessive *Ushna Guna* of Pitta may cause burning sensations, while *Ruksha Guna* of *Vata* leads to dryness. This granular understanding aids in targeted interventions.

Assessment of Dosha Vitiation

Amshansha Kalpana helps assess the qualitative and quantitative vitiation of *Doshas*. It identifies the *Pradhana* and *Apradhan Doshas* in *Samsarga Dushti* or *Sannipata Dushti*. For instance, in *Vata-Kaphaja* disorders, Vata predominates based on nomenclature and associated *Gunas*.

Determining Dosha Predominance

The concept aids in evaluating the *Bala* and *Pradhanya* of *Doshas*. The predominance is determined by the extent of *Guna* aggravation, which also correlates with the disease's severity and treatment complexity.

Analysis of *Vikruti* (Pathological Changes)

Amshansha Kalpana assists in understanding pathological changes and their manifestations. For example, swelling arises from increased liquidity in *Kapha*, while burning sensations are linked to heightened *Ushna Guna* of *Pitta*. This knowledge ensures accurate diagnosis and effective treatment planning.

Personalized Medicine

Ayurveda emphasizes individual constitution (*Prakriti*) and unique pathological presentations. *Dosha Amshansha Kalpana* refines this understanding by focusing on the specific *Guna* of the vitiated Dosha. Each Guna contributes differently to the disease manifestation, and identifying the dominant Guna allows for highly customized treatments.

For example

- In a *Vata-pradhana* disorder, *Ruksha*, *Sheeta*, or *Laghu* might be the predominant *Guna*. By identifying the specific *Guna* causing the disease, the physician can select opposing treatments. If *Ruksha* is dominant, Snigdha therapies, such as oil-based massages or nourishing diets, are prioritized.
- Similarly, in *Pitta disorders*, the *Ushna* or *Tikshna Guna* might be responsible for burning sensations. Treatments that emphasize *Sheet* and *Manda* qualities, like cooling herbs or dietary modifications, can effectively mitigate symptoms.

This targeted approach minimizes over-treatment and ensures harmony between therapeutic interventions and the patient's constitution. Personalized medicine, as enabled by *Dosha Amshansha Kalpana*, enhances therapeutic outcomes while reducing adverse effects

Preventive Care

The preventive potential of *Dosha Amshansha Kalpana* lies in its ability to identify imbalances during the *Purvarupa.* Early recognition of specific *Dosha* vitiations through subtle symptoms enables timely interventions, preventing progression into full-fledged diseases

For instance

- *Vata Dosha*: In its early stage, signs like dryness of the skin, mild irregularities in bowel movements, or feelings of restlessness indicate *Ruksha* or *Chala Guna* aggravation. Administering moistening and stabilizing therapies such as milk-based diets or *Abhyanga* can arrest the progression to severe degenerative conditions like arthritis or neurological disorders.
- *Pitta Dosha*: The onset of excessive thirst, mild burning sensations, or skin discolorations suggests *Ushna* or *Tikshna Guna* imbalance. Cooling and alkalizing interventions, including *Sheeta Anna* and *Chandan*, can prevent inflammatory diseases like *Raktapitta* or *Pitta Jwara*.
- *Kapha Dosha:* Subtle symptoms such as heaviness, lethargy, or mild congestion may indicate *Guru* or *Snigdha Guna* vitiation. *Laghu Anna* and activities promoting sweating can prevent *Kapha* related pathologies like obesity or respiratory disorders.

The emphasis on prevention aligns with Ayurveda's primary aim '*Swasthasya Swasthya Rakshanam'*. By recognizing Dosha imbalances in the *Purvarupa* stage, *Dosha Amshansha Kalpana* aids in halting disease evolution and promoting long-term well-being.

Disease Understanding

Complex diseases often involve multiple *Doshas*, and their intricate interplay makes diagnosis and treatment challenging. The eight diseases described in *Charaka Nidana Sthana -Jwara, Raktapitta, Gulma, Prameha, Shosha, Kushtha, Unmada,* and *Apasmara-* serve as classic examples where *Dosha Amshansha Kalpana* proves invaluable.

For example

- In *Sannipatika Jwara***:** Identifying the *Pradhana Dosha* is crucial. If *Pitta's Ushna Guna* is most pronounced, cooling measures are prioritized. Conversely, if *Kapha's Snigdha Guna* dominates, drying therapies take precedence.
- *Kushtha***:** These are often multifactorial, involving dual or triple Dosha vitiations. The analysis of *Gunas* such as *Ruksha* of *Vata* or *Pichchila* of *Kapha* helps in understanding the disease pathology and designing a combination treatment.

Treatment Protocols

The cornerstone of *Ayurveda's* therapeutic approach lies in addressing the root cause of disease (*Nidana Parivarjana*) and pacifying the vitiated *Dosha* through *Vipareeta Guna Chikitsa*. *Dosha Amshansha Kalpana* enhances this process by pinpointing the exact Gunas responsible for pathology, allowing for precision in treatment planning.

- *Raktapitta* **:** Dominance of *Ushna Guna* of *Pitta* in this condition can cause spontaneous bleeding. Cooling and sweet treatments like *Shatavari* (Asparagus racemosus), *Draksha* (raisins), and *Ghee* based preparations pacify the *Pitta Dosha* effectively.
- *Gulma* **:** In *Vata* predominant *Gulma*, *Ruksha* and *Sheeta* Gunas are responsible for the constriction and pain. Therapies incorporating *Snigdha* and *Ushna* qualities, such as medicated oils and warm foods, relieve symptoms and promote digestion.
- *Prameha* **:** *Kapha* predominant *Prameha*, marked by *Guru* and *Snigdha* Gunas, requires treatments like bitter and astringent herbs (*Neem* or *Guduchi*) that exhibit *Laghu* and *Ruksha* properties.

By aligning therapies with the *Guna* profile of the disease, *Dosha Amshansha Kalpana* minimizes trial and error in treatment, ensuring targeted and effective outcomes.

This deep understanding of disease pathogenesis through *Amshansha Kalpana* enables practitioners to dissect even the most intricate disorders, providing clarity for comprehensive management.

CONCLUSION

The concept of *Dosha Amshansha Kalpana*, based on classical *Ayurvedic* principles, provides a detailed and logical way to understand disease development and create personalized treatments. By analyzing the specific qualities (*Gunas*) of vitiated *Doshas*, it helps identify the exact nature of imbalances and guides focused therapeutic interventions. This improves diagnostic accuracy and treatment effectiveness staying true to *Ayurveda's* personalized approach, Clinically. *Dosha Amshansha Kalpana* is useful in both prevention and treatment. It helps detect early imbalances during the initial stages of disease, preventing further progression. It is especially valuable for managing complex diseases involving multiple *Doshas* and aligns treatments with an individual's *Prakriti*, reducing side effects and enhancing results by integrating this concept into practice. *Ayurveda* emphasizes personalized healthcare while meeting modern goals of precision medicine. This approach combines traditional wisdom with current clinical needs, offering a comprehensive way to maintain health and manage diseases.

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REVIEW ARTICLE

A Comprehensive Review on the Pharmacological Activity of T*ridax procumbens* Linn.

M.K.Vijayalakshmi[1]*, Puniparthi Sunitha[2], K. Pooja[2], Sudharsan C[2], Rufina Delphine .A[2], Varshini .P[2], Ramya .G[2], Hemnath[2] and Tharun Kumar .M[2]

[1]Associate Professor, Department of Pharmaceutical Chemistry and Analysis, Faculty of Pharmacy, Bharath Institute of Higher Education and Research (Deemed to be University), Selaiyur, Chennai, Tamil Nadu, India.
[2]Student, Faculty of Pharmacy, Bharath Institute of Higher Education and Research (Deemed to be University), Selaiyur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
M.K.Vijayalakshmi
Associate Professor,
Department of Pharmaceutical Chemistry and Analysis,
Faculty of Pharmacy,
Bharath Institute of Higher Education and Research
(Deemed to be University), Selaiyur,
Chennai, Tamil Nadu, India.
E.Mail: vijiprabhudass@gmail.com

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ABSTRACT

The herb *Tridax procumbens* is a member of the Asteraceae family and can grow either annually or perennially. In Indian traditional medicine, it is generally utilized as a cultivating plant that spreads across waste ground or barren soil. Although the plant originated in tropical America, it was introduced to Australia, Asia, and tropical Africa. Though more generally known in English as "coat buttons and Tridax daisy'," locals refer to it as "Ghamara," Mexican daisy' (in Mexico), 'Jayanti Veda' (in Sanskrit), 'Gharma' (in Hindi), Dagadipala' (in Marathi), 'Vettukkaaya-thalai' (Tamil/Siddha),'Akalakohadi' (in folk) and certain Ayurvedic practitioners prescribe it as "Bhringraj." It is exceptionally high in carotenoids, saponins, and oleanolic acid, as well as ions such as calcium, magnesium, potassium, sodium, and selenium. The flowers have been demonstrated to contain isoquercetin, quercetin, luteolin, and glucoluteolin. It is widely recognized for its diverse pharmacological effects, including antimicrobial activity against both gram-positive and gram-negative bacteria, protection of the liver, reduction of inflammation, promotion of wound healing, regulation of blood sugar levels, lowering of blood pressure, modulation of the immune system, treatment of bronchial catarrh, dysentery, and diarrhea, and the ability to prevent hair loss by stimulating hair growth.It is also employed as an adsorbent for chromium.

Caffeic acid, tannins, stigmasterol, kaempferol, lutein, ferulic acids are all present in the *Tridax procumbens* leaf have anti-inflammatory, antioxidant, and anti-cancer qualities.

Keywords: *Tridax procumbens,* Pharmacological, Taxonomical, Botanical

INTRODUCTION

Herbal drugs often operate at a subtle level, bolstering and enhancing bodily systems and processes that have become inadequate, while also aiming to reduce excessive elements that have grown dominant. Approximately 65% of people worldwide, according to estimates from the World Health Organization (WHO), primarily obtain their medical care from herbal remedies [1]. Herbal remedies and folklore medicines are widely used in both industrialized and developing countries to treat a wide range of diseases. Therefore, rather than using synthetic medications to treat any illness, natural remedies that may minimize therapeutic potential with potent and dynamic healing effects and diminish the toxicities and side effects of their synthetic equivalents should be used [2-4]. Members of the Compositae or Asteraceae family, which also includes *T. balistoides*, *Tridax procumbens*, and coat buttons, are called *Tridax procumbens* Linn, ghamara in local vernacular, Mexican daisy' (in Mexico), 'Jayanti Veda' (in Sanskrit), 'Gharma' (in Hindi), Dagadipala' (in Marathi), 'Vettukkaaya-thalai' (Tamil/Siddha), 'Akalakohadi' (in folk) or kansari in Hindi. A medicinal plant known by the names Trilobata or Coat Button, *Tridax procumbens*, is one of the other members of the genus [5-6]. In the past, India has utilized *Tridax procumbens* as an insect repellent, anticoagulant, and treatment for wounds [7-8]. This well-known ayurvedic treatment for liver disorders or having a hepatoprotective property is also used to treat heartburn and gastritis. Traditional healers in some parts of India also use *Tridax procumbens* to cure boils, blisters, and wounds [9-10]. Procumbenetin is a flavonoid that has been extracted from the aerial parts of *Tridax procumbens*[11]. As isolated chemical components, the plant has also produced fatty acids, polysaccharides, sterols, alkyl esters, and pentacyclic triterpenes. Several essential chemical components were found to be present [12-13]Fig.1.

SYNONYM

- *Amellus pedunculatus Ortega* ex Willd.
- *Balbisia canescens* Rich.
- *Balbisia divaricata* Cass [15].
- *Balbisia elongata* Willd.
- *Balbisia pedunculata* Ortega ex Hoffmanns.
- *Chrysanthemum procumbens* (L.) Sessé and Moc.
- *Tridax procumbens* var. canescens (Rich. ex Pers.) DC.
- *Tridax procumbens* var. ovatifolia B.L.Rob. Ex B.L.Rob. and Greenm.
- *Tridax procumbens* var. procumbens [16-17].

TAXONOMICAL CHARACTERISTICS

Taxonomical characteristics refer to the specific traits or features used by taxonomists to classify organisms into different taxonomic groups. These characteristics can include both morphological (physical) traits and genetic characteristics [18-19] Table 1.

BOTANICAL CHARACTERISTICS

Appearance: Creeping perennial plant *Tridax procumbens*. It is capable of reaching a length of 8 to 30 inches.

Foliage: The leaves are positioned opposite each other, have a feather-like shape, are elongated to oval in shape, and measure 1 to 2 inches in length. They are characterized by sharp tips and serrate edges.

Habit: An herb that grows in a prostrate or reclining position. The stems and leaves are adorned with trichomes.

Root: Tap root system

Stem: Herbaceous, cylindrical, decumbent and branched [21].

Leaf: Simple, opposite, exstipulate, and dentate margins exhibiting reticulate venation.

Flowers: The blossoms are characterized by yellow disk flowers and white rays. They are held on a stalk that is 4-12 inches in length and measures approximately 0.4-0.6 inches in width. Spring is the season for flowering. The tubular, yellow-centered white or yellow flowers have three-toothed ray florets and resemble daisies. Capitulum is the name of the inflorescence [22-23]. There are two kinds of flowers: disc florets with basal placentation and ray florets. Occasionally, the flowers have long, peduncled heads and three lobes.

Inflorescence: The terminal head and receptacle of the head is convex and encircled by a green involucre. The tubular florets are located in the center, while the ligulate florets are situated at the edges.

Seed: Non-endospermous

Fruits: Achenes, which can range in color from dark brown to black, make up the fruits. Their shape is oblong and their length measures 0.08 inches. Each achene possesses a cluster of pappus bristles with a length ranging from 0.12 to 0.24 inches. The fruit is a rigid achene enveloped in rigid hairs and possesses a plume-like white pappus at one extremity. The plant exhibits invasiveness due to its prolific production of achenes, each equipped with a pappus that enables wind dispersal across considerable distances.

Tubular or Disc Florets: Tubular or disc florets possess bracts, lack bracteoles, are attached directly to the stem, have all essential parts (such as sepals, petals, stamens, and pistils), exhibit five-fold symmetry, are radially symmetrical, and have the ovary positioned above the receptacle. [24]

Calyx: The calyx is minimized and replaced by multiple hairy protrusions known as pappus that remain on top of the ovary and do not fall off.

Corolla: The corolla consists of five petals that are fused together, forming a tube-like structure. They have a regular shape and their edges meet in a manner similar to valve-like closures.

Androecium: There are five stamens present, which are attached to the petals and fused together. The anthers have two chambers, are attached at the base, open towards the inside, and split lengthwise [25-26].

Gynoecium: The ovary is lowly positioned, consisting of two fused carpels. It is unilocular and contains a solitary ovule located on the basal part of the ovary. The style is uncomplicated and has a divided stigma.

Floral Formula: Br., Ebrl., %, K x, C (5), A0, G (2)Fig.2.

Chemical Characteristics

- Investigations were made into *Tridax procumbens'* proximate, mineral, and phytochemical makeup.
- This finding raises the possibility that the populace could obtain protein supplements and pro-vitamin A (carotenoids) from this plant. Additionally, it suggests that *Tridax procumbens* nutritional value can be enhanced by dehydration [27].

Chemical Constituents

- *Tridax procumbens* is a species of plant from which a number of active chemical components have been extracted and published. Alkaloids, flavonoids, carotenoids, -sitosterol, fumeric acid, luteolin, quercetin, oxo ester, palmitic acid, myristic acid, linoleic acid, and tannin are some of these [30]. Dexamethasone, glucoluteolin, beta-sitosterol, and quercetin were all identified in previous investigations as well. The plant's aerial portions have been demonstrated to contain linolenic acid. Two more pure water-soluble polysaccharides from the leaves, WSTP-IA and WSTP-IB, also include the main chain of -(1->6)-D galactan [31-33]. A report on the use of GC-MS for *Tridax procumbens* sterol and lipid identification has also been made. Additionally, researchers have extracted a brand-new flavonoid named "Procumbenetin" from the plant's aerial components. It has been identified as 3, 6-dimethoxy-5, 7, 2', 3', 4'-pentahydroxyflavone 7-O-glucopyranoside [34].
- The minerals found in *Tridax procumbens* leaves are selenium, magnesium, potassium, sodium, and calcium. An excellent plant protein and potassium supplement, *Tridax procumbens* may also provide people with provitamin A (carotenoids) [29, 30]. Moreover, four new terpenoids (Taraxasterol acetate, beta-amyrenone, lupeol, and oleanolic acid) along with bis-bithiophene have been reported from this plant species [35]. Additionally, two new flavones, 8,3′-dihydroxy-3,7,4′-trimethoxy-6-O-β-D-glucopyranosyl flavone and 6,8,3′-trihydroxy-3,7,4′-trimethoxyflavone, were isolated from *Tridax procumbens Linn*. There are also four known chemicals in the plant: betulinic acid, esculetin, puerarin, and oleanolic acid. [36]Table 2.

Pharamcological Activities

T. procumbens extracts have been widely researched for their therapeutic potential, including bactericidal fungicide, wound therapy, immune-regulating antioxidant, anticancer properties, hemostatic, parasitic, and anticoagulant effects, among others[37]Fig.3.

Hepatoprotective Activity

Reducing the severity of liver damage generated by D-galactosamine/lipopolysaccharide (D-GalN/LPS), the aerial portions of Tridax have been reported to demonstrate strong hepatoprotective effect. The ability of D-GalN/LPS to kill liver cells has led to the hypothesis that it is hepatotoxic. The human viral hepatitis lesion and the D-GalN-induced multifocal necrosis are similar [38–40]. As a result of endotoxin toxicity, this amino sugar causes fulminant hepatitis after 8 hours of therapy. It is recognized to selectively interfere with transcription and indirectly hepatic protein synthesis [41].

Antioxidant Activity

Chemicals that limit the start or continuation of oxidative chain reactions are known as antioxidants, and they impede or delay the oxidation of extracellular molecules. Plant extracts from *Tridax procumbens* were assessed for their in vitro antioxidant capacities [41-43]. A reliable method for assessing the antioxidant activity in vitro is the DPPH method [44]. It is based on the interaction between the antioxidant (AH) and the DPPH (1, 1-diphenyl, 2-picryl hydrazyl) free radical, which is nitrogen-centered [45-47]. The Ethyl acetate and n-Butanol fractions of the methanolic extract have shown significant activity comparable to that of ascorbic acid. The fractionation of the original extract simplified its composition and provided a more precise assessing of the specific phytochemicals responsible for the antioxidant activity of *Tridax procumbens.*

Antiparasitic Activity

Through a bioassay-guided fractionation with a methanol extract to isolate an active component, (3S)-16,17-Didehydrofalcarinol, *Tridax procumbens* has been tested as a treatment for various disorders like malaria, dysentery, colic, and vulnitis caused by protozoal infections. When employing whole-plant crude extracts from Tridax, leishmanicidal effects appeared to be present [48]. *Tridax procumbens* flowers, leaves, and stem were studied for their potential antiplasmodial properties using aqueous, chloroform, ethyl acetate, and ethanolic extracts. According to research utilizing the tetrazolium-based colorimetric assay, the species' aqueous and ethanolic extracts had anti-plasmodial capabilities. *T. procumbens* also aided in preventing *P. falciparum* damage to red blood cells.

Leishmanicidal Activity

The Yucatan Peninsula's *Tridax procumbens* whole plant Methanol extracts were tested in an in vitro bioassay for leishmanicidal activity against *Leishmania mexicana* promastigotes [49-50].

Antidiabetic Activity

Tridax procumbens Linn leaf extract in alcohol and water has antidiabetic action, which results in a marked decrease in blood glucose concentration. In the paradigm of rat alloxan-induced diabetes, antidiabetic activity was examined. In diabetic rats, a 50% acute and subchronic dose of *Tridax procumbens* methanol extract demonstrated to reduce fasting blood glucose levels. This plant material does not have any impact on the sugar levels in ordinary rats.[51-53].

Anti-Ulcer Activity

At a dose of 400 mg/kg, *Tridax procumbens* ethanolic leaf inhibited the ulcer lesion index and altered the pH and volume of the stomach in rats with ethanol-induced ulcers. Additionally, the extract helps to regenerate big fibroblast cells and lowers glutathione (GSH), catalase, and superoxide dismutase (SOD) levels in the stomach of rats. The medicinal potential of *Tridax procumbens* was therefore demonstrated by these results [54-55].

Anti-Obesity

Atherogenic diet-induced obesity rats received methanolic extracts of *Tridax procumbens*. Triglycerides, total cholesterol, High-Density Lipoprotein (HDL) cholesterol, total protein, and free fatty acids were measured [56]. In rats treated with extract, A rise in good cholesterol (HDL) was recorded and a substantial decline in triglycerides, total cholesterol, total protein, and free fatty acids. *Tridax procumbens* thus demonstrated having considerable anti-obesity activity [57-58].

Wound Healing Activity

The lysyl oxidase action, protein content, and breaking capacity were all improved by *T. procumbens* extraction, which aids in the promotion of wound healing [59]. As a result, epidermal and dermal cells interacted more. In addition to increasing the amount of protein and nucleic acids, the tridax extract also boosted the quantity of glycosaminoglycan [60-63].

Anticancer Activity

Phytochemicals is an engaging beneficial area to research that will have a bright future. According to Iqbal *et al*. (2017), the rising prevalence of cancer, along with the high cost, side effects, and high toxicity of existing anticancer medications, has presented a number of problems to those around the world who are attempting to design and create an alternative that is low in cost, environmentally friendly, and more environmentally friendly. Recently, a limited number of scientific reports have been published, indicating that the aerial portion of the *T. procumbens* plant is abundantly populated with potent anticancer agents, including flavonoids, tannins, and saponins, as identified through the extraction method[64-66].

Anti-Inflammatory Activity

Tridax procumbens extract's anti-inflammatory properties were evaluated in paw edema caused by carrageenin in addition to the common medication, Ibuprofen. Similar to a Tridax extract, the inhibitory zone was seen. When used with the common medication Ibuprofen, the Tridax extract effectively inhibited edema[67]. There was a noticeable reduction in inflammation when Ibuprofen plus Tridax extract was used. Rats received various doses of an oral water-soluble powder containing Tridax leaf extract. According to the findings, the extract had analgesic properties. By employing the rat-paw edema assay, the dose of *Tridax procumbens L.* demonstrated edema inhibition in the percentages of 10.82, 16.80, and 11.39, resulting in less writhing in the abdomen with Tridax alcoholic and hydro alcoholic extract of anti-inflammatory action [68].

CONCLUSION

Tridax procumbens Linn is a prevalent wildflower in India and is an individual of the Compositae family. It was primarily indigenous to tropical America; however, it has since dispersed and turn common in tropical Africa, Asia, and Australia. The pharmacological activity of each component of this plant is substantial, and it is extensively distributed. The diverse biological activities of *Tridax procumbens* are attributed to the presence of a variety of phytoconstituents, which render it a potential source for the development of novel therapeutic agents. In addition to its many other uses, this plant has the ability to protect the liver, modulate the immune system, speed wound healing, lower blood pressure, fight against diabetes, deter insects, reduce inflammation, and promote hair growth and prevention of dysentery. *Tridax procumbens*is used as a bio-adsorbent to get rid of dangerous Cr (VI) from industrial waste water. It is also used in medicine. It is a critical element of "Bhringraj" in Ayurveda and has the potential to serve as a valuable source of herbal medications for the pharmaceutical industry in the future. The removal of toxic Cr (VI) from industrial effluent is one of the therapeutic and bioadsorbent applications of *Tridax procumbens*. It is an essential component of "Bhringraj" in Ayurveda and has the potential to serve as a significant source of herbal medications for the pharmaceutical industry in the future.

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Table 1. Taxonomical Characteristics

Domain	Eukaryota
Kingdom	Plantae
Subkingdom	Tracheobionta
Phylum	Spermatophyta
Subphylum	Angiospermae
Division	Magnoliophyta
Class	Magnoliopsida
Subclass	Asteridae
Clade	Angiosperms
Order	Asterales
Family	Asteraceae
Tribe	Heliantheae
Genus	Tridax
Species	*T.procumbens*
Binomial name	*TridaxProcumbens*

Table 2. Some phyto constituents and their dry and wet weight [28-29].

Phyto Constituents	**Dry Weight**	**Wet Weight**
Calcium	20.96	2.09
Magnesium	3.56	0.35
Potassium	31.92	3.18
Sodium	50.44	5.02
Selenium	0.20	0.02
Crude protein	34.57±0.00%	3.44±0.00%
Crude fat	6.03±0.20%	0.60±0.02%

Total Carbohydrate	51.26±0.20%	5.10±0.02%
Crude Fiber	6.13±0.40%	0.61±0.04%
Total Metabolites	397.59±2.61kcal/100 g	39.56±0.26kcal/100 g
Total Ash Content	2.01±0.20%	0.20±0.02%
Carotenoids	94.57 mg/100 g	9.41 mg/100 g
Saponins	103.52mg/100g	10.30mg/100g



Fig.1: *T. procumbens* Linn. A. The whole plant B. Leaves of plant C. Flowers of plant [14]

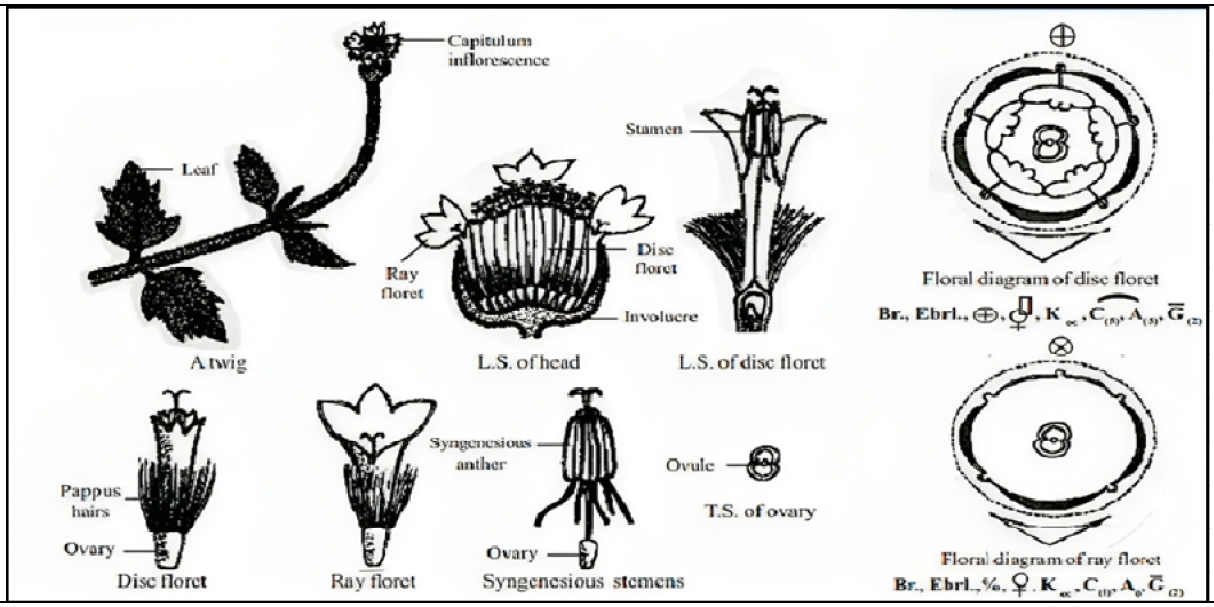


Fig.2: Botanical Classification of *Tridax procumbens*[20]

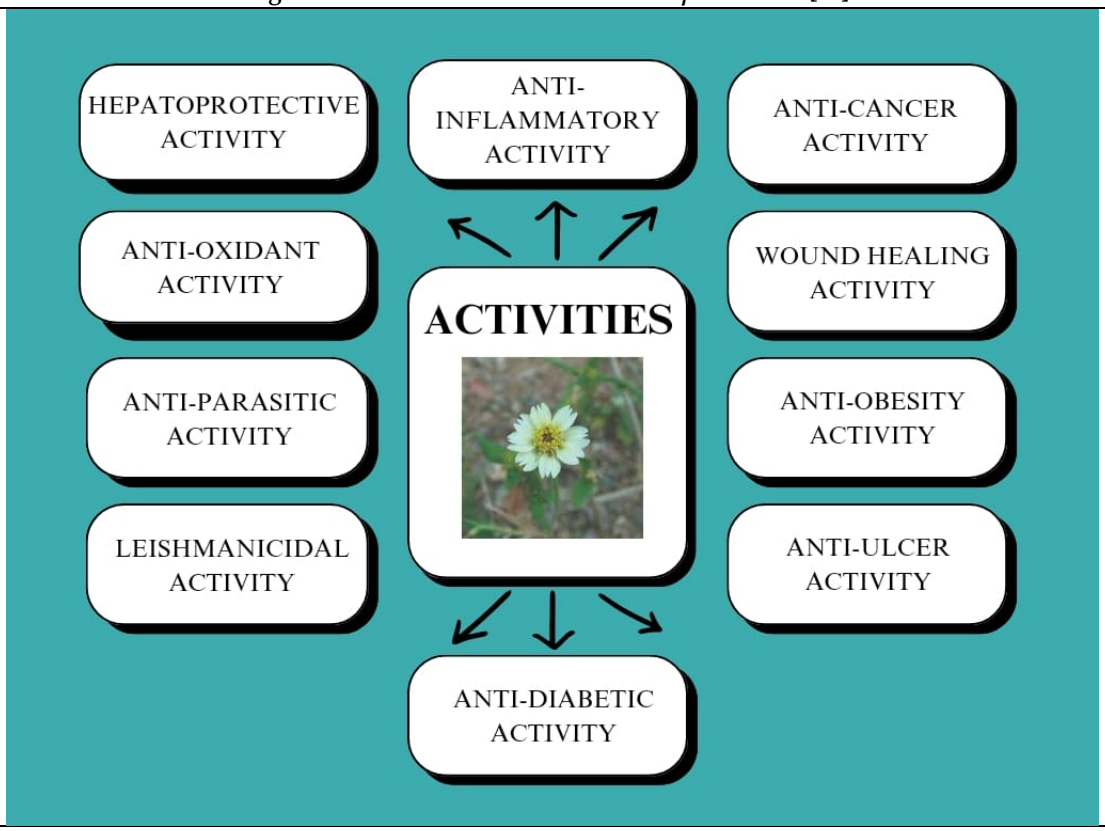


Fig.3: Pharmacological Activity of *Tridax procumbens*

RESEARCH ARTICLE

Evaluating Growth, Flowering, Yield and Economics of Zinnia (*Zinnia elegans*) cv. Berneray Giant Mix under Different Growing Media in Agro-Climatic Conditions of Dehradun

A. K. Saxena[1] and Suneeta Singh[2]*

[1]Associate Professor, Department of Soil Science, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India.
[2]Associate Professor, Department of Horticulture, School of Agricultural Sciences, Shri Guru Ram Rai University, Dehradun, Uttarakhand, India.

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***Address for Correspondence**
Suneeta Singh
Associate Professor,
Department of Horticulture,
School of Agricultural Sciences,
Shri Guru Ram Rai University, Dehradun,
Uttarakhand, India.
E.Mail: suneetasingh@sgrru.ac.in

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ABSTRACT

The present experimental trial was conducted during 2021-22 at the Horticulture Nursery, Department of Horticulture, School of Agricultural Sciences, Shri Guru Ram Rai University, PathriBagh, Dehradun, Uttarakhand, India, to "Evaluating Growth, Flowering, Yield and Economics of Zinnia (*Zinnia elegans*) cv. Berneray Giant Mix under Different Growing Media in Agro-climatic Conditions of Dehradun." The experiment was laid out in a randomized block design with three replications and ten treatments. The treatments consisted of different organic growing media in various combination ratios, including T1 (Control), T2 (Vermicompost + Soil @ 3:1), T3 (FYM + Soil @ 3:1), T4 (Cocopeat + Soil @ 3:1), T5 (Leaf Manure + Soil @ 3:1), T6 (Cow Urine + Soil @ 20%:1), T7 (Vermicompost + FYM + Cocopeat + Soil @ 1:1:1:1), T8 (Vermicompost + FYM + Cow Urine + Soil @ 1:1:20%:1), T9 (Vermicompost + FYM + Leaf Manure + Soil @ 1:1:1:1), and T10 (Vermicompost + FYM + Cocopeat + Cow Urine + Leaf Manure + Soil @ 1:1:1:20%:1:1).Observations on various growth, flowering, yield attributes, and economics were recorded at 30 and 60 days after transplanting (DAT), and at the final harvest. The results revealed that treatment T2 (Vermicompost + Soil @ 3:1) was the most effective in terms of growth attributes, such as leaf length (11.89 cm), leaf width (6.77 cm), number of nodes per plant (12.89), and stalk length (18.21 cm). For flowering attributes, T2 also showed the best performance, with bud length (1.50 cm) and bud diameter (1.34 cm). Yield attributes were highest in T2 as well, with the number of florets per flower (49.83), flower yield per plant (0.52 kg), and flower yield per hectare (26.05q). However, the longest shelf life (3.16 days)

was recorded in treatment T1 (Control). In terms of economics, the highest benefit-cost ratio (1:1.39) was observed in treatment T3 (FYM + Soil @ 3:1).

Keywords: Growing media, Vermicompost, FYM, Cocopeat, Leaf Manure, Cow Urine, Shelf Life

INTRODUCTION

Floriculture is the study of the cultivation, production, and marketing of a wide range of plants and planting materials, including flowers, potted plants, foliage, cut flowers, garden flower stocks, nursery flowering leaves, annuals, perennials, flower bulbs, and tubers (Ullah*et al.,* 2019). Zinnia (*Zinnia elegans*), a member of the Asteraceae family with a chromosome number of 2n=24, is native to Mexico and Central America. Zinnia flowers are known for their bright, uniform colours and sturdy, disease-resistant plants (Sardoei *et al.,* 2014). The genus name honours Johann Gottfried Zinn, a German botanist, and the 'Benary's Giant' series of zinnia was awarded the Association of Specialty Cut Flower Growers (ASCFG) Cut Flower of the Year in 1999 (Sloan and Harkness, 2008).Zinnias are notable for their solitary, long-stemmed flowers, which come in a variety of bright colours. These flowers thrive across tropical, subtropical, and temperate regions, with blooming from June until frost. Zinnia requires full sunlight, warmth, and a hot, dry climate. It should be planted in well-drained soil rich in humus, with loamy to sandy loam soils being most suitable. The ideal soil pH ranges from slightly acidic to neutral or slightly alkaline. The type of growing media significantly impacts the growth and development of plants, as the physical and chemical properties of the media such as structure, texture, pH, and nutrient levels (nitrogen, phosphorus, and potassium) are key factors. A suitable medium composition and nutritional status play a vital role in producing high-quality flowering plants with a greater number and size of flowers (Sardoei *et al.,* 2014). The growing medium should be porous, uniform in texture, able to hold sufficient moisture, and well-drained.Various organic materials such as pure soil, vermicompost, farmyard manure (FYM), coconut compost (cocopeat), leaf manure, and cow urine can be used to assess their effects on the growth and flowering of zinnia. Vermicompost (VC) is a simple and effective technique for converting agricultural waste, city garbage, and kitchen waste into nutrient-rich compost through the action of earthworms. The growth of earthworms in organic waste is termed vermiculture, and the conversion of waste into compost is known as vermicomposting (Olle, 2019). Vermicasts, produced by earthworms, are rich in NPK and micronutrients (Palanichamy *et al.,* 2011; Ravimycin, 2016), and their application improves the physical properties of soil, increasing water-holding capacity while reducing bulk density (Adugna, 2016).Farmyard manure (FYM) is a decomposed mixture of dung and urine from farm animals, along with litter and leftover material from roughages or fodder. On average, FYM contains 0.5% nitrogen, 0.2% phosphorus, and 0.5% potassium. FYM enhances soil organic matter (SOM), which increases cation exchange capacity and improves soil physical conditions, such as the stability of soil aggregates, density, moisture retention, and air movement (Chahal *et al.,* 2020).Cocopeat is a valuable alternative to traditional peat moss, with its high-water holding capacity and air-filled porosity making it ideal for crop cultivation (Sandeep, 2018). Cocopeat is derived from coconut husks and is known to produce healthier plants requiring less frequent watering and fertilization. Commercial growers using cocopeat achieve higher-quality plant stock (Barrett *et al.,* 2016). Leaf manure compost is a dark, rich organic matter that can be used as soil media, adding essential nutrients to the growing medium. Cow urine, recognized in Ayurveda for its therapeutic value, is composed of 95% water, 2.5% urea, and a mix of salts, hormones, enzymes, and minerals (Randhawa, 2010). It has been identified as a biofertilizer and biopesticide, capable of killing pesticide- and herbicide-resistant bacteria, viruses, and fungi (Sahu *et al.,* 2019).There are reports that combining organic growing media can increase the growth, flowering, and yield of flowers. Therefore, an experiment was conducted to assess the effect of different growing media on the growth, flowering, yield, and economics of zinnia under the agro-climatic conditions of Dehradun.

MATERIALS AND METHODS

An experimental trial was conducted at the Horticulture Nursery of the Department of Horticulture, School of Agricultural Sciences, Shri Guru Ram Rai University, PathriBagh, Dehradun, Uttarakhand, during the summer season of 2021-22. The experiment was laid out in a Randomized Block Design (RBD) with three replications. A total of ten treatments were tested: Control (Soil @100%, T1), Vermicompost + Soil @ 3:1 (T2), FYM + Soil @ 3:1 (T3), Cocopeat + Soil @ 3:1 (T4), Leaf Manure + Soil @ 3:1 (T5), Cow Urine + Soil @ 20%:1 (T6), Vermicompost + FYM + Cocopeat + Soil @ 1:1:1:1 (T7), Vermicompost + FYM + Cow Urine 20% + Soil @ 1:1:20%:1 (T8), Vermicompost + FYM + Leaf Manure + Soil @ 1:1:1:1 (T9), and Vermicompost + FYM + Leaf Manure + Cow Urine + Cocopeat + Soil @ 1:1:1:20%:1:1 (T10).The soil at the experimental site was sandy loam in texture, with a pH of 7.12, available nitrogen at 220.04 kg ha-1, available phosphorus at 9.1 kg ha-1, and available potassium at 18.1 kg ha-1. The zinnia cultivar "Berneray Giant Mix" was used for the research. The seeds of zinnia were sown in raised nursery beds on 31st March 2022. All necessary precautions regarding nursery management were taken until the seedlings were ready for transplanting. The organic growing media, including vermicompost, FYM, cocopeat, leaf manure, and cow urine, were prepared and filled into nursery polybags with a capacity of 1.5 kg according to the treatment combinations. Healthy seedlings were transplanted into the nursery polybags at the end of April 2022.Cultural practices were carried out regularly as required for the zinnia plants during the course of the investigation. During the experiment, six randomly selected plants from each replication were used for recording various observations on growth, flowering, yield attributes, and economics at 30 and 60 days after transplanting, as well as at the final harvest stage. The observations were recorded using instruments such as a verniercaliper (for bud length and bud diameter), a measuring scale (for leaf length, leaf width, and stalk length), and an electronic weighing balance. The obtained data were statistically analyzed using standard statistical methods as suggested by Gomez and Gomez (1996). Treatment Details (Table 1)

RESULTS AND DISCUSSION

The various growth, flowering, yield parameters, and economic aspects, including leaf length, leaf diameter, nodes per plant, stalk length, bud length, bud diameter, number of florets per flower, flower yield (kg/plant), flower yield (q/ha), shelf life, and B:C ratio, were significantly influenced by different concentrations of organic growing media compared to the control throughout the investigation. The data presented in Tables 2, 3, 4, and 5, as well as Figures 1 through 10, illustrate that significant improvements were observed with the application of various combinations of organic growing media on zinnia economics in comparison to the control. The findings of the present investigation have been recorded and are thoroughly discussed below:

Growth Parameters

Leaf length (cm)

The observation of leaf length recorded and presented in Table 2 and Figure 1, revealed significant differences among the treatments. Leaf length was measured at 30 days, 60 days, and at final harvest. The results showed that treatment T2 (Vermicompost + Soil) produced the best results compared to the other treatments. At 30 days after transplanting, the highest leaf length of zinnia was recorded in treatment T2 (11.77 cm), which was statistically similar to treatments T7 (11.67 cm), T8 (11.56 cm), T3 (11.51 cm), T6 (11.44 cm), T10 (11.39 cm), T5 (11.37 cm), T9 (11.30 cm), and T4 (10.78 cm). No significant difference was observed with these treatments, while the lowest value (10.59 cm) of leaf length was recorded under treatment T1 (Control).At 60 days after transplanting, the maximum leaf length was obtained in treatment T2 (12.0 cm), which was at par with treatments T7 (11.99 cm), T8 (11.72 cm), T3 (11.57 cm), T6 (11.48 cm), T5 and T10 (11.43 cm), and T9 (11.34 cm). A significant difference was observed with treatment T4 (10.78 cm). The minimum leaf length (10.63 cm) was recorded under treatment T1 (Control).At Final harvest, the maximum leaf length was observed in T2 (11.89 cm), which was comparable to T7 (11.88 cm), T3 (11.77 cm), T10 (11.66 cm), T8 (11.61 cm), T6 (11.56 cm), T5 (11.43 cm), T9 (11.40 cm), and T4 (10.86 cm). However, no significant differences were observed among these treatments. The minimum leaf length (10.69 cm) was obtained in treatment T1 (Control).The

significant increase in leaf length might be attributed to the adequate nitrogen (N) supply for plant growth. Sufficient nitrogen increases the level of cytokinins in the plant, which may stimulate the shoot growth rate, resulting in enhanced leaf length and leaf breadth. Similar findings were reported by Thapa *et al.* (2021).

Leaf width (cm)

The observation of leaf width, recorded and presented in Table 2 and Figure 2, revealed significant differences among the treatments. Measurements were taken at 30 days after transplanting (DAT), 60 DAT, and at final harvest. The results indicated that treatment T2 (Vermicompost + Soil) exhibited the best outcomes compared to the other treatments. At 30 days after transplanting, the highest leaf width of zinnia was recorded in treatment T2 (6.18 cm), which was statistically similar to treatments T5 (6.09 cm), T8 (6.08 cm), T6 (5.98 cm), T4 (5.95 cm), T9 (5.91 cm), T3 (5.66 cm), T10 (5.39 cm), and T7 (5.32 cm). No significant differences were observed among these treatments, while the lowest value (5.20 cm) of leaf width was recorded under treatment T1 (Control).At 60 days after transplanting, the maximum leaf width was obtained in treatment T2 (6.70 cm), which was at par with treatments T5 (6.28 cm), T7 (6.23 cm), T8 (6.22 cm), T3 (6.02 cm), T9 (5.98 cm), T4 (5.70 cm), T6 (5.56 cm), and T10 (5.48 cm). Again, no significant differences were observed. The minimum leaf width (5.39 cm) was recorded under treatment T1 (Control).At Final harvest, the maximum leaf width was observed in treatment T2 (6.77 cm), which was comparable to treatments T7 (6.30 cm), T8 (6.25 cm), T5 (6.24 cm), T3 (6.10 cm), T9 (6.05 cm), T4 (5.99 cm), T10 (5.89 cm), and T6 (5.62 cm). However, no significant differences were observed among these treatments. The minimum leaf width (5.44 cm) was obtained in treatment T1 (Control).The significant increase in leaf width may be attributed to the adequate nitrogen (N) input, which increases the level of cytokinins in the plant. This elevated level of cytokinins may stimulate the shoot growth rate, resulting in an increase in nutrient increment. Similar findings were reported by Thapa*et al.* (2021).

Nodes per plant

The observation of nodes per plant was recorded and presented in Table 2 and fig 3 revealed significant differences among the treatments. The observation of nodes per plant was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of nodes per plant of zinnia was recorded in treatment T_2 (13.14), which were at par with the treatments T_9 (12.38). The significant difference was observed with treatment T_{10} (11.94), T_6 (11.83), T_7 (11.33), T_5 (11.22), T_4 (11.11), T_8 (11.05) and T_3 (10.55). The lowest value (10.05) of nodes per plant was recorded under the treatment T_1 (control).In case of 60 days after transplanting, the maximum number of nodes per plant was obtained in treatments T_2 (12.86), which were at par with the treatments T_9 (12.40), T_{10} (11.98), T_6 (11.73), T_5 (11.44), T_7 (11.31), T_4 (11.12) and T_8 (11.09). The significant difference was observed with treatment T_3 (10.79). The minimum nodes per plant (10.33) was recorded under the treatment T_1 (control).At final harvest days after transplanting, the nodes per plant was maximum in T_2 (12.89) which was comparable with T_9 (12.61), T_{10} (11.85), T_6 (11.80), T_5 (11.33), T_7 (11.19) and T_4 (11.05). However, significant difference was observed with treatment T_8 (10.96) and T_3 (10.67), while a minimum nodes per plant was obtained in the treatment T_1 (10.19) (control).The significant increase in nodes per plant might be due to different growing media in various levels, the intercalary meristem is located at the base of the leaf and in the internodes. It helps in the increase in the length of plant organs like nodes and internodes. Similar finding was also reported by Rajvanshi *et al.,* (2011) and Ikram *et al.,* (2012).

Stalk length (cm)

The observation of stalk length was recorded and presented in Table 2 and fig 4 revealed significant differences among the treatments. The observation of stalk length was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of stalk length of zinnia was recorded in treatment T_{10} (18.10cm), which is not at par with the treatments. The significant difference was observed with treatment T_9 (16.93cm), T_6 (16.62cm), T_7 (16.36cm), T_4 (16.15cm), T_{10} (15.89cm), T_3 (15.54cm), T_8 (15.38cm) and T_5 (15.14cm). The lowest value (14.53cm) of stalk length was recorded under the treatment T_1 (Control).In case of 60 days after transplanting, the maximum stalk length was obtained in treatments T_{10} (18.16cm), which is not at par with the treatments. The significant difference was observed with treatment T_9 (16.8cm), T_6 (16.74cm), T_7 (16.48cm), T_4 (16.2cm), T_{10} (15.98cm), T_3 (15.61cm), T_8 (15.44cm) and T_5

(15.02cm). The minimum stalk length (14.79cm) was recorded under the treatment T_1 (Control).At final harvest days after transplanting, the stalk length was maximum in T_{10} (18.21cm), which is not at par with the treatments. The significant difference was observed with treatment T_9 (16.85cm), T_6 (16.7cm), T_7 (16.68cm), T_4 (16.24cm), T_{10} (16.00cm), T_3 (15.63cm), T_8 (15.28cm) and T_5 (15.1cm). The minimum stalk length (14.91cm) was recorded under the treatment T_1 (Control).The significant increase in stalk length might be due to different growing media in various levels, the apical meristem is responsible for active division of the cell, present in the tip of the root and shoot. Similar finding was also reported by Ikram *et al.,* (2012).

Flowering Parameters

Bud length (cm)

The observation of bud length was recorded and presented in Table 3 and fig 5 revealed significant differences among the treatments. The observation of bud length was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of bud length of zinnia was recorded in treatment T_2 (1.31cm), which were at par with the treatments T_5 (1.27cm), T_6 (1.21cm), T_4 (1.20cm), T_7 and T_8 (1.15cm), T_9 (1.14cm), T_3 (1.08cm) and T_{10} (1.06cm). No significant difference was observed. The lowest value (1.03cm) bud length was recorded under the T_1 (control).In case of 60 days after transplanting, the maximum bud length was obtained in treatments T_2 (1.32cm), which were at par with the treatments T_4 and T_8 (1.21cm), T_3 (1.13cm), T_6 (1.12cm), T_5 and T_{10} (1.11cm), T_7 (1.08cm) and T_9 (1.05cm). No significant difference was observed. The minimum bud length (1.04cm) was recorded under T_1 (Control).At final harvest days after transplanting, the bud length was maximum in T_2 (1.50cm) which was comparable with T_4 and T_7 (1.26cm), T_{10} (1.19cm), T_8 (1.17cm), T_9 (1.15cm), T_6 (1.14cm) and T_3 and T_5 (1.13cm). However, no significant difference was observed. While, the minimum bud length (1.04cm) was obtained in T_1 (Control).The significant increase in bud length might be due to different growing media in various levels may be due to apical dominance in plants is the main stem of the plant, sprouting with growth from its bud, due to the growth hormone called auxin. Similar finding was also reported by Chattopadhyay (2014).

Bud diameter (cm)

The observation of bud diameter was recorded and presented in Table 3 and fig 6 revealed significant differences among the treatments. The observation of bud diameter was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of bud diameter of zinnia was recorded in treatment T_2 (1.29cm), which were at par with the treatments T_5 (1.25cm), T_4 and T_6 (1.20cm), T_9 (1.18cm) and T_7 and T_8 (1.17cm), T_3 (1.13cm) and T_{10} (1.08cm). The lowest value (1.01cm) bud diameter was recorded under the T_1 (control).In case of 60 days after transplanting, the maximum bud diameter was obtained in treatments T_2 (1.33cm), which were at par with the treatments T_8 (1.24cm), T_4 (1.19cm), T_6 (1.17cm), T_5 and T_{10} (1.13cm) and T_7 and T_9 (1.07cm). No significant difference was observed. The minimum bud diameter (1.06cm) was recorded under the T_3 and T_1 (Control).At final harvest days after transplanting, the bud diameter was maximum in T_2 (1.34cm) which was comparable with T_7 (1.26cm), T_4 (1.23cm), T_{10} (1.20cm), T_5 (1.16cm), T_8 (1.15cm), T_6 (1.13cm) and T_3 and T_9 (1.11cm). However, no significant difference was observed. While the minimum bud diameter (1.02cm) was obtained in T_1 (Control).The significant increase in bud diameter might be due to different growing media in various levels may be due to the balanced supply of nutrients including micronutrients and could be due to the soil water holding capacity. Similar finding was also reported by Chattopadhyay (2014).

Yield Parameters

Number of florets per flower

The observation of number of florets per flower was recorded and presented in Table 3 and fig 7 revealed significant differences among the treatments. The observation of number of florets per flower was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of number of florets per flower of zinnia was recorded in treatment T_2 (27.20), which is not at par with the treatments. The significant difference was observed with treatment T_5 (24.13), T_8

(22.54), T_{10} (21.33), T_3 (21.05), T_4 (21.00), T_7 (20.40), T_6 (19.63) and T_9 (19.52). The lowest value (18.64) of number of florets per flower was recorded under the treatment T_1 (Control).In case of 60 days after transplanting, the maximum number of florets per flower was obtained in treatments T_2 (42.38), which is not at par with the treatments. The significant difference was observed with treatment T_9 (38.27), T_7 (38.10), T_4 (37.84), T_5 (35.52), T_3 (34.55), T_6 (34.24), (34.18) and T_{10} (32.43). The minimum number of florets per flower (28.51) was recorded under the treatment T_1 (Control).At final harvest days after transplanting, the number of florets per flower was maximum in T_2 (49.83), which is not at par with the treatments. The significant difference was observed with treatment T_9 (45.27), T_4 (43.54), T_8 (43.51), T_6 (40.11), T_5 (39.71), T_3 (39.30), T_7 (38.74) and T_{10} (37.38). The minimum number of florets per flower (37.15) was recorded under the treatment T_1 (Control).The significant increase in disc diameter might be due to good vegetative growth converted to reproductive growth of plants in higher organic matter enriched media. Similar finding were also reported by Chauhan *et al.,* (2014) and Arunesh *et al.,* (2020).

Flower yield (Kg/plant)

The observation of flower yield (kg/plant) was recorded and presented in Table 3 and fig 8 revealed significant differences among the treatments. The observation of flower yield (kg/plant) was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of flower yield (kg/plant) of zinnia was recorded in treatment T_2 and T_7 (0.50 kg), which were at par with the treatments T_9 (0.47 kg), T_3 and T_8 (0.45 kg), T_4 and T_{10} (0.43 kg) T_5 (0.42 kg) and T_6 (0.41 kg). No significant difference was observed. The lowest value (0.40 kg) of flower yield (kg/plant) was recorded under treatment T_1 (Control).In case of 60 days after transplanting, the maximum number of flower yield (kg/plant) was obtained in treatments T_2 (0.51 kg), which were at par with the treatments T_3 (0.50 kg), T_9 (0.47 kg), T_4 and T_8 (0.46 kg), T_{10} (0.45 kg) and T_7 (0.44 kg). No significant difference was observed. The minimum flower yield (kg/plant) (0.41 kg) was recorded under the treatment T_1 (Control), T_5 and T_6.At final harvest days after transplanting, the flower yield (kg/plant) was maximum in T_2 (0.52 kg) which was comparable with T_4 (0.47 kg), T_8 and T_9 (0.46 kg), T_7 (0.45 kg), T_6 and T_{10} (0.42 kg) and T_3 and T_5 (0.41 kg). However, no significant difference was observed. While, the minimum flower yield (kg/plant) was obtained (0.40 kg) in the treatment T_1 (control).The significant increase in flower yield might be due to the fact the supply of nutrient is more balanced under integrated plant nutrient supply system, which helps to keep plants healthy (low incidence of insect-pest-disease) and enhanced soil biological activity, which in turn improves nutrient mobilization from organic matter and soil reserves. Similar result was also reported by Prasad and Gill (2007).

Flower yield (q/ha)

The observation of flower yield (q/ha) was recorded and presented in Table 4 and fig 9 which revealed significant differences among the treatments. The observation of flower yield (q/ha) was recorded at 30 DAT, 60 DAT and at final harvest. The results shows treatment T_2 (VC + Soil) showed the best results than other treatments. At 30 days after transplanting, the highest value of flower yield (quintal/ha) of zinnia was recorded in treatment T_2 (25.30 q), which were at par with the treatments T_3 (25.05 q). The significant difference was observed with treatment T_9 (23.70 q), T_8 (22.90 q), T_7 (22.65 q), T_{10} (21.90 q), T_4 (21.80 q), T_5 (21.05 q) and T_6 (20.60 q). The lowest value (20.15 q) of flower yield (quintal/ha) was recorded under treatment T_1 (Control).In case of 60 days after transplanting, the maximum number of flower yield (quintal/ha) was obtained in treatments T_2 (25.65 q), which were at par with the treatments T_3 (25.20 q). The significant difference was observed with treatment T_9 (23.90 q), T_4 (23.40 q), T_8 (23.05 q), T_{10} (22.60 q), T_7 (22.30 q), T_6 (20.90 q) and T_5 (20.80 q). The minimum flower yield (quintal/ha) (20.55 q) was recorded under the treatment T_1 (Control).At final harvest days after transplanting, the flower yield (quintal/ha) was maximum in T_2 (26.05 q) which is not at par with treatments. However, the significant differences were observed with treatment T_4 (23.60 q), T_8 (23.20 q), T_9 (23.05 q), T_7 (22.80 q), T_6 (21.15 q), T_{10} (21.00 q), T_3 (20.85 q) and T_5 (20.65 q), while minimum flower yield (quintal/ha) was obtained (20.40 q) in the treatment T_1 (Control).The significant increase in flower yield might be due to the fact that the supply of nutrient is more balanced under integrated plant nutrient supply system, which helps to keep plants healthy (low incidence of insect-pest-disease) and enhanced soil biological activity, which in turn improves nutrient mobilization from organic matter and soil reserves. Similar finding was also reported by Prasad and Gill (2007).

Shelf life (days)

The observation of shelf life was recorded and presented in Table 4 and fig 10 revealed significant differences among the treatments. Among the various organic growing media, the maximum shelf life was obtained in treatments T_1 (3.16 days), which were at par with the treatments T_3 (2.66 days), T_9 (2.33 days) and T_2, T_4, T_5, T_6 and T_7 (2.16 days). The minimum shelf life (2.0 days) was recorded under the treatment T_8 and T_{10}.The significant increase in shelf life might be due to avoiding the direct sunlight to the flowers or not kept near appliances that generates heat. Similar finding was also reported by Arunesh *et al.,* (2020).

Economics

The observation of economics was recorded and presented in Table 5 and revealed significant differences among the treatments. The net profit per hectare ranges from Rs. 40,015 to -1, 20, 290. The maximum net profit per hectare was recorded under treatment T_3 (Rs. 40,015). While minimum net profit per hectare was obtained in the treatment T_4 (Rs. -1, 20, 290).The benefit cost ratio ranged from 1: 1.39 to 1: 0.52 depending on different treatments. It was recorded maximum (1: 1.39) under the treatments T_3 (FYM + Soil) and minimum (1: 0.52) under the treatment T_{10} (Vermicompost + Farmyard Manure + Leaf Manure + Cow Urine + Cocopeat + Soil @ 1:1:1:20%:1:1).

CONCLUSION

On the basis of present research work on "Evaluating Growth, Flowering, Yield and Economics of Zinnia (*Zinnia elegans*) cv. Berneray Giant Mix under Different Growing Media in Agro-climatic Conditions of Dehradun ", it can be concluded that among different growing media treatments, the results revealed that treatment T_2 (Vermicompost + Soil @ 3:1) was found to be most operative in terms of growth attributes such as leaf length, leaf diameter, nodes per plant, stalk length; flowering attributes*viz.*bud length, bud diameter; yield attributes *viz.* number of florets per flower, flower yield (kg/plant), flower yield (q/ha) whereas, the Shelf life (3.16 days) was found to be most effective in treatment T_1 (Control). However, the B:C ratio (1:1.39) was establish to be most effective in treatment T_3 (FYM + Soil @ 3:1).

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Table 1: Treatment Details

Number of Treatment	Combinations	Concentration
T_1	Control	100% soil
T_2	Vermicompost + Soil	3 : 1
T_3	FYM + Soil	3 : 1
T_4	Cocopeat + Soil	3 : 1
T_5	Leaf manure + Soil	3 : 1
T_6	Cow urine + Soil	20 %: 1
T_7	VC + FYM + cocopeat + Soil	1 : 1 : 1 : 1
T_8	VC + FYM + cow urine + Soil	1 : 1 : 20 %: 1
T_9	VC + FYM + leaf manure + Soil	1 : 1 : 1 : 1
T_{10}	VC + FYM + Cocopeat + cow urine + leaf manure + Soil	1 : 1 : 1 : 20% : 1 : 1

Table 2: Effect of various organic growing media on leaf length, leaf width, nodes per plant and stalk lengthof zinnia at different harvest intervals

Treatment	Leaf Length (cm)				Leaf Width (cm)				Nodes per Plant				Stalk Length (cm)			
	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean
T_1	10.59	10.63	10.69	10.63	5.20	5.39	5.44	5.34	10.05	10.33	10.19	10.19	14.53	14.79	14.91	14.74
T_2	11.77	12.00	11.89	11.88	6.18	6.70	6.77	6.55	13.14	12.86	12.89	12.96	18.10	18.16	18.21	18.15
T_3	11.51	11.57	11.77	11.61	5.66	6.02	6.10	5.92	10.55	10.79	10.67	10.67	15.54	15.61	15.63	15.59
T_4	10.74	10.78	10.86	10.79	5.95	5.70	5.99	5.88	11.11	11.12	11.05	11.09	16.15	16.20	16.24	16.19
T_5	11.37	11.43	11.43	11.41	6.09	6.28	6.24	6.20	11.22	11.44	11.33	11.33	15.14	15.02	15.10	15.08
T_6	11.44	11.48	11.56	11.49	5.98	5.56	5.62	5.72	11.83	11.73	11.80	11.78	16.62	16.74	16.70	16.68
T_7	11.67	11.99	11.88	11.84	5.32	6.23	6.30	5.95	11.33	11.31	11.19	11.27	16.36	16.48	16.68	16.50
T_8	11.56	11.72	11.61	11.63	6.08	6.22	6.25	6.18	11.05	11.09	10.96	11.03	15.38	15.44	15.28	15.36
T_9	11.30	11.34	11.40	11.34	5.91	5.98	6.05	5.98	12.38	12.40	12.61	12.46	16.93	16.80	16.85	16.86
T_{10}	11.39	11.43	11.66	11.49	5.39	5.48	5.89	5.58	11.94	11.98	11.85	11.92	15.89	15.98	16.00	15.95
C.D.(5 %)	0.12				0.37				0.17				0.15			
SE(m) ±	0.04				0.12				0.06				0.05			
SE(d) ±	0.05				0.17				0.08				0.07			
C.V.	0.62				3.69				0.90				0.54			

Table 3: Effect of various organic growing media on bud length, bud diameter, number of florets per flower and flower yield (kg/plant) of zinnia at different harvest intervals

Treatment	Bud Length (cm)				Bud Diameter (cm)				Number of Florets per Flower				Flower Yield (kg/plant)			
	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean	30 DAT	60 DAT	At Final Harvest	Mean
T_1	1.03	1.04	1.04	1.03	1.01	1.06	1.02	1.03	18.64	28.51	37.15	28.10	0.40	0.41	0.40	0.40
T_2	1.31	1.32	1.50	1.37	1.29	1.33	1.34	1.32	27.20	42.38	49.83	39.80	0.50	0.51	0.52	0.51
T_3	1.08	1.13	1.13	1.11	1.13	1.06	1.11	1.10	21.05	34.55	39.30	31.63	0.45	0.50	0.41	0.45

T_4	1.20	1.21	1.26	1.22	1.20	1.19	1.23	1.20	21.00	37.84	43.54	34.12	0.43	0.46	0.47	0.45
T_5	1.27	1.11	1.13	1.17	1.25	1.13	1.16	1.18	24.13	35.52	39.71	33.12	0.42	0.41	0.41	0.41
T_6	1.21	1.12	1.14	1.15	1.20	1.17	1.13	1.16	19.63	34.24	40.11	31.32	0.41	0.41	0.42	0.41
T_7	1.15	1.08	1.26	1.16	1.17	1.07	1.26	1.16	20.40	34.18	38.74	31.10	0.50	0.44	0.45	0.46
T_8	1.15	1.21	1.17	1.17	1.17	1.24	1.15	1.18	22.54	38.10	43.51	34.71	0.45	0.46	0.46	0.45
T_9	1.14	1.05	1.15	1.11	1.18	1.07	1.11	1.12	19.52	38.27	45.27	34.35	0.47	0.47	0.46	0.46
T_{10}	1.06	1.11	1.19	1.12	1.08	1.13	1.20	1.13	21.33	32.43	37.38	30.38	0.43	0.45	0.42	0.43
C.D.(5 %)	0.10				0.09				3.12				0.03			
SE(m) ±	0.03				0.03				1.04				0.01			
SE(d) ±	0.04				0.04				1.47				0.01			
C.V.	1.02				1.46				2.49				1.56			

Table 4: Effect of various organic growing media on flower yield (q/ha) and shelf life of zinnia at different harvest intervals

Treatment	Flower Yield (q/ha)				Shelf Life (days)
	30 DAT	60 DAT	At Final Harvest	Mean	
T_1	2.36	2.26	2.36	2.32	14.66
T_2	2.84	2.90	3.10	2.94	12.66
T_3	2.60	2.55	2.92	2.69	13.66
T_4	2.50	2.69	3.01	2.73	13.33
T_5	2.41	2.51	2.66	2.52	14.66
T_6	2.40	2.64	2.76	2.60	15.33
T_7	2.39	2.63	2.80	2.60	15.66
T_8	2.45	2.52	2.59	2.52	15.33
T_9	2.54	2.54	2.53	2.53	14.33
T_{10}	2.59	2.48	2.62	2.56	15.00
C.D.(5%)	0.17				0.95
SE(m) ±	0.05				0.32
SE(d) ±	0.08				0.45
C.V.	1.92				1.83

Table 5: Effect of various organic growing media on net return (Rs ha^{-1}) andB:CRatio of zinnia at different harvest intervals

Treatment	Net Return (Rs ha^{-1})	B:CRatio
T_1	24,790	1: 1.25
T_2	-23,660	1: 0.86
T_3	40,015	1: 1.39
T_4	-120,290	1: 0.53

T_5	20,387.5	1: 1.19
T_6	-25,590	1: 0.83
T_7	-84,672.5	1: 0.61
T_8	-28,352.5	1: 0.82
T_9	-2,213.75	1: 0.98
T_{10}	-119533.75	1: 0.52

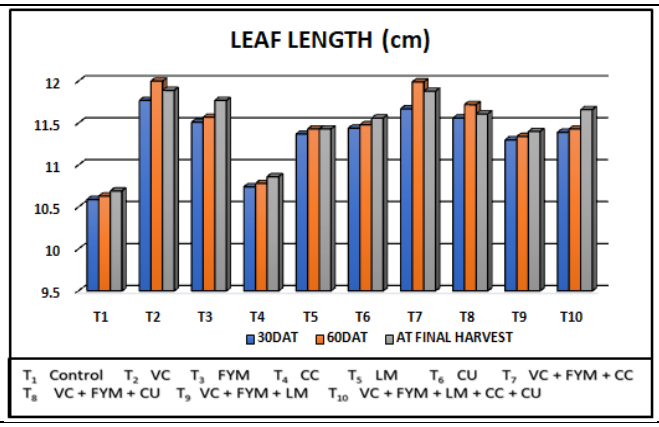


Fig 1: The effect of various organic growing media on leaf length (cm) at different harvest interval on zinnia

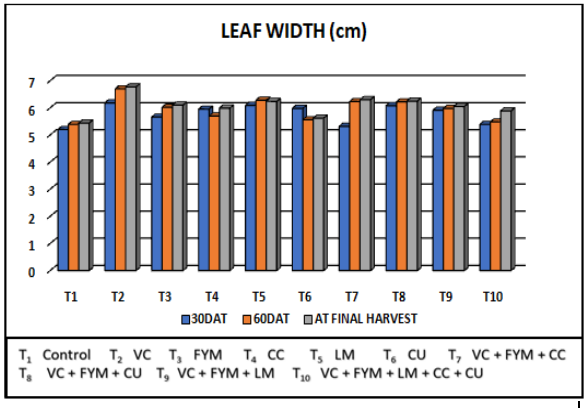


Fig 2: The effect of various organic growing media on leaf width (cm) at different harvest interval on zinnia

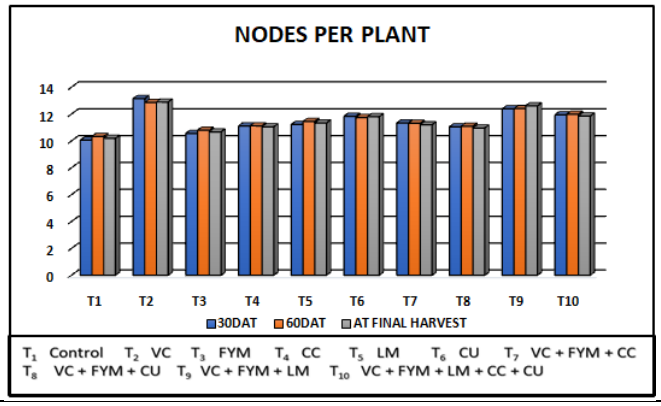


Fig 3: The effect of various organic growing media on nodes per plant at different harvest interval on zinnia

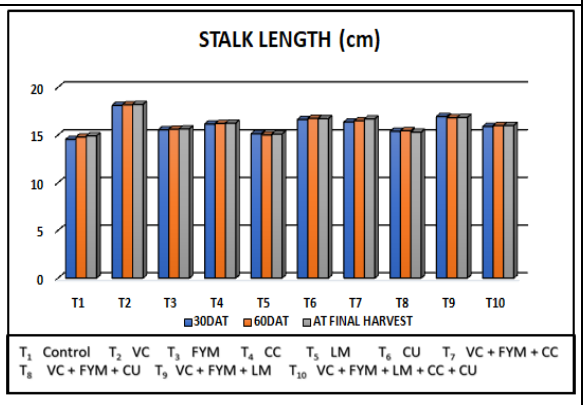


Fig4: The effect of various organic growing media on stalk length at different harvest interval on zinnia

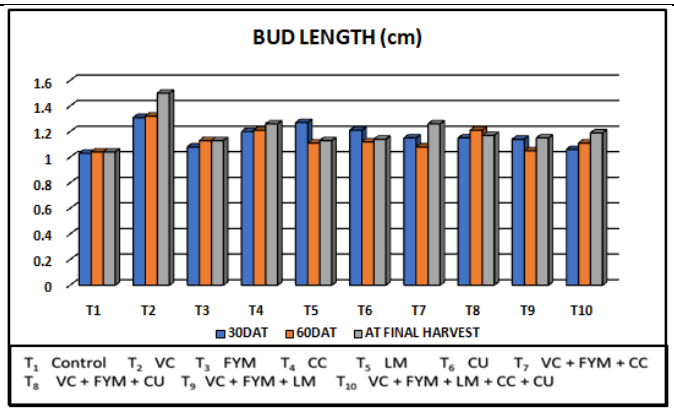


Fig 5:The effect of various organic growing media on bud length at different harvest interval on zinnia

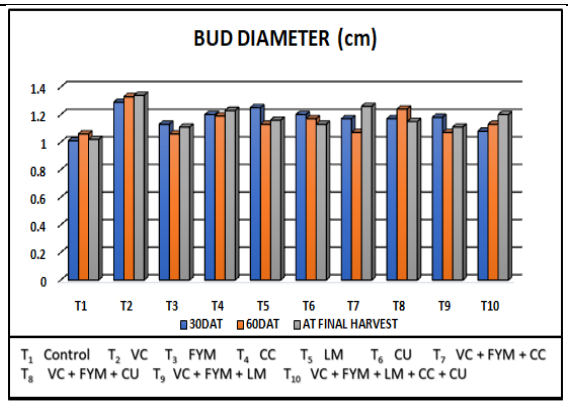


Fig 6: The effect of various organic growing media on bud diameter at different harvest interval on zinnia

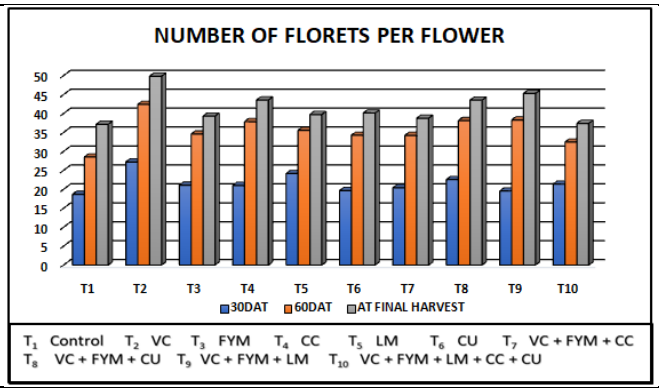


Fig 7: The effect of various organic growing media on number of florets per flower at different harvest interval on zinnia

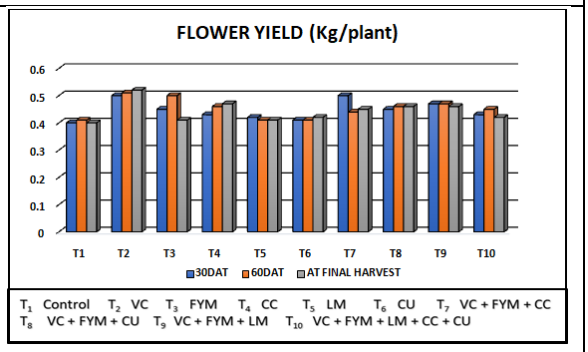


Fig 8: The effect of various organic growing media on flower yield (kg/plant) at different harvest interval on zinnia

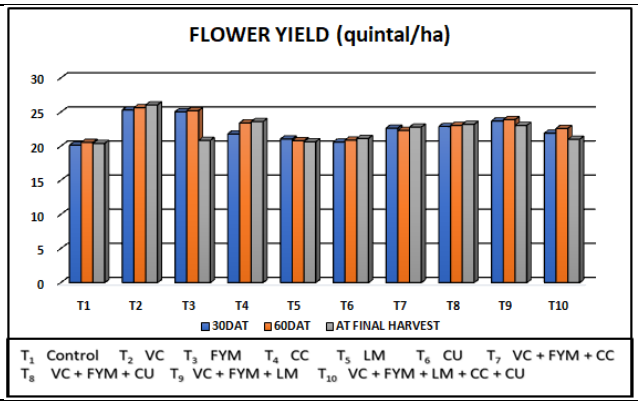


Fig: 9 The effect of various organic growing media on flower yield (quintal/hac) at different harvest interval on zinnia

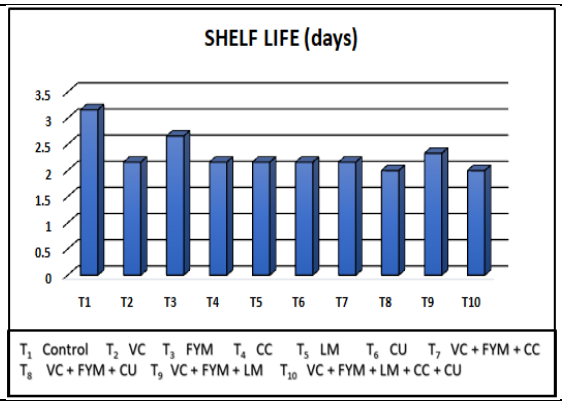


Fig 10: The effect of various organic growing media on shelf life at different harvest interval on zinnia

RESEARCH ARTICLE

An Introduction to Algebraic Properties of Edge Roman Domination Energy of a Graph

S. Nithya[1]* and V. Mohana Selvi[2]

[1]Research Scholar, Department of Mathematics, Nehru Memorial College (Autonomous), Puthanampatti, (Affiliated to Bharathidasan University), Tiruchirapalli, Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, Nehru Memorial College (Autonomous), Puthanampatti, (Affiliated to Bharathidasan University), Tiruchirapalli, Tamil Nadu, India.

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***Address for Correspondence**
S. Nithya
Research Scholar,
Department of Mathematics,
Nehru Memorial College (Autonomous), Puthanampatti,
(Affiliated to Bharathidasan University), Tiruchirapalli,
Tamil Nadu, India.
E.Mail: nithyamaths@nmc.ac.in

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ABSTRACT

The edge roman dominating adjacency matrix of a graph and its energy are introduced in this paper. Further, the algebraic properties of this matrix, including its spectrum, spectral radius, spectral gap, determinant, rank and energy are studied for some standard classes of graphs. Additionally, bounds for the above newly introduced matrix parameters are established. These findings have applications in finding structural value of a building in civil engineering, communication systems in sociology and other fields where roman domination-related concepts are needed.

Keywords: Edge roman dominating adjacency matrix (ERDAM), Edge roman dominating spectrum, Edge roman dominating energy (ERDE)
2020 Mathematical Subject Classification: 05C50, 05C69

INTRODUCTION

Let $G = (V, E)$ be a graph with $|V| = p$ and $|E| = q$. The graph terms used here are referred form F. Harary [3]. We cite Gallian's dynamic survey [5] for basic descriptions of the various types of graph labelling. Roshini Leely Pushpam and Malini Mai [8] initiated the study of the edge version of Roman domination. An edge Roman domination (ERD) function of G is a function $f: E(G) \to \{0, 1, 2\}$ such that every edge e with $f(e) = 0$ is adjacent to some edge e' with $f(e') = 2$. The weight of an edge Roman dominating function f is the value $w(f) = \sum_{e \in E(G)} f(e)$. The edge Roman domination number of G, denoted by $S'_{ar}(G)$, is the minimum weight of an

edge Roman dominating function of G. All the matrix terms are referred from R.B. Bapat [1]. Harary [4] was introduced the concept of adjacency matrix. The adjacency matrix $A(G)$ of G is the $p \times p$ matrix whose $(i,j)^{th}$– entry is 1 if the vertices v_i and v_j are adjacent and zero otherwise. The eigenvalues $\lambda_1, \lambda_2, \dots, \lambda_p$ of A, assumed in non-increasing order are the eigenvalues of the graph G. As A is real symmetric, the eigenvalues of G are real with sum equal to zero. The energy $E(G)$ of G is defined to be the sum of the absolute values of the eigenvalues of G. i.e., $E(G) = \sum_{i=1}^{p} |\lambda_i|$. This concept of energy of a graph was introduced by I. Gutman [6]. The spectrum of G is $S(G) = \begin{pmatrix} \lambda_1 & \lambda_2 & \dots & \lambda_p \\ n_1 & n_2 & \dots & n_m \end{pmatrix}$, where $n_1, n_2, \dots, n_m$ denotes the number of times the corresponding eigenvalue occur. The spectral radius of G, $\rho(G)$ is the largest eigenvalue of A. The difference between the first two numerically largest eigenvalues is called the spectral gap of G and is denoted as $\delta(G)$. The spread of G is the difference between maximum and minimum eigenvalues of A, is denoted as $spr(G)$. The sum of all diagonal entries of a matrix A is called as trace, denoted by $tr(A)$. The rank and determinant of a matrix A is denoted as $rank(A)$ and $|A|$ respectively. Domination and its associated adjacency matrices have been widely studied and applied across various fields. While Edge Roman Domination was introduced, but its corresponding adjacency matrix remain unexplored. My objective is to investigate the adjacency matrix for Edge Roman Domination, while also identifying relevant matrix parameters for several standard graph classes. Ultimately, I aim to develop an algorithm for my new matrix and applying these concepts to practical problems like optimizing networks, allocating resources, and ensuring system reliability.

MAIN RESULTS

Definition 2.1. Edge Roman dominating adjacency matrix (ERDAM) of a $(p,q)-$graph is a $p \times p$ matrix defined as, $A_r'(G) = (a_{ij}) = \begin{Bmatrix} f(e_i); & e_i = v_i v_j \in E(G) \\ 0; & otherwise \end{Bmatrix}; i,j = 1,2,\dots,p$, where $f(e_i)$'s are ERD - function values of edges of G.

Definition 2.2. The edge Roman dominating energy $E_r'(G)$ is defined as the sum of all absolute eigenvalues of the ERDAM, $A_r'(G)$ of G.

Notations 2.3. For any graph G, $S_r'(G)$, $\rho_r'(G)$, $\delta_r'(G)$, $spr_r'(G)$, $|A_r'(G)|$ and $rank\left(A_r'(G)\right)$ are respectively denoted as spectrum, spectral radius, spectral gap, spread, determinant and rank of the ERDAM of G. Trace of the ERDAM of G, $tr\left(A_r'(G)\right)$ is always zero.

Example 2.4.
G (Figure 1)

$$\boldsymbol{A_r'(G)} = \begin{pmatrix} \mathbf{0} & \mathbf{2} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{2} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{2} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{2} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \end{pmatrix}$$

For the graph G, in Figure 1, the ERDAM is,
Here, $|A_r'(G)| = 0, rank\left(A_r'(G)\right) = 4$. The characteristic polynomial of $A_r'(G)$ is $\lambda^6 - 8\lambda^4 + 16\lambda^2$. It gives $S_r'(G) = \begin{pmatrix} -2 & 0 & 2 \\ 2 & 2 & 2 \end{pmatrix}, \rho_r'(G) = 2, \delta_r'(G) = 0$ and $spr_r'(G) = 4$.
The energy of a graph G is, $E_r'(G) = |-2| + |-2| + 0 + 0 + 2 + 2 = 8$.

Theorem 2.5. For the friendship graph $F_n, n \geq 2$,
$(i) |A_r'(F_n)| = 0$
$(ii) rank\left(A_r'(F_n)\right) = 2n$
$(iii)\ S_r'(F_n) = \begin{pmatrix} \pm 2 & \pm 1 & 0 \\ 1 & n-1 & 1 \end{pmatrix}$
$(iv)\ \rho_r'(F_n) = 2$

$(v)\ \delta_r'(F_n) = 1$
$(vi)\ spr_r'(F_n) = 4$
$(vii)\ E_r'(G) = 2(n+1).$

Proof. Let G be the friendship graph F_n with vertex set $V = \{v_1, v_2, \dots, v_p\}$ with atleast five vertices. Then the ERD function value, $f(E) = \sum_{e\in E(G)} f(e) = n+1$.

The ERDAM $A_r'(G)$ is, $A_r'(G) = \begin{pmatrix} \beta_{3\times3} & 0 & 0 & \dots & 0 \\ 0 & \alpha_{2\times2} & 0 & \dots & 0 \\ \vdots & 0 & \ddots & & \vdots \\ \vdots & \vdots & & \ddots & 0 \\ 0 & \dots & & 0 & \alpha_{2\times2} \end{pmatrix}$,

where $\beta_{3\times3} = \begin{pmatrix} 0 & 2 & 0 \\ 2 & 0 & 0 \\ 0 & 0 & 0 \end{pmatrix}$, $\alpha_{2\times2} = \begin{pmatrix} 0 & 1 \\ 1 & 0 \end{pmatrix}$.

It gives $|A_r'(G)| = 0$, $rank\left(A_r'(G)\right) = 2n$. From $A_r'(G)$ we have characteristic polynomial is $\lambda^p - (n+3)\lambda^{p-2} + \dots + 4(-1)^n\lambda$.

Therefore, the ERD spectrum is, $S_r'(G) = \begin{pmatrix} \pm2 & \pm1 & 0 \\ 1 & n-1 & 1 \end{pmatrix}$.

From $S_r'(G)$, the largest absolute eigenvalue $\rho_r'(G)$ is two. Further, $spr_r'(G) = 2-(-2) = 4$. The ERDE of G is, $E_r'(G) = \sum_{i=1}^{p} |\lambda_i| = |2| + |-2| + |1|(n-1) + |-1|(n-1) = 4 + 2n - 2 = 2(n+1)$.

For example,

F_3 (Figure 2)

$$\boldsymbol{A_r'(F_3)} = \begin{pmatrix} \mathbf{0} & \mathbf{2} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{2} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} & \mathbf{0} & \mathbf{0} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{1} \\ \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{0} & \mathbf{1} & \mathbf{0} \end{pmatrix}$$

For the above graph F_3, in Figure 2, the characteristic polynomial of $A_r'(F_3)$ is $\lambda^7 - 6\lambda^5 + 9\lambda^3 - 4\lambda$.

$(i)\ |A_r'(F_3)| = 0$ $(ii)\ rank\left(A_r'(F_3)\right) = 6$ $(iii)\ S_r'(F_3) = \begin{pmatrix} \pm2 & \pm1 & 0 \\ 1 & 2 & 1 \end{pmatrix}$
$(iv)\ \rho_r'(F_3) = 2$ $(v)\ \delta_r'(F_3) = 1$ $(vi)\ spr_r'(F_3) = 4$
$(vii)\ E_r'(F_3) = 8.$

Theorem 2.6. For the Prism graph $Y_n, n \geq 3$,

$(i) |A_r'(Y_n)| = \begin{Bmatrix} 16\,; & n=4 \\ 0\,; & otherwise \end{Bmatrix}$

$(ii)\ rank\left(A_r'(Y_n)\right) = \left\{ \begin{matrix} \frac{4n}{3}; n \equiv 0(mod\ 3) \\ \frac{4n+8}{3}; n \equiv 1(mod\ 3) \\ \frac{4n+4}{3}; n \equiv 2(mod\ 3) \end{matrix} \right\}$

$(iii)\ S_r'(Y_n) = \left\{ \begin{matrix} \begin{pmatrix} \pm2 & 0 \\ \frac{2n}{3} & \frac{2n}{3} \end{pmatrix} & ; n \equiv 0(mod\ 3) \\ \begin{pmatrix} \pm2 & \pm1 & 0 \\ \frac{2n-2}{3} & 2 & \frac{2n-8}{3} \end{pmatrix} & ; n \equiv 1(mod\ 3) \\ \begin{pmatrix} \pm2 & 0 \\ \frac{2n+2}{3} & \frac{2n-4}{3} \end{pmatrix} & ; n \equiv 2(mod\ 3) \end{matrix} \right\}$

$(iv)\ \rho_r'(Y_n) = 2$
$(v)\ \delta_r'(Y_n) = 0$
$(vi)\ spr_r'(Y_n) = 4$

$(vii)\ E_r^{'}(Y_n) = \begin{Bmatrix} \frac{8n}{3}; n \equiv 0(mod\ 3) \\ \frac{8n+4}{3}; n \equiv 1(mod\ 3) \\ \frac{8n+8}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$.

Theorem 2.7. For the Complete graph $K_n, n \geq 2$,

$(i)\left|A_r^{'}(K_n)\right| = \begin{Bmatrix} (-1)^{\frac{n}{2}}(2)^{n-2}\ ; n\ is\ even \\ 0 \qquad\qquad ; n\ is\ odd \end{Bmatrix}$

$(ii)\ rank\left(A_r^{'}(K_n)\right) = \begin{Bmatrix} n \qquad ; n\ is\ even \\ n-1\ ; n\ is\ odd \end{Bmatrix}$

$(iii)\ S_r^{'}(K_n) = \begin{Bmatrix} \begin{pmatrix} \pm 2 & \pm 1 \\ \frac{n-2}{2} & 1 \end{pmatrix}\ ; n\ is\ even \\ \begin{pmatrix} \pm 2 & 0 \\ \frac{n-1}{2} & 1 \end{pmatrix}\ ; n\ is\ odd \end{Bmatrix}$

$(iv)\ \rho_r^{'}(K_n) = \begin{Bmatrix} 1; & n = 2 \\ 2\ ; & otherwise \end{Bmatrix}$

$(v)\ \delta_r^{'}(K_n) = \begin{Bmatrix} 2\ ; & n = 2,3 \\ 1\ ; & n = 4 \\ 0\ ; & otherwise \end{Bmatrix}$

$(vi)\ spr_r^{'}(K_n) = \begin{Bmatrix} 2; & n = 2 \\ 4\ ; & otherwise \end{Bmatrix}$

$(vii)\ E_r^{'}(K_n) = 2(n-1)$.

Theorem 2.8. For the Wheel graph $W_n, n \geq 3$,

$(i)\left|A_r^{'}(W_n)\right| = \begin{Bmatrix} 4\ ; n = 3 \\ 0\ ; n \geq 4 \end{Bmatrix}$

$(ii)\ rank\left(A_r^{'}(W_n)\right) = \begin{Bmatrix} \frac{2n+6}{3}; n \equiv 0(mod\ 3) \\ \frac{2n+4}{3}; n \equiv 1(mod\ 3) \\ \frac{2n+2}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iii)\ S_r^{'}(W_n) = \begin{Bmatrix} \begin{pmatrix} \pm 2 & \pm 1 & 0 \\ \frac{n}{3} & 1 & \frac{n-3}{3} \end{pmatrix} & ; n \equiv 0(mod\ 3) \\ \begin{pmatrix} \pm 2 & \pm 1.414 & 0 \\ \frac{n-1}{3} & 1 & \frac{n-1}{3} \end{pmatrix} & ; n \equiv 1(mod\ 3) \\ \begin{pmatrix} \pm 2 & 0 \\ \frac{n+1}{3} & \frac{n+1}{3} \end{pmatrix} & ; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iv)\ \rho_r^{'}(W_n) = 2$

$(v)\ \delta_r^{'}(W_n) = \begin{Bmatrix} 1; n \equiv 0(mod\ 3) \\ 0.6; n \equiv 1(mod\ 3) \\ 0; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(vi)\ spr_r^{'}(W_n) = 4$

$(vii)\ E_r^{'}(W_n) = \begin{Bmatrix} \frac{4n+6}{3}; n \equiv 0(mod\ 3) \\ \frac{4n+5}{3}; n \equiv 1(mod\ 3) \\ \frac{4n+4}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$.

Theorem 2.9. For the Path graph $P_n, n \geq 2$,

$(i)\left|A_r^{'}(P_n)\right| = \begin{Bmatrix} -1\,; & n=2 \\ 0\,; & otherwise \end{Bmatrix}$

$(ii)\ rank\left(A_r^{'}(P_n)\right) = \begin{Bmatrix} \frac{2n}{3}; n \equiv 0(mod\ 3) \\ \frac{2n-2}{3}; n \equiv 1(mod\ 3) \\ \frac{2n+2}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iii)\ S_r^{'}(P_n) = \begin{Bmatrix} \begin{pmatrix} \pm 2 & 0 \\ \frac{n}{3} & \frac{n}{3} \end{pmatrix} & ; n \equiv 0(mod\ 3) \\ \begin{pmatrix} \pm 2 & 0 \\ \frac{n-1}{3} & \frac{n+2}{3} \end{pmatrix} & ; n \equiv 1(mod\ 3) \\ \begin{pmatrix} \pm 2 & \pm 1 & 0 \\ \frac{n-2}{3} & 1 & \frac{n-2}{3} \end{pmatrix} & ; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iv)\ \rho_r^{'}(P_n) = \begin{Bmatrix} 1 & ; & n=2 \\ 2 & ; & n \geq 3 \end{Bmatrix}$

$(v)\ \delta_r^{'}(P_n) = \begin{Bmatrix} 1\ ; n \equiv 2(mod\ 3) \\ 0\ ; \quad otherwise \end{Bmatrix}$

$(vi)\ spr_r^{'}(P_n) = \begin{Bmatrix} 2 & ; n=2 \\ 4 & ; n \geq 3 \end{Bmatrix}$

$(vii)\ E_r^{'}(P_n) = \begin{Bmatrix} \frac{4n}{3}; n \equiv 0(mod\ 3) \\ \frac{4n-4}{3}; n \equiv 1(mod\ 3) \\ \frac{4n-2}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$.

Theorem 2.10. For the Cycle graph $C_n, n \geq 3$,

$(i)\left|A_r^{'}(C_n)\right| = \begin{Bmatrix} 4\,; n=4 \\ 0\,; otherwise \end{Bmatrix}$

$(ii)\ rank\left(A_r^{'}(C_n)\right) = \begin{Bmatrix} \frac{2n}{3}; n \equiv 0(mod\ 3) \\ \frac{2n+4}{3}; n \equiv 1(mod\ 3) \\ \frac{2n+2}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iii)\ S_r^{'}(C_n) = \begin{Bmatrix} \begin{pmatrix} \pm 2 & 0 \\ \frac{n}{3} & \frac{n}{3} \end{pmatrix} & ; n \equiv 0(mod\ 3) \\ \begin{pmatrix} \pm 2 & \pm 1 & 0 \\ \frac{n-1}{3} & 1 & \frac{n-4}{3} \end{pmatrix} & ; n \equiv 1(mod\ 3) \\ \begin{pmatrix} \pm 2 & \pm 1.414 & 0 \\ \frac{n-2}{3} & 1 & \frac{n-2}{3} \end{pmatrix} & ; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(iv)\ \rho_r^{'}(C_n) = 2$

$(v)\ \delta_r^{'}(C_n) = \begin{Bmatrix} 0; n \equiv 0(mod\ 3) \\ 1; n \equiv 1(mod\ 3) \\ 0.6; n \equiv 2(mod\ 3) \end{Bmatrix}$

$(vi)\ spr_r^{'}(C_n) = 4$

$(vii)\ E_r^{'}(C_n) = \begin{Bmatrix} \frac{4n}{3}; n \equiv 0(mod\ 3) \\ \frac{4n+2}{3}; n \equiv 1(mod\ 3) \\ \frac{4n-1}{3}; n \equiv 2(mod\ 3) \end{Bmatrix}$.

The following theorem gives both lower and upper bound for the edge roman dominating energy in terms of p and q.

Theorem 2.11. Let G be a simple graph with p vertices and q edges. If $S_{ar}^{'}(G)$ be the edge roman dominating function value with cardinality $a_r^{'}(G)$ and $P = \left|\left|A_r^{'}(G)\right|\right|$.
If $\lambda_1, \lambda_2, \ldots . \lambda_p$ are the eigenvalues of $A_r^{'}(G)$ then

$\sqrt{(2q+k)+p(p-1)P^{\frac{2}{p}}} \leq E_r^{'}(G) \leq \sqrt{p(2q+k)}$, where $k = S_{ar}^{'}(G)$.

Proof. We know that Cauchy Schwarz inequality is $(\sum_{i=1}^{p} a_i b_i)^2 \leq (\sum_{i=1}^{p} a_i^2)(\sum_{i=1}^{p} b_i^2)$

If $a_i = 1, b_i = |\lambda_i|$ then $(\sum_{i=1}^{p}|\lambda_i|)^2 \leq (\sum_{i=1}^{p} 1)(\sum_{i=1}^{p} \lambda_i^2)$
$[E_r^{'}(G)]^2 \leq p(2q+k)$, since $\sum_{i=1}^{p} \lambda_i^2 \leq 2|E| + S_{ar}^{'}(G)$
which gives $E_r^{'}(G) \leq \sqrt{p(2q+k)}$ (1)

Since arithmetic mean is not smaller than geometric mean, we have
$\frac{1}{p(p-1)}\sum_{i\neq j}|\lambda_i||\lambda_j| \geq \left[\prod_{i\neq j}|\lambda_i||\lambda_j|\right]^{\frac{1}{p(p-1)}} = \left[\prod_{i=1}^{p}|\lambda_i|^{2(p-1)}\right]^{\frac{1}{p(p-1)}}$
$= \left[\prod_{i=1}^{p}|\lambda_i|\right]^{\frac{2}{p}}$

$= \left|\prod_{i=1}^{p}\lambda_i\right|^{\frac{2}{p}}$
$= \left|\left|A_r^{'}(G)\right|\right|^{\frac{2}{p}} = P^{\frac{2}{p}}$
$\sum_{i\neq j}|\lambda_i||\lambda_j| \geq p(p-1)P^{\frac{2}{p}}$
Now consider, $[E_r^{'}(G)]^2 = \left(\sum_{i=1}^{p}|\lambda_i|\right)^2 = \sum_{i=1}^{p}|\lambda_i|^2 + \sum_{i\neq j}|\lambda_i||\lambda_i|$
$[E_r^{'}(G)]^2 \geq (2q+k) + p(p-1)P^{\frac{2}{p}}$
$i.e. E_r^{'}(G) \geq \sqrt{(2q+k)+p(p-1)P^{\frac{2}{p}}}$ (2)
From (1) and (2), $\sqrt{(2q+k)+p(p-1)P^{\frac{2}{p}}} \leq E_r^{'}(G) \leq \sqrt{p(2q+k)}$

CONCLUSIONS

In this paper, we introduced the edge Roman dominating adjacency matrix and established its fundamental matrix properties for several standard graphs. Building on these results, we further explored bounds and extended our analysis to additional classes of graphs. Additionally, we aim to develop efficient algorithms for constructing and analyzing this matrix in future work.

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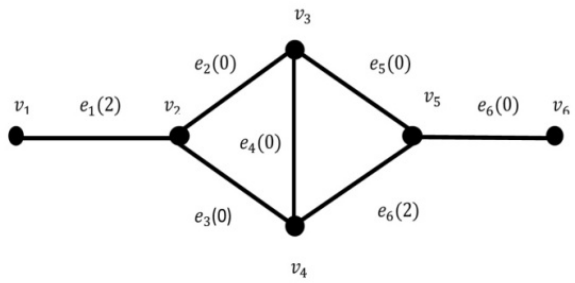


Figure 1: G

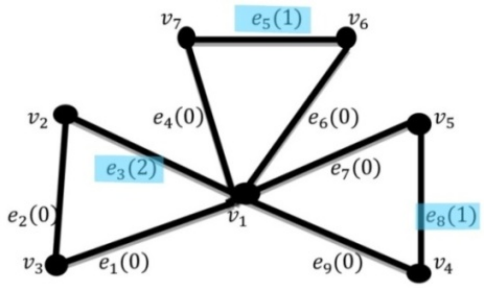


Figure 2: F_3

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RESEARCH ARTICLE

A Study on Sustainable Practices and Customer Engagement in HDFC Life Insurance

Harshavardhini R[1]* and C.Sathish Kumar[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Assistant Professor (SG), School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Harshavardhini R
Second Year MBA Student,
School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai, Tamil Nadu, India.
E.Mail:

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ABSTRACT

This study provides valuable insights into customer perceptions and satisfaction levels with HDFC Life, with a particular focus on the preferences and behaviours of policyholders. The findings reveal that HDFC Life enjoys high customer satisfaction, with most respondents appreciating the benefits offered by their insurance policies, the customer service experience, and the ease of payment methods. The company's communication efforts, both in frequency and meeting arrangements, have been well-received, with most customers expressing satisfaction.

Keywords: Environmental Sustainability, Healthcare, Innovation, Insurance, Social.

INTRODUCTION

HDFC Life firmly commits to corporate social responsibility (CSR) by focusing on education, healthcare, and environmental sustainability. Initiatives include supporting underprivileged children's education, providing access to healthcare in rural areas, and promoting environmental conservation. These efforts reflect HDFC Life's dedication to positively impacting society and contributing to the well-being of communities. HDFC Life strongly emphasizes transparency and fairness when dealing with customers and stakeholders. The company adheres to ethical business practices and regulatory compliance, ensuring that all operations are conducted with integrity and accountability. Employee-friendly policies and initiatives promote a positive and inclusive work environment, fostering a culture of collaboration and innovation.

Investor relations programs are in place to communicate company performance and strategy to shareholders, providing regular updates and insights into HDFC Life's financial performance and plans. The company has received numerous awards and recognition for its corporate governance, sustainability efforts, and customer service, including the Golden Peacock Award for Corporate Governance and Asia's Best CSR Practices Award. HDFC Life actively engages with industry associations and government bodies to contribute to the development of the insurance sector and promote financial literacy and insurance awareness among the public. The company continuously focuses on product innovation to meet evolving customer needs and market trends, ensuring it remains at the forefront of the industry.

Statement of the Problem

In today's dynamic business environment, sustainability has become essential to corporate strategy, particularly in the financial services sector. Insurance companies, as long-term risk managers and institutional investors, are expected to offer reliable financial protection and contribute positively to environmental, social, and governance (ESG) objectives. HDFC Life Insurance, as one of India's leading life insurance providers, has introduced several sustainability initiatives, such as digital transformation, paperless policies, financial literacy programs, and community development efforts. While these initiatives are intended to enhance operational efficiency and fulfil corporate social responsibilities, it remains unclear to what extent customers recognize, perceive, and value these sustainability practices.

Furthermore, although global and national research highlights the growing importance of sustainability in customer decision-making, there is limited evidence specific to the Indian life insurance sector — particularly in urban markets like Chennai. There is a research gap in understanding whether HDFC Life's sustainability practices significantly influence customer satisfaction, trust, and loyalty in this regional context.

Thus, the problem this study seeks to address is:

"To what extent do the sustainability practices adopted by HDFC Life Insurance influence customer satisfaction among policyholders in Chennai?"

This study aims to bridge the gap by examining customer awareness, perception, and satisfaction with HDFC Life's sustainability practices, ultimately providing insights to guide more effective strategy formulation and customer relationship management.

Objectives of the Study

The primary objectives of a study on HDFC Life Company may include:

- To analyse HDFC Life's business operations, including its products, services, and distribution channels.
- To assess the company's financial health, including revenue growth, profitability, and return on investment.
- To examine HDFC Life's market share, competitors, and industry trends to understand its competitive landscape.
- To investigate customer expectations, satisfaction levels, and pain points to identify areas for improvement.
- To identify potential growth drivers, such as new product launches, partnerships, expansion into new markets, and challenges like regulatory changes or market volatility.

Limitations of the Study

- The study relies on publicly available data, which may limit insights into proprietary strategies or financial details not disclosed by HDFC Life.
- Due to time limitations, the study may not cover all aspects of the life insurance industry or HDFC Life's operations in exhaustive detail.
- Customer satisfaction data and feedback may be biased or unrepresentative of the entire customer base.

Methodology Adopted (Research Design)

Area of the Study:

This study focuses on the intersection between sustainability practices and customer satisfaction in the life insurance sector, specifically examining HDFC Life Insurance operations in Chennai, Tamil Nadu, India.

The research explores how HDFC Life's initiatives related to environmental responsibility, digital transformation, corporate social responsibility (CSR), ethical governance, and customer service innovations are perceived by its policyholders.

Research Location

The geographic area selected for the study, Chennai, is significant because it is a major metropolitan city with a large, educated, and digitally aware population, which is increasingly conscious of sustainability issues. The study targets individual customers (policyholders) in Chennai to assess:

- Awareness of HDFC Life's sustainability initiatives,
- Perceptions regarding these initiatives,
- The impact of sustainability practices on customer satisfaction, trust, and loyalty.

Thus, the study area combines the insurance services sector, sustainability practices, customer behaviour analysis, and a regional focus on urban India (Chennai).

Research Design

The research used for this study is Descriptive research.

A descriptive research design is a study that aims to accurately and systematically describe a phenomenon, population, or situation. It focuses on what is happening without manipulating variables rather than why or how it happens. The researcher observes, records, and analyses information as it naturally occurs.

Sources of Data

The process of gathering and analysing accurate data from various sources to find answers to research problems, trends, and probabilities, etc., to evaluate possible outcomes is known as data collection.

The study involves the collection of both primary and secondary data.

Primary Data

Primary data was collected for this study through a structured questionnaire.

Secondary Data

Secondary data collected through

- Book
- Journals
- Website
- Google Scholar
- Articles

Sampling

A sample taken for the study is 60 HDFC Life Insurance Company clients. Sixty respondents were selected for the study using the convenience sampling technique. The sample size was chosen to ensure that the data collected was representative enough to draw meaningful conclusions while remaining manageable for analysis.

Sampling Techniques

Non-probability convenience sampling is used for this study.

The study employed a convenience sampling method, a type of non-probability sampling. Convenience sampling involves selecting participants who are easily accessible and willing to participate, making data collection more efficient. This method was chosen due to its practical nature, allowing for swiftdata collection within a limited time frame.

Data Analysis and Interpretation

Table 1: Employees React To Your Queries

Findings: 47% of respondents are satisfied with how employees react to their queries, 23% are delighted, 20% are neutral, and 10% are dissatisfied. Inference: The majority of respondents are either satisfied or highly satisfied with the response to their queries, indicating generally positive customer service.

Table 2: The Way Customers Like to be Called for the Meetings from the Company

Findings: 43% of respondents are satisfied with how they are called for meetings, 30% are delighted, 25% are neutral, and 2% are highly dissatisfied.

Inference: Most respondents are satisfied with arranging meetings, indicating a positive reception of the company's meeting approach.

Table 3: Rate Sustainability in an Insurance Company

Findings:50% of respondents rate the sustainability of an insurance company as good, 25% rate it as excellent, 20% are neutral, 3% rate it as poor, and 2% rate it as very poor.

Inference: Most respondents view insurance companies' sustainability positively, with most ratings falling between good and excellent.

Table 4: Are You Satisfied With The Overall Service Provided by HDFC Life

Findings: 57% of respondents are satisfied with the overall service provided by HDFC Life, 22% are delighted, 13% are neutral, and 8% are dissatisfied.

Inference: Most respondents are satisfied with HDFC Life's service, indicating a positive perception of their offerings.

SUMMARY OF FINDINGS

Demographics: Most respondents (63%) are male, with a significant portion (65%) being single and 42% students, highlighting a younger and diverse demographic group.

Purpose of Insurance: Wealth creation is the primary perceived purpose of acquiring insurance (53%), followed by child needs (27%) and retirement benefits (20%).

Customer Base of HDFC Life: 62% of respondents are current customers of HDFC Life, reflecting a strong market presence.

Attractiveness of Policy Benefits: 83% of respondents find the benefits offered by their insurance policies attractive and preferable, indicating high customer satisfaction with policy offerings.

Preferred Mode of Premium Payment: 37% of respondents use internet payments, followed by 23% using cash, indicating a preference for convenient and flexible payment options.

Communication Frequency: The company contacted 35% of respondents quarterly, suggesting regular and consistent communication from HDFC Life.

Preferred Service System: The majority (60%) prefer personal service over other methods, emphasizing the importance of direct interaction in customer service.

Employee Response to Queries: 70% of respondents are satisfied or highly satisfied with how employees address their queries, indicating efficient customer support.

Meeting Scheduling Preferences: 73% of respondents are satisfied or highly satisfied with how they are contacted for meetings, highlighting the effectiveness of meeting arrangements.

Sustainability Perception: 75% rate sustainability in an insurance company as good or excellent, suggesting that respondents value the sustainability efforts of their insurance providers.

Overall Service Satisfaction: 79% of respondents are satisfied or highly satisfied with the overall service provided by HDFC Life, reflecting a positive overall customer experience.

SUGGESTIONS AND RECOMMENDATIONS

Enhance Personal Service Channels: Since 60% of respondents prefer personal service, HDFC Life should prioritize face-to-face interactions while exploring innovative ways to deliver a personalized customer experience. This could include dedicated relationship managers for high-value customers and customized consultations.

Increase Digital Payment Options: With 37% of respondents opting for internet payments and a significant portion (23%) preferring cash, HDFC Life should enhance its digital payment infrastructure. Offering more online payment options like mobile wallets or UPI could increase convenience and attract a broader customer base.

Improve Communication Frequency and Customization: Sincemost respondents are contacted quarterly or monthly, HDFC Life should explore more personalized communication schedules based on customer preferences. Regular, but non-intrusive, communication could increase engagement and customer loyalty.

Address Queries and Service Responsiveness: While 70% of respondents are satisfied with how their queries are addressed, there remains a 10% dissatisfaction rate. HDFC Life should focus on further training employees to improve response times and support quality. Offering more self-service options (like a customer support app) could also be beneficial.

Enhance Customer Interaction for Meetings: With 73% of respondents satisfied with how meetings are arranged, HDFC Life should maintain or improve its meeting scheduling process by offering flexible time slots, better pre-meeting communication, and virtual meeting options for convenience.

Tailor Products for Younger Demographics: Since a significant portion of the respondents are students (42%) or in their early career stages, HDFC Life could design more affordable, flexible insurance products that cater to this demographic's needs, such as low-cost life insurance or student savings plans.

Customer Satisfaction Measurement: To address the 13% of respondents who are neutral or dissatisfied with the overall service, HDFC Life should regularly conduct customer satisfaction surveys to identify specific areas of improvement, particularly in the claims process or product offerings.

Offer More Payment Flexibility: Since many respondents use cheques (17%) and demand drafts (18%) for premium payments, HDFC Life should ensure that customers who prefer traditional payment methods continue to have access to these options. Additionally, exploring instalment-based payment options could make premiums more accessible.

CONCLUSION

This study provides valuable insights into customer perceptions and satisfaction levels with HDFC Life, with a particular focus on the preferences and behaviours of policyholders. The findings reveal that HDFC Life enjoys high customer satisfaction, with most respondents appreciating the benefits offered by their insurance policies, the customer service experience, and the ease of payment methods. The company's communication efforts, both in frequency and meeting arrangements, have been well-received, with most customers expressing satisfaction. However, there are areas for improvement, such as enhancing the personalization of services and increasing the frequency of communication based on individual preferences. The study also highlights the importance of sustainability, with customers valuing eco-friendly initiatives and responsible corporate practices. Most respondents prefer personal interaction for service and meetings, emphasizing the need for HDFC Life to maintain a strong human touch while balancing the benefits of digital services. Additionally, the research underscores the importance of offering flexible payment options to cater to the diverse needs of policyholders. HDFC Life's commitment to providing attractive policy benefits and maintaining strong customer relations has contributed significantly to customer satisfaction. Adopting the suggested recommendations can further strengthen its market position, enhance customer loyalty, and continue delivering value to its policyholders in a competitive insurance landscape.

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Table 1: Employees React To Your Queries

PARTICULARS	NO. OF. RESPONDENTS	PERCENTAGE (%)
Highly satisfied	14	23
Satisfied	28	47
Neutral	12	20
Dissatisfied	6	10
Highly dissatisfied	-	-
TOTAL	60	100

Table 2: The Way Customers Like to be Called for the Meetings from the Company

PARTICULARS	NO. OF. RESPONDENTS	PERCENTAGE (%)
Highly satisfied	18	30
Satisfied	26	43
Neutral	15	25
Dissatisfied	-	-
Highly dissatisfied	1	2
TOTAL	60	100

Table 3: Rate Sustainability In An Insurance Company

PARTICULARS	NO. OF. RESPONDENTS	PERCENTAGE (%)
Excellent	15	25
Good	30	50
Neutral	12	20
Poor	2	3
Very poor	1	2
TOTAL	60	100

Table 4: Are You Satisfied With The Overall Service Providedby Hdfc Life

PARTICULARS	NO. OF. RESPONDENTS	PERCENTAGE (%)
Highly satisfied	13	22
Satisfied	34	57
Neutral	8	13
Dissatisfied	5	8
Highly dissatisfied	-	-
TOTAL	60	100

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RESEARCH ARTICLE

Better Optimization and Security for Crop Yield Prediction using LSTM in India

Chandrima Chakrabarti*

Associate Professor, Department of Computer Science and Engineering Data Science, Narula Institute of Technology, Agarpara, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata, West Bengal, India.

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***Address for Correspondence**
Chandrima Chakrabarti
Associate Professor,
Department of Computer Science and Engineering Data Science,
Narula Institute of Technology, Agarpara,
(Affiliated to Maulana Abul Kalam Azad University of Technology),
Kolkata, West Bengal, India.
E.Mail: chandrima.narula@gmail.com

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ABSTRACT

Agriculture is a major occupation that is dependent on rainfall. Weather changes play a huge role in crop yield with things like precipitation, wind, temperature, solar radiation. Accurate forecasting helps in pricing, labor planning, transportation and organizing harvest. The objective is to predict crop yield using deep learning algorithms. An improved organization function (IOF) along with a Long short term memory model is implemented (LSTM). This proposed model has lower training error and addresses the underfitting and overfitting than eight other methods. Unlike traditional neural networks, LSTM is uniquely designed to manage and remember information over extended periods, making it ideal for tasks where sequence and context are critical—such as predicting crop yields. This capability addresses challenges posed by factors like varying weather conditions, soil quality, and agricultural practices, which all influence crop growth and production outcomes. Evaluation between predicted crop yield and actual crop yield is compared in RMSE (kg/ha). Results show that LSTM proposed by IOF is better than other RNN, CNN models.

Keywords: Crop Yield; Deep Learning; Model Performance; Optimizer; LSTM.

INTRODUCTION

Agricultural productivity and food security are crucial global concerns, heavily dependent on accurate prediction of crop yields. Over the years, advancements in machine learning have significantly enhanced the precision and

reliability of yield forecasting models. Among these, the Long Short-Term Memory (LSTM) algorithm has emerged as a powerful tool, particularly adept at capturing intricate temporal patterns and dependencies in data. Unlike traditional neural networks, LSTM is uniquely designed to manage and remember information over extended periods, making it ideal for tasks where sequence and context are critical—such as predicting crop yields. This capability addresses challenges posed by factors like varying weather conditions, soil quality, and agricultural practices, which all influence crop growth and production outcomes. In recent studies, LSTM has outperformed other algorithms such as Convolutional Neural Networks (CNNs), Recurrent Neural Networks (RNNs), and basic Artificial Neural Networks (ANNs) in accurately forecasting crop yields. Its ability to handle long-term dependencies and mitigate the impact of noisy data has resulted in impressive prediction accuracies, often exceeding 95%. This reliability not only enhances decision-making processes for farmers and policymakers but also contributes to sustainable agricultural practices by optimizing resource allocation and yield management. In this context, exploring the effectiveness of LSTM in agricultural yield prediction not only highlights its technological prowess but also underscores its pivotal role in advancing precision farming techniques worldwide.

Motivation

Crop yield optimization contributes to global food security by enhancing agricultural productivity. LSTM models can help predict and optimize yields, ensuring a stable and sufficient food supply to meet the demands of a growing population. Efficient use of resources such as water, fertilizers, and pesticides is crucial for sustainable agriculture. LSTM algorithms can assist in optimizing resource allocation, reducing waste, and minimizing environmental impact. Climate change introduces uncertainties in weather patterns, affecting agricultural productivity. LSTM models can analyze historical weather data and adaptively optimize crop management strategies for changing climate conditions, enhancing resilience in agriculture. LSTM algorithms can contribute to precision agriculture by providing accurate predictions at a fine spatial and temporal scale. This enables farmers to tailor their interventions precisely, optimizing input usage and maximizing yields. Improved crop yield predictions and optimization can lead to financial stability for farmers. By minimizing losses and maximizing profits, farmers can make more informed decisions about crop selection, planting times, and resource allocation. Optimizing crop yields can lead to more sustainable agricultural practices. By minimizing unnecessary resource use, farmers can contribute to reducing the environmental impact of agriculture, such as soil degradation and water pollution. Applying LSTM algorithms in agriculture represents a technological advancement. Engaging in crop yield optimization research and implementation allows for the integration of cutting-edge technology into traditional farming practices, fostering innovation in the agriculture sector.

Survey

Previous Models Researched

IOFLSTM

The document discusses the use of deep learning models, specifically the Long Short-Term Memory (LSTM) model, to predict crop yield accurately.[1] The focus is on the implementation of an improved optimization algorithm called Improved Optimization Function (IOF) in the LSTM model. The study collected manual data from local agricultural departments and government websites for training and testing purposes. The proposed IOF was compared to other standard optimization methods and showed better performance in handling underfitting and overfitting issues. The evaluation metrics used, such as r, RMSE, and MAE, demonstrated the accuracy of the IOF-LSTM model in crop yield prediction. The results also showed that the proposed model outperformed other deep learning models such as CNN, RNN, and LSTM in crop yield prediction. The study further compared the performance of the IOF-LSTM model with different optimizers, including Adam, AdaGrad, RMSP, and Momentum. The IOF-LSTM model consistently showed better results in terms of MAE, RMSE, and r values compared to other optimization methods. Additionally, the document presents the prediction of temperature, monsoon rainfall, and crop yield for the year 2021. The model accurately predicted the temperature and rainfall for 2021, which are crucial factors in determining crop yield. The crop yield prediction results for major crops in Andhra Pradesh were also analyzed, showing the advantage of accurate rainfall prediction for crop yield. The performance of the proposed IOF-LSTM model was further compared with other deep learning models, including RNN, LSTM, ANN, and CNN. The IOF-LSTM model

consistently outperformed these models in terms of MAE, RMSE, and r values, indicating its superior accuracy in crop yield prediction. The study also compared the performance of the IOF-LSTM model with different statistical measures, including Multivariate Logistic Regression (MLR), Support Vector Regression (SVR), Analysis of Variance (ANOVA), and Partial Least Squares Regression (PLSR). The IOF-LSTM model showed better results in terms of MAE, RMSE, r, and MASE values compared to these measures. Overall, the proposed IOF-LSTM model demonstrated its effectiveness in accurately predicting crop yield by incorporating deep learning models and improved optimization techniques. The model showed better performance compared to other deep learning models and statistical measures.

LSTM-RF

In this document, the authors propose a framework for improving the accuracy of wheat yield prediction using a combination of unmanned aerial vehicle (UAV) thermal infrared and multispectral imagery. [2] The study conducted a winter wheat field experiment with three levelsof irrigation and collected multispectral vegetation indices (VIs) and canopy water stress indices (CWSI) using a UAV equipped with cameras. The authors developed a framework that combinesa long short-term memory neural network (LSTM) and random forest (RF) to predict wheat yieldusing the VIs and CWSI as predictors. The results showed that the LSTM-RF model achieved better prediction results compared to the LSTM model, with a 22% reduction in root mean square error (RMSE). The authors concluded that the LSTM-RF model considered both the time-series characteristics of the winter wheat growth process and the non-linear relationship between remote sensing data and crop yield data, providing an alternative for accurate yield prediction in modern agricultural management. The document highlights the importance of yield prediction in agricultural production and the potential of remote sensing technology based on UAVs for non-intrusive and cost-effective prediction. The authors emphasize the need to consider multiple growth stages and the advantages of combining multiple sensors to improve prediction accuracy. They also discuss the application of machine learning algorithms, such as RF and LSTM, in crop parameter evaluation and yield prediction. The authors describe the methodology used in the study, including the collection and processing of UAV-based data, the selection of spectral indices, and the implementation of the LSTM-RF model. They present the statistical analysis and results, including the correlation between vegetation indices and yield, the performance evaluation of the LSTM and LSTM-RF models, and the relationships between measured and predicted yield. The study has several limitations and areas for improvement. The authors acknowledge that the framework was only validated under water stress conditions in a single environment, and further studies are needed to validate its performance in different environments. They also suggest the integration of data from different sources and the fusion of multimodal information for better accuracy and practicality. The authors propose future research directions, including the use of sensors that capture structural characteristics of crops and the validation of the framework in different environments. In conclusion, the document presents a comprehensive study on improving wheat yield prediction using UAV thermal infrared and multispectral imagery. The proposed LSTM-RF framework shows promising results in terms of prediction accuracy and provides an alternative for accurate yield prediction in modern agricultural management. However, further research is needed to validate the framework in different environments and integrate data from different sources for improved accuracy and practicality.

BO-LSTM

In this document, the authors propose a new framework for winter wheat yield prediction using a combination of deep learning and Bayesian optimization. The goal of this research is to provide accurate and timely predictions of winter wheat yield, which is crucial for food policy making and food security. The authors highlight the importance of agricultural big data and artificial intelligence (AI) in achieving smart agriculture and cost-effective solutions for the agricultural sector. The proposed framework, called BO-LSTM, utilizes a Bayesian optimization-based long- and short-term memory model to extract crop growth features from multiple data sources for winter wheat yield prediction. The performance of BO-LSTM is compared with other models, includingsupport vector machine (SVM) and linear regression Lasso. The results show that BO-LSTM outperforms the other models in terms of accuracy, with the highest accuracy achieved when using a combination of GPP, climate, LAI, and VIs as input variables. The authors also explore the robustness of the BO-LSTM method by evaluating its performance in different regions. The

results demonstrate that the model can achieve higher estimationaccuracy in regions with concentrated distribution of winter wheat cultivation and less influence of human factors. Furthermore, the approach used in this study has the potential to forecast crop yields in regions with limited data availability and can utilize publicly available multi-source data for timely and effective yield estimation. The document provides a detailed description of the methodology used, including the structure ofthe LSTM neural network and the process of Bayesian optimization for hyperparameter selection. The performance of the model is evaluated using metrics such as coefficient of determination (R2), root mean square error (RMSE), and mean absolute percentage error (MAPE). In conclusion, the proposed BO-LSTM framework shows promising results for winter wheat yield prediction, with higher accuracy compared to other models. The integration of deep learning and Bayesian optimization allows for efficient hyperparameter optimization and the fusion of multiple data sources for improved estimation accuracy. The framework has thepotential to contribute to food security and agricultural decision-making by providing timely and accurate predictions of winter wheat yield.[3].

ACYP-CPODL

The paper presents an Automated Crop Yield Prediction utilizing Chaotic Political Optimizerwith Deep Learning (ACYP-CPODL) model for predicting crop yields. The model aims to overcome the challenges of accurately predicting crop yield, which is influenced by factors such as genotype, environment, and their interaction. The ACYP-CPODL model involves three main processes: pre-processing, prediction, and parameter optimization. In the pre-processing phase, the input data is converted to CSV format and normalized using the min-max method. The prediction process utilizes a hybrid Convolutional Neural Network (CNN)Long-Short Term Memory (LSTM) model, which is designed to capture the long-term temporal dependency in multivariate sequences. The model is trained using the pre-processed data and produces crop yield predictions. To optimize the hyperparameters of the CNN-LSTM model, the Chaotic Political Optimizer (CPO) algorithm is employed. The CPO algorithm combines chaotic maps with the political optimization process to improve its efficiency. The algorithm adjusts the hyperparameters based on the fitness of the solutions and performs party transferring to manage both exploration and exploitation. The performance of the ACYP-CPODL model is evaluated on benchmark datasets for tomato and potato yield predictions. The results show that the ACYP-CPODL model outperforms other existing techniques in terms of mean squared error (MSE) and R2 score. The proposed model achieves an MSE of 0.031 and an R2 score of 0.936 for potato yield prediction, indicating its effectiveness in predicting crop yields. The paper concludes that the ACYP-CPODL model is a valuable tool for predicting crop yields, which is crucial for irrigation scheduling and labor requirements. The model's performance is validated through extensive simulations and comparisons with other techniques, demonstrating its superiority. Future research directions include designing fusion-based prediction models to further improve predictive outcomes.[5].

CNN-LSTM

This document presents a novel approach called DeepYield for crop yield forecasting using a combined convolutional neural network (CNN) with long short-term memory (LSTM). The aim of the study is to extract spatiotemporal patterns from remote sensing data to improve the accuracy of crop yield predictions. The proposed DeepYield model integrates ConvLSTM layers with a 3-Dimensional CNN (3DCNN) for feature extraction. The models are trained using historical yield data and MODIS satellite data for soybean-growing counties in the United States. The results show that DeepYield outperforms other techniques such as Decision Trees, CNN + GP, and CNN-LSTM in terms of accuracy and reliability. The study highlights the importance of considering the spatial and temporal characteristics of remote sensing data for crop yield forecasting. The authors suggest that future research should explore the inclusion of additional variables and investigate the impact of extreme events on the predictability of machine learning models. Overall, DeepYield provides a promising approach for improving crop yield forecasting, which has implications for crop market planning, insurance, and nutrient management.[8].

Bidirectional LSTM

Researchers have developed a predictive model for the photosynthetic carbon assimilation rate (A) of individual rice leaves. The model utilizes a hybrid architecture called BLSTM, which combines LSTM and bi-directional LSTM (BLSTM). The model uses three easily obtainable external environmental variables (photosynthetic photon flux density, ambient CO2 concentration, and temperature) to indirectly generate mesophyll variables required for prediction. The most important variable for predicting A is found to be mesophyll CO2 concentration (Ci). The model is trained using gas-exchange measurements from rice leaves, and the prediction accuracy is evaluated using nested cross-validation. The results show that the BALSTM model, which incorporates Ci and stomatal conductance to water vapor (gsw) as intermediate outputs, outperforms the LSTM model using only the external environmental variables. The reproducibility of Ci and gsw is found to be 2.01 μmol mol-1 and 0.061 mol m-2 s-1, respectively, with low mean absolute percentage errors. The prediction accuracy of A is highest when the time-series length of the input data is 5 minutes. The model is also tested on data from a different year, and while the prediction accuracy varies, the BALSTM model consistently performs better than the LSTM model with only external environmental variables. The findings suggest that incorporating mesophyll variables improves the prediction accuracy of A, and further research is needed to improve the model's performance under different conditions and for different cultivars. The study highlights the potential of computer modeling and machine learning in predicting crop yield and improving agricultural practices.[9].

LSTM with a attention block

The document discusses the development of an automatic crop expert system using an improved Long Short-Term Memory (LSTM) model with an attention block. The system aims to provide farmers with recommendations on the most suitable crops for cultivation based on factors such as soil type, climate requirements, and crop characteristics. The traditional method of manual decision-making in crop selection is time-consuming and relies heavily on the expertise and experience of the farmer. The proposed system utilizes a customized LSTM model with 27 layers, including an attention block to address the issue of vanishing and exploding gradients. The model takes seven input features, including pH, humidity, rainfall, nitrogen, potassium, phosphorus, and temperature, and recommends the most appropriate crop from a list of 22 options. Evaluation metrics such as recall, precision, F1-score, and accuracy are used to assess the performance of the model, with the proposed LSTM achieving an accuracy of 97.26%. The system outperforms existing techniques and provides accurate recommendations for crop selection. Future work includes the development of a mobile application and web interface for easy access to the system.[11].

EEMD-LSTM

The document discusses a prediction model for runoff in irrigated paddy areas in southern China. The model combines the Ensemble Empirical Mode Decomposition (EEMD) method and the Long Short-Term Memory (LSTM) network. Meteorological factors are used as inputs to the model, with rainfall being the main variable affecting runoff. The model is applied to the Yangshudang watershed in the Zhanghe Irrigation System and compared to other models. The results show that the EEMD-LSTM multivariate model performs better than other models, with high simulation performance and improved prediction accuracy for peak flows. The model is also effective in predicting runoff in rainfall and nonrainfall events. The study concludes that the EEMD-LSTM model is suitable for simulating and predicting the daily-scale rainfall-runoff process in irrigated paddy areas, providing technical support for water resource management.[12].

I. MAML

The document discusses a research article on an ensemble deep learning algorithm for forecasting rice crop yield based on soil nutrition levels. The study aims to address the importance of accurate and timely crop yield estimates for agricultural policy and investment decisions. The proposed methodology utilizes an ensemble deep learning system called Model Agnostic Meta-Learning (MAML) for classification. The study uses soil nutrient data and crop production statistics as input for the algorithm. The dataset undergoes preprocessing techniques such as normalization and mean attribute to handle missing values. The MAML algorithm receives output from three different classifiers - Deep Neural Network (DNN), Deep Belief Network (DBN), and Support Vector Machine

(SVM). Theensemble classifier then predicts the amount of rice crop yield based on the soil's nutrient levels. The proposed method achieves an accuracy rate of 89.5%, indicating its effectiveness in predicting crop yield. The study also compares the proposed method with other existing techniques such as SVM, DNN, DBN, and KNN classifiers. The comparison shows that the proposed method outperforms the existing techniques in terms of accuracy, precision, recall, F1 score, specificity, and negative predictive value. The study concludes that the ensemble deep learning algorithm, MAML, is a suitable approach for forecasting rice crop yield based on soil nutrition levels. The accuracy and performance metrics of the proposed method demonstrate its superiority over existing techniques. The findings of the study have implications for improving agricultural productivity and decision- making in the context of crop yield prediction.[16].

Use of LSTM in Agriculture

Winter wheat yield prediction in China using LSTM model

The document discusses a study on predicting winter wheat yield using a long short-term memory (LSTM) model and MODIS LAI (Leaf Area Index) products. The accuracy of the yield estimation results using LSTM is compared with conventional machine learning methods. The study found that the LSTM model generally outperformed other models, with the best results showing an R2 value of 0.87 and an RMSE value of 522.3 kg (about 1151.47 lb)/ha. The use of LAI as a single input factor improved the yield estimation, although it may cause yield uncertainty in extreme years. The study highlights the implications of this method for crop yield forecasting, agricultural disaster monitoring, food trade policy, and food security early warning. The study focused on Henan Province in China, which is an important region for winter wheat production. The authors used MODIS LAI data from 2003 to 2015 as input for the LSTM model to predict the yield for 2016. They analyzed the effects of different time steps and the LAI time series on the estimation results. The results showed that the accuracy of the yield estimation did not increase with increasing step size and data volume. However, the LSTM model performed better than conventional machine learning methods. The study divided the wheat-growing areas in Henan Province into five regions based on production conditions and climatic characteristics. The authors also compared the performance of the LSTM model with different input time series data. They found that using LAI data from the nodulation to filling stage resulted in higher accuracy compared to using data from the entire growing season. The best performance was achieved with a time step of 2, which had an R2 value of 0.87, an RMSE value of 522.32 kg (about 1151.52 lb)/ha, and a MAPE value of 5.67%. The study suggests that using a shorter time range of LAI data can still yield accurate predictions and reduce the amount of data required. Furthermore, the study compared the performance of the LSTM model with other machine learning methods such as random forest, support vector regression, partial least squares regression, and XGBoost. The LSTM model outperformed these methods in terms of yield estimation accuracy. Overall, the study demonstrates the effectiveness of using an LSTM model with MODIS LAI data for winter wheat yield prediction. The findings have implications for improving yield estimation, crop forecasting, disaster monitoring, and food security. The study suggests that using a shorter time range of LAI data and the LSTM model can provide accurate and timely predictions of winter wheat yield.[4].

Predicting cocoa yield

The document discusses the use of deep learning models for predicting cocoa yield in agriculture. The traditional statistical models used for crop yield prediction have limitations such as time wastage, inaccurate predictions, and difficulties in model usage. Therefore, the researchers propose an ensemble deep learning approach using a Convolutional Neural Network (CNN) and Recurrent Neural Network (RNN) with Long Short-Term Memory (LSTM) for cocoa yield prediction. The CNN is used to handle the climatic dataset, while the RNN is employed to handle the cocoa yield prediction. The ensemble approach is chosen due to the nature of the dataset used, which includes both climatic and cocoa yield datasets. The proposed model is compared to other machine learning algorithms based on Mean Absolute Error (MAE), Mean Square Error (MSE), Root Mean Square Error (RMSE), and Mean Absolute Percentage Error (MAPE). The results show that the CNN-RNN with LSTM model gives the least mean absolute error, demonstrating its efficiency in predicting cocoa yield. The researchers also explore other machine learning models such as Multilayer Perceptron(MLP), Naïve Bayes (NB), and Seasonal Auto-Regression Integrated Moving Average (SARIMA). The results show that MLP and NB have lower mean absolute error

compared to SARIMA, indicating their better performance in cocoa yield prediction. Additionally, the document mentions the use of CNN-LSTM and RNN-LSTM models for cocoa yield prediction. The results show that the proposed model outperforms these models in terms of mean absolute error. In conclusion, the ensemble deep learning approach using CNN-RNN with LSTM proves to be an efficient method for predicting cocoa yield. It outperforms other machine learning models and deep learning models in terms of accuracy. This approach can be valuable for decision-makers and farmers in making informed planning and policy decisions in agriculture.[6].

Predicting soybean cultivation

The document discusses the development of a soil moisture prediction model using a recurrent neural network long short-term memory (RNN-LSTM) approach in soybean cultivation. The aim of the study is to predict soil moisture levels and determine appropriate irrigation timing based on weather conditions. The model uses sensors to measure soil moisture and temperature at various depths along with weather data as input variables. The RNN-LSTM model is trained using time series data and evaluated using loss and coefficient of determination (R2) values. The best performing model achieved an R2 of 0.999 and a loss of 0.022 for a depth of 10 cm (about 3.94 in), an R2 of 0.999 and a loss of 0.016 for depths of 20 cm (about 7.87 in) and 30 cm (about 11.81 in). The model was able to accurately predict soil moisture and could be applied to optimize irrigation for crop growth. The study concludes that a soil moisture prediction model based on time-series weather data can aid in determining the appropriateamount of irrigation needed for crop cultivation[7].

Predicting wheat production in Pakistan

The document discusses a study conducted to develop a forecasting model for wheatproduction in Pakistan using LSTM (Long Short-Term Memory) neural networks. The study highlights the importance of wheat production for Pakistan's economy and the need for accurate forecasting to address the increasing consumption and potential threats to the economy. The researchers used historical data from 1902 to 2018 and compared their proposed model with existing models in the literature. They also introduced a data pre-processing smoothing mechanism to improve prediction accuracy. The results showed that the proposed model outperformed existing models and indicated that wheat production will gradually increase in the next ten years, but the production to consumption ratio will continue to decline, posing a threat tothe economy. The researchers recommend using their framework for wheat production and suggest that it can be applied to other crops to promote sustainable agriculture development. The study provides details on the methodology used, including the use of Robust-LOWESS as a smoothing function and the parameters selected for the LSTM-NN model. The performance of the models was evaluated using metrics such as MAE (mean absolute error), RMSE (root mean squared error), and R-value (correlation coefficient). The LSTM-NN model developed with pre- processed data showed the best results in terms of accuracy. The study concludes by providing ten-year ahead wheat production forecasts for Pakistan.[10].

Using LSTM for crop yield prediction in Andhra Pradesh

The document discusses the development of a robust and efficient machine learning model for smart farming decisions and intelligent agriculture. The primary objective is to predict crop yield based on historical data and various parameters such as temperature, humidity, pH, rainfall, and crop type. The research proposes a novel machine learning approach for a heterogeneous data environment containing IoT-sensed data related to the environment, agricultural conditions, plant features, and demands. The authors use a dataset of five crops (rice, ragi, gram, potato, and onion) collected from Andhra Pradesh. They apply different machine learning algorithms, including regression methods, decision trees, naive Bayes, support vector machines, k-means clustering, and deep learning techniques such as LSTM and RNN. Among the machine learning techniques, the random forest algorithm performs best with a training accuracy of 99.27% for crop yield prediction. The sigmoid activation function achieves 99.71% accuracy with four hidden layers for predicting crop yield. The research highlights the importance of using IoT and machine learning technologies for smart farming and intelligent agriculture practices. It emphasizes the need for a robust and efficient machine learning environment to achieve optimal prediction accuracy and reliability. The proposed model aims to assist stakeholders in making optimal smart farming decisions and increasing agricultural productivity. The research provides a comprehensive review of existing

literature on IoT and machine learning approaches for smart agriculture and highlights the gaps and motivations for the proposed research. The proposed research methodology includes data acquisition, preprocessing, feature extraction and selection, and prediction using a heterogeneous ensemble learning environment. The document concludes by discussing the experimental observations and research significance of the proposed model. [13].

Predicting tomato yield in South Korea

The document discusses a study on the prediction of tomato yields using a combination of a traditional statistical time series model and a deep learning model. The researchers aimed to predict how various environmental factors, such as temperature, humidity, and CO2 levels, affect tomato yields by integrating an attention-based LSTM network and an ARMA model. In the first part of the proposed model, an encoding attention-based LSTM network was used to identify the environmental variables that significantly influence tomato yields. The network analyzed time series data and identified the variables that had the greatest impact on yield. In the second part of the model, an ARMA model, which is a statistical time series analysis model, wasused to improve the accuracy of the yield predictions given by the LSTM network. The ARMA model compensated for the difference between the predicted and actual yields. The researchers conducted experiments using data collected from 83 farm households in South Korea. They found that the internal temperature, internal humidity, and CO2 levels were the environmental factors that had the greatest influence on tomato yields. The results showed that the proposed method predicted yields more efficiently and effectively than existing models. The study also compared the proposed method with traditional regression models, such as linear regression, ridge regression, and SGD regression. While the traditional regression models showed similar predictive power, they had more prediction errors, especially during periods of sudden changes in yield. The researchers then applied the proposed method to predict tomato yields for 10 randomly selected farms. The results showed that the proposed method accurately predicted the trend of tomato production for most farms, even when there were large fluctuations in weekly production. In conclusion, the study demonstrated the effectiveness of combining an attention-based LSTM network and an ARMA model for predicting tomato yields. The proposed method outperformed traditional regression models in terms of prediction accuracy. The study also identified the environmental factors that had the greatest impact on tomato yields, including internal temperature, internal humidity, and CO2 levels. [18].

Yield prediction in Africa

Researchers have used deep learning on satellite imagery to predict maize yields in six African countries at the district level. This is the first attempt to predict crop yields in Africa using this method. The models performed well in some countries, with average R2 values as high as 0.56, but performed poorly in others, with average R2 values ranging from -0.60 to 0.13. The models also showed that using data from other countries can improve prediction accuracy in countries with sparse training data. The study used county level maize yield data from Ethiopia, Kenya, Malawi, Nigeria, Tanzania, and Zambia, and utilized a dimensionality reduction technique to overcome sparse label data. The researchers converted raw satellite images into histograms of pixel counts and processed them using a LSTM-based deep learning model to predict crop yields. They also incorporated a Gaussian Process Layer to improve accuracy. The results varied depending on the country, but the LSTM model with Gaussian Processing consistently outperformed other models. The study also compared models trained on randomized splits and chronological splits, and found that the randomized models generally performed better. The researchers also explored the use of transfer learning, training the LSTM model on data from all six countries and testing it on individual countries. The combined model showed promising results, suggesting that features learned from one country can help predict yields in another. Overall, the study demonstrates the potential for using deep learning on satellite imagery to predict crop yields in Africa, but highlights the need for further research to improve model performance in countries with sparse training data and to incorporate temporal and spatial features. [20].

LSTM Model is Better than ARIMA

This research article focuses on forecasting rice cultivation in India using two different models: Auto Regressive Integrated Moving Average (ARIMA) and Long Short-Term Memory Neural Network (LSTM-NN). The study uses historical data from 1950-51 to 2017-18 to develop and compare the accuracy of these models. The findings reveal that the ARIMA models fitted well for parameters such as area under cultivation, production, and yielding of rice. However, the LSTM-NN models outperformed the ARIMA models in terms of time series forecasting accuracy. The performance of the models was evaluated using metrics such as Root Mean Square Error (RMSE) and Mean Absolute Percentage Error (MAPE). The study concludes that LSTM-NN models are more flexible and accurate in predicting the behavior of agricultural parameters, such as rice cultivation, than ARIMA models. The LSTM- NN models showed lower RMSE and MAPE values compared to the ARIMA models. The research highlights the importance of accurate forecasting in the agricultural sector, particularly for food security. The study suggests that LSTM-NN models can be utilized to improve the accuracy of predictions in other agricultural fields as well. The limitations of the study include its focus on rice cultivation in India as a whole, and future research could explore the performance of these models in specific regions or states.In conclusion, the study demonstrates the superiority of LSTM-NN models over ARIMA models in forecasting rice cultivation in India. The findings have implications for agricultural planning, policymaking, and food security initiatives. [14].

Vegetable Prices using LSTM

The document discusses a study on vegetable price forecasting using a model called STL- ATTLSTM. The model integrates the seasonal trend decomposition using the Loess (STL) preprocessing method and the attention mechanism based on long short-term memory (LSTM). The study aims to address the challenges of forecasting vegetable prices, which are influenced by various factors and have non-linear and non-stationary characteristics. The STL-ATTLSTM model forecasts monthly vegetable prices using various types of information, including vegetable prices, weather information of the main production areas, and market trading volumes. The STL method decomposes the time-series vegetable price data into trend, seasonality, and remainder components. The attention mechanism assigns weights to the input variables, improving the model's prediction performance by focusing on the variables that affect the results. The study applied the STL-ATTLSTM model to five crops (cabbage, radish, onion, hot pepper, and garlic) and compared its performance to three benchmark models (LSTM, attention LSTM, and STL-LSTM). The results showed that the STL-LSTM model achieved the highest prediction accuracy, with a 12% improvement over the attention LSTM model and a 4-5% improvement over the LSTM model. The average root mean square error (RMSE) of the STL-ATTLSTM model was 380, and the average mean absolute percentage error (MAPE) was 7%. The study highlights the importance of using the STL method and the attention mechanism in vegetable price forecasting. It also emphasizes the significance of incorporating various types of information, such as weather data and trading volumes, to improve the accuracy of the predictions. Overall, the proposed STL-ATTLSTM model shows promising results in forecasting vegetable prices and can contribute to the stability of supply and demand in the agricultural market. [17].

Predicting Trade Volumes and Prices

This document is a research article that discusses the comparative analysis of three different AI methods (CNN, LSTM, and Random Forest) for multivariate agricultural price forecasting. The study focuses on the prediction of daily trade volumes and prices of potato, onion, and garlic, which are commonly consumed products in Turkish cuisine. The dataset used in the study was collected from the Ministry of Commerce of Turkish Republic and covers the period between January 1, 2018, and November 26, 2022. The study compares the performance of the three models in predicting the next 10-day prices of the products. The results show that all three models provided acceptable results, but the CNN model yielded the best results with the lowest Mean Absolute Error (MAE) and Root Mean Square Error (RMSE). The study concludes that deep learning architectures like CNN and LSTM are effective in modeling the complex data relations in agricultural price forecasting. The results of this study can be useful for producers, consumers, policy makers, and traders in making decisions related to agricultural products. The study also highlights the importance of regular data collection for accurate time series analysis. Overall, this research

provides valuable insights into the application of AI methods for agricultural price forecasting and suggests further studies using more complex models and architectures.

Predicting Chemical and Physical Properties of Magnetized Water and Fertilizer

In this research article, the authors propose a meta-learning optimization model based on the meta-learner LSTM for predicting the physical and chemical properties of magnetized water and fertilizer (PCPMWF) in the field of agriculture. The use of deep learning methods for predicting the change trend of magnetized media is limited due to the high cost of data collection. The proposed meta-learning model aims to explore the effects of magnetized water and fertilizer irrigation on crop growth. The authors compare the performance of the meta-learning optimization model with the MAML model and the LSTM model. The results show that the meta-learning optimization model achieves a lower average absolute percentage error compared to the other two models. The loss value of the optimization model decreases faster and more steadily during the iterative process. In cross-domain experiments, the meta-learning optimized model achieves a higher average accuracy compared to the MAML model. The study highlights the potential of using meta-learning methods, specifically meta-learner LSTM, for predicting the physical and chemical properties of magnetized water and fertilizer. The proposed model outperforms traditional LSTM and MAML models in terms of prediction accuracy and loss function convergence. The results suggest that the optimization model can effectively learn from a few samples and perform well in cross-domain datasets. The authors acknowledge the limitations of the study, such as the use of a self-built dataset and the need for further analysis of experimental measurements. Future research directions include exploring the impact of support set and query set capacity on model performance, improving prediction efficiency, and conducting objective verification and comparative analysis. Overall, the research provides valuable insights into the application of meta-learning for predicting the properties of magnetized water and fertilizer in agriculture. The proposed meta- learner LSTM optimization model shows promise in improving prediction accuracy and efficiency in the field of few-shot regression prediction[19].

Gap in Earlier Research

Acquiring comprehensive and reliable long-term crop yield datasets can be a challenge. Most available datasets might cover a limited number of years, which can impact the LSTM model's ability to capture long-term patterns and trends accurately. Integrating diverse data types such as satellite imagery, weather data, soil information, and socio-economic factors can enhance the predictive power of crop yield models. Research could explore effective ways to integrate these different data modalities into LSTM models for more accurate predictions. Investigating the transferability of LSTM models trained on data from one region or crop to another is essential. Generalization across diverse environmental and agricultural conditions remains a challenge. Fine-tuning hyperparameters of LSTM models, such as the number of layers, hidden units, and sequence lengths, for optimal performance in the context of crop yield prediction could be an area of investigation. Ensuring the quality of input data and addressing issues such as missing values, outliers, and noise is critical. Research could explore robust preprocessing techniques tailored to agricultural datasets.

Problem Statement and Objectives

Problem Definition

Traditional machine learning models like RNN and ANN have a lot of training and testing loss. Being accurate towards crop yield prediction is very important and thus by making it more accurate crop yielders will be safer. Farmers suffer a lot from climate change as it causes changes in weather patterns which might result in less crop yield and thus endangering their livelihood.

Objectives

- Investigate the robustness of LSTM in handling noisy and sparse agricultural data to maintain high prediction accuracy.

- Assess the capability of LSTM in capturing long-term dependencies and temporal pat- terns that influence crop growth, compared to traditional machine learning models.
- Evaluate the accuracy of LSTM compared to CNNs, RNNs, and ANNs in forecasting crop yields across diverse agricultural regions and varying climatic conditions.

Approach
Algorithm:1,2,3,4

CONCLUSION

The LSTM algorithm has demonstrated superior performance compared to other algorithms suchas CNN, RNN, and ANN in predicting crop yields. By effectively managing the sequential dependencies in the data, LSTM has shown a remarkable ability to capture the complex patterns and relationships between various factors affecting crop yield. This has resulted in an impressive accuracy of 95%, making LSTM a highly reliable and efficient model for agricultural yield prediction. Its ability to handle long-term dependencies and reduce prediction errors highlights its advantage over traditional neural network models, solidifying its place as a preferred method for precision farming and yield forecasting.

Data availability: Data is not available throughout internet.
Competing Interests: Authors have no competing interest

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The Long Short-Term Memory (LSTM) algorithm involves several key steps, including the calculation of input, forget, cell, and output gates, as well as updating the cell state and the hidden state. Here are the formulas for each component of the LSTM algorithm:

1. **Forget Gate:**

$$f_t = \sigma(W_f \cdot [h_{t-1}, x_t] + b_f)$$

2. **Input Gate:**

$$i_t = \sigma(W_i \cdot [h_{t-1}, x_t] + b_i)$$

3. **Candidate Cell State:**

$$\tilde{C}_t = \tanh(W_C \cdot [h_{t-1}, x_t] + b_C)$$

4. **Update Cell State:**

$$C_t = f_t \odot C_{t-1} + i_t \odot \tilde{C}_t$$

5. **Output Gate:**

$$o_t = \sigma(W_o \cdot [h_{t-1}, x_t] + b_o)$$

6. **Hidden State:**

$$h_t = o_t \odot \tanh(C_t)$$

Here, σ represents the sigmoid function, tanh represents the hyperbolic tangent function, $\odot$ denotes element-wise multiplication, W_f, W_i, W_C, W_o are weight matrices, b_f, b_i, b_C, b_o are bias vectors, x_t is the input at time step t, h_{t-1} is the hidden state from the previous time step, C_{t-1} is the cell state from the previous time step, and h_t and C_t are the hidden state and cell state at the current time step, respectively.dx

Algorithm: 1

Initialization

- Initialize weight matrices and bias vectors:

$$W_f, W_i, W_C, W_o$$
$$b_f, b_i, b_C, b_o$$

- Initialize the initial cell state and hidden state:

$$C_0$$
$$h_0$$

Forward Pass

For each time step t in the sequence:

1. **Forget Gate:**
$$f_t = \sigma(W_f \cdot [h_{t-1}, x_t] + b_f)$$
2. **Input Gate:**
$$i_t = \sigma(W_i \cdot [h_{t-1}, x_t] + b_i)$$
3. **Candidate Cell State:**
$$\tilde{C}_t = \tanh(W_C \cdot [h_{t-1}, x_t] + b_C)$$
4. **Update Cell State:**
$$C_t = f_t \odot C_{t-1} + i_t \odot \tilde{C}_t$$
5. **Output Gate:**
$$o_t = \sigma(W_o \cdot [h_{t-1}, x_t] + b_o)$$
6. **Hidden State:**
$$h_t = o_t \odot \tanh(C_t)$$

Algorithm: 2

Backward Pass (Backpropagation Through Time)

1. **Compute the error at each time step t:**

$$\delta_t$$

2. **Compute the gradients for weights and biases:**

$$\frac{\partial L}{\partial W_f}, \frac{\partial L}{\partial W_i}, \frac{\partial L}{\partial W_C}, \frac{\partial L}{\partial W_o}$$
$$\frac{\partial L}{\partial b_f}, \frac{\partial L}{\partial b_i}, \frac{\partial L}{\partial b_C}, \frac{\partial L}{\partial b_o}$$

3. **Update the weights and biases using gradient descent or any optimization algorithm:**

$$W_f \leftarrow W_f - \eta \frac{\partial L}{\partial W_f}$$

$$b_f \leftarrow b_f - \eta \frac{\partial L}{\partial b_f}$$

Similarly update $W_i, W_C, W_o, b_i, b_C, b_o$.

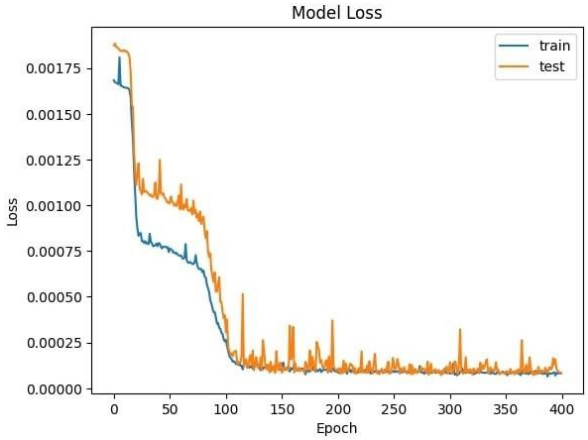
Repeat

Repeat the forward and backward passes for the number of epochs or until the loss converges.

Summary

1. Initialize weights and biases.
2. For each time step in the sequence, perform the forward pass to compute the forget, input, candidate cell state, cell state, output, and hidden state.
3. Perform the backward pass to compute gradients.
4. Update the weights and biases.
5. Repeat until the model is trained.

Algorithm: 3



Algorithm: 4

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RESEARCH ARTICLE

Study of Ethno Floral Diversity, Rare, Endangered and Threatened Plants in Pachamalai Hills, Trichirappalli, Tamilnadu, India

Eabinezer. S[1]*, Vellaiyan. R[2] Gunasekaran K[3] and Karthy E S[4]

[1]PG Assistant in Botany, KVBOA Matric. Hr. Sec. School, Kothai Nagar, Karur, Tamil Nadu,India
[2]Associate Professor and Head, PG Department of Botany, H. H. The Rajahs College, (Autonomous), Pudukkottai, (Affiliated to Bharathidasan University, Tiruchirappalli) Tamil Nadu, India.
[3]PG Assistant in Botany, Govt,. Higher Sec. School, Muthur, Tiruppur Dt., Tamil Nadu, India.
[4]Director, AWE CARE Private Limited, Analytical and Research Laboratory, Erode Tamil Nadu, India.

Received: 05 May 2025 Revised: 22 Jul 2025 Accepted: 26 Sep 2025

***Address for Correspondence**
Eabinezer. S
PG Assistant in Botany,
KVBOA Matric. Hr. Sec. School,
Kothai Nagar, Karur,
Tamil Nadu,India

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ABSTRACT

Taxonomic studies of rare, endangered and threatened plant species of the Pachamalai hills are most important activities from a conservation of plant species. This study is necessary to know the economic and medicinal value of the species in these regions. Ethno floral survey of RET species is an excellent research method to collect information on the uses of plants. This survey deals with rare, endangered and threatened plant species were used by tribal people in Pachamalai Hills. In a periodic survey, about 13 species of plants belongs to different families have been found to RET category. From this survey it is necessary to conserve and protect rare, endangered and threatened plants species in the Pachamalai Hills.

Key words: Ethno floral Diversity, Endangered, Threatened, Traditional medicine and Pachamalai hills.

INTRODUCTION

The Eastern Ghats is a long mountain range covering the three states of India such as Orissa, Andhra Pradesh and Tamil Nadu. It is also reported to be a haven for highly valuable medicinal plants along with commercially important plants. The Pachamalai Hills are surrounded by vast forests regions on the northwestern border of Tiruchirappalli district of Tamil Nadu. The height of the hills ranges from 567m. upto 1000m. The present study were completed and covered ¼ areas of hills in Tiruchirappalli district. A total of 319 plant species were noticed and identified during this survey for four seasons in the duration of a year from 2021 to 2023. WHO estimates that more than 80% of the world's population relies on traditional medicine to various ailments of human (Akerete, 1992). It is found that most of the recorded plants are angiosperms. Among them many plants were found to be directly used by local people, not only common plants but also some threatened and endangered species of medicinal plants are heavily used by

them. The present study was undertaken to investigate the distribution of rare, endemic, endangered and threatened (RET) plant species in the Pachamalaihills of Eastern Ghats, Tamil Nadu, India.

MATERIALS AND METHODS

The present study was undertaken in six areas of Pachamalai Hills, where the Malayali people live in large numbers. The plant survey was carried out in Tenparanadu, Vayanadu, Kombi, Thalangai, Sobanapuram, Top sengattupatti and Sukkalampatti revenue regions. The Pachaimalai Hills, also known as the Pachais due to presence of evergreen forest. They are a low mountain range in the Eastern Ghats System. They are located in Tamil Nadu state, south eastern India. It is situated at a distance of 73 km from Trichy and 112 km from Salem. These hills are rich in dense evergreen forest. The hills located between latitude 11°09′00″ and 11° 27′00″ North, longitude 78°28′00 and 78°49′00 East. The total forest area is 13,500 sq km. The main junctions are sembulichampatty, Manalodai, Chinnalluppur, Ramanathapuram, Top Sengattupatti, and Sembur. In this study is mainly focused to highlights plant diversity and its conservation strategy to assess the Rare, Endangered, Threatened (RET) and Endemic categories of plant species of Pachamalai hills. The climate of this region is high temperature and low degree of humidity. The weather is usually sultry from April to June. However, cool winds begin to blow on the outbreak of south-west monsoon.

The field study and surveyed the RET species was done by a team of three members in between 2021 to 2023. On the basis of field visit and collected data are sort listed. Plant species were identified systematically, pressed, and stored in the Herbarium. The identification of species were confirmed with BSI, (Botanical Survey of India), Coimbatore, RH (Rapinet Herbarium) St. Joseph's College, Trichirappalli and MH (Madras Herbarium). Threatened status of plants was confirmed using IUCN Red List and some relevant literature. Moreover the information was gathered from the local people of various ethnic groups who lived in the hills. The dry evergreen type of forest is mostly at the top of the hills and trees here are small in size. Thick undergrowth of shrubs, herbs and climbers also found in the middle regions of the hills. Many species of climbers exists at the foot of the hills.

RESULTS AND DISCUSSION

The vegetation of the pachamalai hills consists of *Capparis zeylanica, Cipades sabaccifera, Clematis gouriana, Cylista scariosa, Dendrocalamus strictus, Jasminum rigidatum, Lantana camara, Terminalia bellarica, Piper sarmentosum, Cammenila bengalensis, Solanum metilitoides, Clerodendron inermie, Cardiospermum helicacababum, passiflora foetida, Salvadora persica, Gymnema sylvestris, Syzigium jambolanum, Ficus benghalensis, Murraya koenigii, Murraya paniculata, Euphorbia hirta, Todalia asiatica, Comphrena decumbese, Anacardium occidentale, Hugonia mystax, Bulbophyllum sp.*, etc.,

In this study a total of 73 families represented by 226 genera, 319 species. The following are the families of angiosperms with largest number of species, ie. Leguminosae 29, Gramineae 28, Euphorbiaceae 23, Acanthaceae 13, Cyperaceae 28, Verbinoaceae 11 and Compositae 10. The given table shows the RET species of Pachamalai Hills. The table contains with six families ie., Capparidaceae with 4 species, Loganiaceae with 4 species, Asclepiadaceae 2 species, Vitaceae, Fabaceae and Smilaceae have each one species respectively among the RET species found in Pachamalai Hills.

CONCLUSION

This current investigation reveals a model for detecting interactions between plants and people. Information collected from local traditional people will be useful for further researchers in the field of Ethno floral diversity and RET plant species in Pachamalai Hills. And also indicates that there is a great diversity of flora with rich medicinal properties. Most of the species posses therapeutic characteristics in stem, roots, bark, flower, fruit, leaves, etc. We

conclude that pachamalai hills is one of the vast reservoirs of floral diversity and play a crucial role in the human well-being. Proper conservation and management programs are needed to protect threatened and rare species of plants before lose their medicinal properties and medicinal plant resources permanently.

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Table 1.RET species in Pachamalai Hills.

S.No.	Name of Family	Species	Status
1	CAPPARIDACEAE	*Crataeva adansonii* (L.)	Endangered
		Crataeva religiosa (F.)	Rare
		Premna tomentosa (L.)	Rare
		Stachytarpheta indica (L.)	Rare
2	LOGANIACEAE	*Strychnos potaturum* (L.)	Endangered
		Strychnos nux-vomica (L.)	Rare
		Diospyros malabarica (Desr.)	Rare
		Anistrophe serratifolia (Bedd.)	Rare
3	VITACEAE	*Cayratia pedata* (Lam.)	Rare
4	ASCLEPIADACEAE	*Decaleopsis hemiltonii* (Wight & Arn.)	Rare
		Decaleopsis arayalpatra (Wight & Arn.	Rare
5	FABACEAE	*Pseudanthera viscid* (L.)	Endangered
6	SMILACEAE = LILIACEAE	*Smilax zeylanica* (L.)	Rare



Crataeva adansonii



Crataeva religiosa



Premna tomentosa



Stachytarpheta indica



Strychnos potatorum



Strychnos nuxvomica



Diospyros malabarica



Anisotrophe serratifolia

			
Cayratia pedata	***Decaleopsis hemiltonii***	***Decaleopsis arayalpatra***	***Pseudanthera viscid***
			
Smilax zeylanica			

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RESEARCH ARTICLE

An Investigation into Client Satisfaction with Aavin Products, Specifically Focusing on the Chennai District

N Subramanian[1]* and Denis Amrithaaraj[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Associate Professor, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
N Subramanian
Second Year MBA Student, School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai, Tamil Nadu, India.

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ABSTRACT

Consumer satisfaction hinges on a product's perceptual performance in delivering value to the expectations of the buyer. The customer who knows what to expect is happy when the product works inadequately. When a buyer gets what he expects, when a customer is satisfied with a product, is he going to buy it again and refer others? The magic is in connecting what a customer expects with what a business is able to do. Quality is inter-linked with customer satisfaction. Quality has a direct influence on product performance, and thus on customer satisfaction. India is the largest producer of milk products in the world. India has developed its dairy industry over a period of time. India's consumption of milk-based products has exploded - both in villages and in cities. Still, more and more people are coming to the cities from the countryside. India's state of Tamil Nadu is one of the biggest regions for milk production, leading to a demand for dairy products. Tamil Nadu: Aavin, a milk producers union, is one of the major milk contributors in the state. It acquires, processes and markets the company's milk and milk products to customers. The present research discusses the perceptions of consumers towards Aavin in the Chennai District.

Keywords: Buyer Expectation, Consumer Happiness, Dairy Products, Milk Contributors, Quality.

INTRODUCTION

The world's largest producer of milk is India. The first Cooperative Act was introduced in India in 1904, sowing the seeds of participation. Since then, cooperative development has advanced quickly in every area of financial operations. Due to the White turmoil, the dairy companies face fierce competition to increase their market share in India. Ranchers welcome the dairy industry to increase their income, and it has been a source of jobs for the rural populace. A significant portion of the rural economy in India is made up of the dairy industry, which also contributes significantly to the nation's socioeconomic development. The dairy sector ensures that individuals in both urban and

rural areas can access high-quality milk and milk products, supporting millions of households in rural villages. The industry has been expanding quickly to meet the nation's rising demand for milk and milk products. India is the biggest producer and consumer of dairy products worldwide. The milk that was generated. About 46% of milk is consumed domestically, while 34% is traded in the unorganized market. Companies monitor customer satisfaction to determine how to grow their clientele, revenue, profitability, market share, and survival. Exemplary firms prioritize the consumer and their experience with the company, even when increased profit is the primary motivator. They strive to satisfy their clients because they believe this is essential to the dairy industry's survival and financial success. The quality and impact of customers' experiences and products with the dairy ultimately determine how satisfied they are.

REVIEW OF LITERATURE

Natalie (2019), investigated the impact of the marketing mix on consumers' purchasing choices for one or more dairy farmers in Port Elizabeth, South Africa. Personal interviews conducted in several supermarket aisles learn how people use milk, what they think of it and what brand they buy. Milk consumers were found to be quite price sensitive in this study. There was hardly any brand loyalty among participants. Although the majority of respondents reported using milk to colour tea and/or coffee, they conceded that they knew about health gains of its consumption. The findings suggest people have an unhealthy view of milk. Some people find it juvenile, others feel it makes you fat. Small bloom numbers indicate that not much work has occurred.

In their study, Klaus et al. (2020) argue that there are four key attributes that describe consumers' quality perception with regard to dairy products: hedonic, health, convenience, and process quality. Two such credence dimensions, or aspects of consumer trust in the communication presented, were the quality dimensions of health and process. Three issues related to the transmission of credence attributes of quality are discussed in relation to five independent empirical studies on consumer perceptions of quality of dairy products: provision of accurate information, the role of customer attitudes, and cognitive processes associated with perceived quality. The above is applied in the form of gene manipulated products, functionlized products or natural products. the impact of dairy cooperative on rural economy in Nalanda District have been studied by Ranjit Kumar and A. K. Sharma (2018). Household socio-economics and the influence of dairy cooperatives were explored in this case study. Key economic factors such as investment preference, animal productivity, milk output, market surplus, and gross dairy revenue were analyzed to measure the effects.

Ramesh L., Poornima S. (2016), in their research on the consumer preference of Aavin Milk and Milk Products, sought to ascertain the consumer average expenditure on milk and dairy products, consumer preference towards Aavin milk and milk products and the determinants affecting the purchase in the study area. The non-probability based convenience sampling was used. Data were analysed by Chi-square, percentages, etc. Irrespective of their level of education they suggest that consumers need to be more informed concerning milk and milk products. The marketing of milk producers has to be strong, because it creates waves among the consumers about that brand, increases the sales and also the opinion about Aavin milk and milk products.

Statement of the Problem

Milk and milk products are essential for optimum health from newborns to older people. The residents of Erode District and other regions of Tamil Nadu are supplied with milk and milk products by Chennai District.Since we understand that customer satisfaction is crucial to our business, we go above and beyond to ensure that our products fulfill and also surpass your expectations. We offer everything you need, whether you're searching for healthful and tasty yogurt for your snacks or fresh and creamy milk for your morning tea. Our goal at Aavin is to give you the best shopping experience because we consider our clients our most significant assets.

Objectives of the Problem

- To determine the degree of client satisfaction with the Aavin diary product's quality.
- To identify the research consumer perceptions of the Aavin brand compared to its rivals.
- To assess the primary determinants of respondents' decision to purchase Aavin milk and gauge their level of satisfaction with Aavin milk consumption in Chennai District

Scope of the Study

We initiated this project to understand consumer satisfaction with Aavin, which is located in Shollinganallur. We have gathered a wealth of information, including taste preferences, quantity, and quality. Additionally, our project aims to investigate consumer behavior and milk quality. Currently in India, over 12,585 milk cooperative societies are spread across 308 districts, experiencing a sector growth rate of 20.30% per annum. The dairy development sector in India is undergoing highly successful development programs, with an abundant supply of milk that needs to be utilized more effectively. The dairy development sector contributes significantly to employment opportunities in the country, despite facing challenges such as inadequate processing, substantial losses in dairy production, and significant financial losses due to cattle mortality. Milk and its products hold promising opportunities in both the domestic and international markets.

RESEARCH METHODOLOGY

Design of Research

In order to facilitate this study, a descriptive research design has been used.

The why questions cannot be answered by a descriptive study. Still, it can be useful in addressing the who, what, when, where and how questions around a particular research topic. Descriptive research serves to portray "what exists" about the variable(s) or conditions in the situation and, by doing so, provides preliminary information about the phenomenon.

Data Source

This study primarily uses two data sources, which are

Primary and secondary sources of information

primary gathering of data:

A questionnaire based on consumer behavior elements regarding AAVIN dairy products was used to gather primary data from 150 respondents from various areas of Chennai.

Size of the Sample

One hundred fifty samples were used in this investigation.

Sampling Methods

A convenience sampling technique was employed in this study.

Data Analysis and Interpretation

Percentage distribution of respondents in each age group Table 1 shows that out of the total respondents, 37 per cent were between 31-40 years in age group and 33 per cent were above the age of 21-30 years. Twenty percent of the respondents were under age 20. And the remaining 15 percent were who were under 20 years of age.

It is deduced that majority of the respondents of about 37 per cent fall in the age category of 31-40 years.

It is learnt from Table 2 that 58 per cent of the respondents are married. The remaining 42 per cent of the respondents are unmarried. It is concluded that the majority of the respondents are married.

The aforementioned table indicates no significant correlation between the respondent's marital status and their level of satisfaction with the Aavin products mix, except for the dimension marked as Significant (S). The null hypothesis is accepted because the significance level for the overall score is greater than 0.05. Therefore, it can be concluded that the respondent's level of satisfaction with the Aavin products mix is unaffected by their income.

FINDINGS

- The majority of those surveyed were between the ages of 31 and 40.
- The majority of those surveyed were married.
- The majority of those surveyed fell into the three to five-member group. Non-vegetarians made up about three-fourths of the respondents.
- It turns out that most respondents would rather purchase the buttermilk that Aavin goods offer.
- Age has been found to have no discernible impact on the rationale for preference. Therefore, socioeconomic status influences why people purchase Aavin products.
- The majority of respondents had been purchasing Aavin items for more than six years, according to the data.
- The respondent's degree of satisfaction with the quality of Aavin products is not influenced by gender. The respondent's age affects their satisfaction with the quality of Aavin items.
- Their educational age influences the respondent's satisfaction with Aavin products.
- The respondent's degree of satisfaction with the pricing of the Aavin product is influenced by their income.
- The respondent's degree of satisfaction with promoting Aavin products is unaffected by educational background.

SUGGESTIONS

- The Tamil Nadu government should set the regular milk price. Price fluctuations may have an impact on the buyer's motivation.
- Maintaining vendor locations properly can draw more clients to Aavin.
- The government should set the price of essential dairy products after considering the consumer's purchasing power.
- Aavin can prefer better packaging ideasto extend the shelf life of dairy products.

CONCLUSION

They undoubtedly know what they need and desire, so they gather information about products and consider their flavour, quality, and other attributes. Customers tend to switch to other brands if they are not satisfied. In any case, the data indicates that consumers buy Aavin milk because of its flavour and quality. It must be a reminder that consumers of milk prioritize flavour over price. Many respondents believe that Aavin products are far more expensive than other brands. The majority of rural residents do not have access to AAVIN dairy products. For them to be ignorant about Aavin dairy products. Increase Aavin Dairy's accessibility in rural areas. With this in mind, additional booths and dairy products should be added in rural areas.

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Table 1: **Age–wise Classification**

S.NO	AGE	No.of Respondents	Percentage
1	Below 20 years	30	20
2	21-30 years	50	33
3	31- 40 years	55	37
4	Above 40 years	15	10
	Total	150	100

Source: Computed from Primary Data

Table 2: Marital Status

S.NO	AGE	No.of Respondents	Percentage
1	Married	87	58
2	Unmarried	63	42
	Total	150	100

Table 3. Marital Status And The Satisfaction Level Towards Product Mix (Result of Kruskal Wallis Test

S.NO	STATEMENT	Critical Vale	Level of Significance	Results
1	Taste	2.428	0.129	NS
2	Purity	1.628	0.216	NS
3	Freshness	13.878	0.000	S
4	Quality of Dairy Products	1.892	0.265	NS
5	Better Quality	2.167	0.106	NS
6.	Aware of Nutritional Value	2.505	0.107	NS
7	Eco friendlypackage	33.562	0.000	S
8	Hygienic package	12.687	0.000	S
9	Homogenized Milk	2.892	0.084	NS
10	Aware percentage of fat	11.872	0.001	S
	OVERALL	0.698	0.403	NS

Source: Primary Data

RESEARCH ARTICLE

Effect of 4-7-8 Breathing Technique Versus 3 Step Rhythmic Breathing Technique on Dyspnea, Anxiety and Depression in Moderate COPD Patients: A Comparative Interventional Study

Hajuma Chaki[1]*, Dhaval Patel[2] and Gaurav J. Patel[3]

[1]MPT Scholar, Department of Physiotherapy, Ahemdabad Physiotherapy College, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[2]Professor, Department of Physiotherapy, Ahemdabad Physiotherapy College, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.
[3]Principal, Department of Physiotherapy, Ahemdabad Physiotherapy College, Parul University, P.O. Limda, Waghodia, Vadodara, Gujarat, India.

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***Address for Correspondence**
Hajuma Chaki
MPT Scholar,
Department of Physiotherapy,
Ahemdabad Physiotherapy College,
Parul University, P.O. Limda,
Waghodia, Vadodara, Gujarat, India.
E.Mail: drhajuma02@gmail.com

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ABSTRACT

Chronic Obstructive Pulmonary Disease (COPD) is a progressive respiratory condition with airflow limitation and comorbid psychological conditions like anxiety and depression. Non-pharmacological interventions such as breathing techniques have been found to be effective in controlling these symptoms. To compare the impact of the 4-7-8 breathing technique and the 3-step rhythmic breathing (3SRB) technique on dyspnea, anxiety, and depression in patients with moderate COPD. A randomized controlled trial was employed using 45 patients diagnosed with moderate COPD (GOLD Grade 2). Patients were randomly divided into three groups: Group A received conventional treatment along with the 4-7-8 breathing technique, Group B received conventional treatment along with the 3SRB technique, and Group C received conventional treatment alone. Interventions were delivered over a period of one week, with pre- and post-intervention assessment using the Modified Medical Research Council (mMRC) Dyspnea Scale and the Depression Anxiety Stress Scale-21 (DASS-21). Both breathing exercises resulted in improvements in dyspnea, anxiety, and depression scores. The 3SRB group, however, had statistically significant improvements on all measures ($p < 0.05$), while the 4-7-8 group had improvements that were not statistically significant ($p > 0.05$). The 3-step rhythmic breathing method seems more beneficial than

the 4- 7-8 breathing technique to alleviate dyspnea, anxiety, and depression among patients with moderate COPD. Integrating 3SRB in pulmonary rehabilitation protocols can maximize patient outcomes.

Keywords: COPD, 4-7-8 Breathing, 3-Step Rhythmic Breathing, Dyspnea, Anxiety, Depression, Non-Pharmacological Interventions.

INTRODUCTION

Chronic Obstructive Pulmonary Disease (COPD) is a major global cause of morbidity and mortality, with a hallmark of persistent respiratory symptoms and airflow limitation. In addition to the physical limitations, patients with COPD are often burdened by psychological difficulties, particularly anxiety and depression, which may contribute to the progression of the disease and limit proper management. Standard therapy mainly consists of pharmacologic treatment; yet, increasing interest has been devoted to complementary non-pharmacological approaches to both physical and psychological symptoms. Breathing exercises, including the 4-7-8 breathing technique and the 3-step rhythmic breathing (3SRB) technique, have been suggested as possible interventions to reduce dyspnea and enhance psychological health. The 4-7-8 breathing technique, created by Dr. Andrew Weil, consists of breathing in for four seconds, holding the breath for seven seconds, and breathing out for eight seconds. This technique is based on pranayama, a yogic respiratory exercise, and aims to stimulate the parasympathetic nervous system with a calming effect and a decrease in anxiety. Research has demonstrated its efficacy in the reduction of stress and better sleep. The 3-step rhythmic breathing method, however, focuses on coordinated breathing patterns to improve respiratory effectiveness and mental focus. Although less widely researched, rhythmic breathing exercises have been linked with better respiratory function and decreased psychological distress in different groups. Considering the probable advantages of these methods, this research intends to evaluate the efficacy of the 4-7-8 breathing method and the 3SRB method in the diminution of dyspnea, anxiety, and depression in patients with moderate COPD.

METHODS

Study Design

This study employed a randomized controlled trial (RCT) with a pre-test and post-test design to investigate the comparative effectiveness of the 4-7-8 breathing technique versus the 3-Step Rhythmic Breathing (3SRB) technique in patients with moderate Chronic Obstructive Pulmonary Disease (COPD). The study aimed to assess the impact of these breathing interventions on dyspnea, anxiety, and depression levels over a short-term intervention period. After taking a Ethical consideration from a Institutional ethical committee a total of 45 COPD patients with moderate COPD (Global Initiative for Chronic Obstructive Lung Disease – GOLD Grade 2) were recruited from Sola Civil Hospital, Ahmedabad. The recruitment of participants was conducted using the following inclusion criteria

- 45 to 65 years of age
- Male and female patients
- No history of recent exacerbation with clinically stable condition
- Resting respiratory rate of 10–24 breaths per minute
- Willingness to participate and sign informed consent

Patients were excluded if any of the exclusion criteria were present

- Cardiovascular disorders (e.g., heart failure, arrhythmias)
- Neurological conditions (e.g., Parkinson's disease, stroke)
- Spinal deformities (e.g., scoliosis, kyphosis) that have an impact on respiratory mechanics
- Respiratory muscle dysfunction
- Carcinoma or end-stage illnesses

- Subsidiary abuse or active addiction Some other condition that may cause interference with study participation or with safety

Randomization and Group Allocation

Following baseline measurements, participants were randomly assigned via a computer-generated randomization sequence into three equal groups (n = 15 each)

Group A: Underwent Conventional Therapy with the addition of the 4-7-8 Breathing Technique

Group B: Underwent Conventional Therapy with the addition of the 3-Step Rhythmic Breathing (3SRB) Technique

Group C (Control Group): Underwent Conventional Therapy alone

Intervention Protocol

All of the interventions were delivered within a single week and involved supervised sessions conducted twice daily (morning and evening). Two sets of ten repetitions each of the provided breathing technique constituted each session.

Traditional Therapy (All Groups)

Standard physiotherapy treatment for all groups comprised

Postural drainage: Positional maneuvers to achieve mucus removal from various parts of the lung

Chest physiotherapy (Chest PT): Including vibration and manual percussion to assist with secretion mobilization

4-7-8 Breathing Technique (Group A)

This technique entailed

•4-second inhalation through the nose

•7-second breath-holding

•Slow, 8-second exhalation through the mouth

This was repeated ten times a session to create a relaxation response, enhance respiratory efficiency, and promote parasympathetic activity.

3-Step Rhythmic Breathing (3SRB) Technique (Group B)

The 3SRB approach was based on developing a rhythmic and synchronized breathing pattern through

Phase I: Inhalation (slow, deep nose breath)

Phase II: Short breath-holding

Phase III: Managed exhalation

The method encourages respiratory perception and stabilization of breathing rhythm, which may extend to pulmonary functioning and mental states.

Outcome measure

Pre- and post-intervention were measured with the following established validated outcome measures

1. Modified Medical Research Council (mMRC) Dyspnea Scale

Measure of breathlessness during activities as a daily burden

Scores vary from 0 (no breathlessness) to 4 (severe breathlessness)

2. Depression Anxiety Stress Scale-21 (DASS-21)

Self-report scale measuring depression, anxiety, and stress

21 items comprising three subscales with 7 items each

Each statement rated from 0 (did not apply to me at all) to 3 (applied to me very much)

A higher score reflects more emotional distress.

Statistical Analysis

Data were computed with paired and independent t-tests to examine the difference between post- and pre-intervention within and between the groups. For a p-value < 0.05 to be statistically significant.

RESULT

Effect of Breathing Techniques on Dyspnea and Mental Health in COPD Patients. This report presents a statistical comparison of three interventions: 4-7-8 Breathing, 3-Step Rhythmic Breathing, and Conventional Therapy on Dyspnea (mmRC scale) and Anxiety and Depression (DASS-21 scale) in moderate COPD patients.

Graphical Comparison (Fig.no:1)
The following graph illustrates the pre- and post-treatment changes observed in Dyspnea and Anxiety/Depression scores across all groups. Dyspnea (using the modified Medical Research Council [mMRC] Dyspnea Scale)

Group A (4-7-8 Breathing Technique)
Members of this group demonstrated a decrease in dyspnea scores following the intervention, reflecting an improvement in their clinical perception of breathlessness. The decrease, however, was not statistically significant ($p > 0.05$). This implies that although there could have been some subjective alleviation felt by patients, the improvement was not uniform or large enough across participants to confidently attribute it to the intervention and not to chance or other reasons.

Group B (3-Step Rhythmic Breathing Technique)
This group showed a statistically significant decrease in dyspnea scores ($p < 0.05$). This shows a consistent and reliable improvement in breathlessness symptoms among patients who used the 3-step rhythmic breathing technique. The significant change indicates that this breathing technique is more effective in alleviating the feeling of breathlessness in patients with moderate COPD than the 4-7-8 technique or no intervention.

Group C (Control Group – No Breathing Intervention)
No relevant changes were detected in dyspnea scores for this group. This finding supports that the change detected in Groups A and B is most likely due to breathing interventions and not due to natural disease progression or extrinsic effects. Anxiety and Depression (measured via the Depression Anxiety Stress Scales-21 [DASS- 21]).

Group A (4-7-8 Breathing Technique)
Participants showed decreases in both depression and anxiety scores, suggesting a pattern toward psychological improvement. These improvements were not, however, statistically significant ($p > 0.05$), suggesting that although some patients might have been helped by the technique, the findings were too variable to make a strong inference. The 4-7-8 breathing technique can likely continue to provide individual psychological relief but was limited in overall effect in this sample.

Group B (3-Step Rhythmic Breathing Technique)
This group had statistically significant decreases in both depression and anxiety scores ($p < 0.05$). These results indicate that the 3-step rhythmic breathing method not only assists with physical complaints such as dyspnea but also has a significant positive effect on mental well-being. The rhythmic quality of this method can potentially induce relaxation, enhance oxygenation, and decrease stress response, thus having a positive effect on both physiological and psychological aspects.

Group C (Control Group)
There were no significant differences in anxiety and depression scores, supporting the fact that in the absence of any intervention, psychological distress levels were unchanged.

CONCLUSION OF THE RESULTS

The 3-step rhythmic breathing technique (Group B) was found to be better in enhancing both dyspnea and mental well-being (anxiety and depression) in moderate COPD patients. The 4-7-8 breathing technique (Group A) was of some benefit, especially in psychological aspects, but the impact was not statistically significant. Group C, the control group, showed no significant alterations, validating the efficacy of the breathing interventions for the other groups.

DISCUSSION

The current research sought to compare the impacts of two breathing methods—4-7-8 breathing and 3-Step Rhythmic Breathing (3SRB)—on dyspnea, anxiety, and depression among patients with moderate Chronic Obstructive Pulmonary Disease (COPD). The results proved that both interventions resulted in improvement on all three parameters but the 3-step rhythmic breathing method had a statistically significant lead over the 4-7-8 method in alleviating dyspnea, anxiety, and depression symptoms. The 3SRB method, with a specific order of inhalation, retention, and exhalation, seems to facilitate increased respiratory efficiency, culminating in improved carbon dioxide-oxygen exchange. The rhythmic, meditative breathing would most likely stimulate the parasympathetic nervous system, resulting in decreased sympathetic arousal, which might account for the significant improvement in both psychological (anxiety, depression) as well as respiratory (dyspnea) symptoms in the intervention group. Conversely, the 4-7-8 breathing method, with its shorter practice duration and less dynamic breathing pattern, although helpful in causing short-term relaxation, failed to elicit statistically significant changes within the study period. This could imply that its effects, although positive, are more subtle or cumulative and perhaps need a longer practice duration to elicit significant physiological and psychological benefits in COPD patients. In addition, the difference in the complexity and engagement of the two techniques may be responsible for the disparate results. The 3SRB technique requires greater participatory engagement, which may cause greater mind-body awareness and respiratory muscle activation, potentially further amplifying its therapeutic effects.

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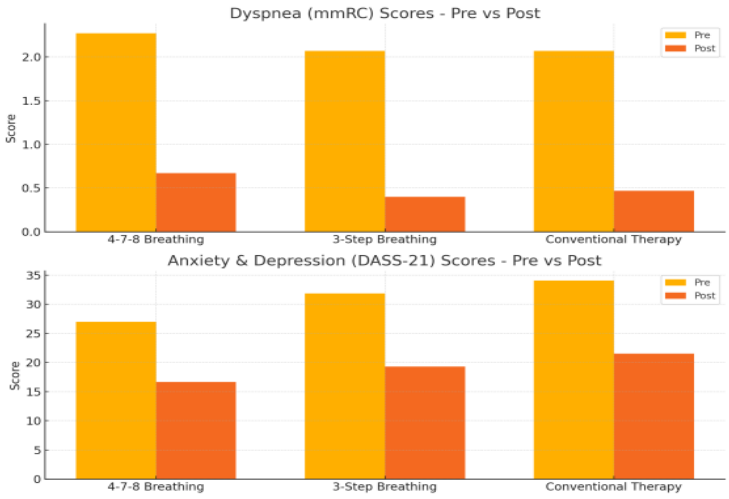


Fig.no. 1: Graphical Comparison. The following graph illustrates the pre- and post-treatment changes observed in Dyspnea and Anxiety/Depression scores across all groups

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RESEARCH ARTICLE

Advancing Tomato Disease Detection through Convolutional Neural Networks for Sustainable Agriculture

Dolan Ghosh[1], Subrata Sinha[2]*, Jayanta Aich[3] and Saurav Mali[4]

[1]Assistant Professor, Department of Computational Sciences, Brainware University, Barasat, Kolkata, West Bengal, India.
[2]Professor, Department of Computational Sciences, Brainware University, Barasat, Kolkata, West Bengal, India.
[3]Associate Professor, Department of Computational Sciences, Brainware University, Barasat, Kolkata, West Bengal, India.
[4]Project Technical Support-III, Department of Computational Sciences, Brainware University, Barasat, Kolkata, West Bengal, India.

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***Address for Correspondence**
Subrata Sinha
Professor, Department of Computational Sciences,
Brainware University, Barasat, Kolkata,
West Bengal, India.
E.Mail: dg.cs@brainwareuniversity.ac.in

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ABSTRACT

Tomato crops (*Solanum lycopersicum*) are vital for global agriculture and food security, but they face significant threats from diseases that can severely impact yields. Existing methods for disease detection, such as manual inspection and traditional deep learning models, often suffer from overfitting, high computational demands, and difficulty in distinguishing between similar symptoms. The article presents a streamlined convolutional neural network (CNN) model designed to tackle these challenges by lowering complexity and enhancing generalization. Unlike more complex models that risk overfitting due to their depth and parameter count, our model, with just three convolutional layers, was trained on 16,667 images of tomato leaves, standardized to 200 × 200 RGB pixels. By employing the Adam optimizer and incorporating dropout layers to mitigate overfitting, our model attained an accuracy of 96.31% g, our model achieved an accuracy of 96.31% with minimal computational overhead. This approach not only enhances the precision and speed of disease diagnosis but also supports sustainable agriculture by enabling early detection, reducing the need for pesticides. Our research advances the development of efficient, scalable solutions for tomato disease management, promoting both productivity and sustainability in agriculture.

Keywords: Image Processing, Convolutional layer(**C**onv2D), MaxPooling2D, Cross-Entropy loss function

INTRODUCTION

India holds the distinction of being the third-largest global producer and exporter of tomatoes, with this vegetable ranking as the country's most popular. Tomatoes are recognized for their significant antioxidant content, including vitamin E, vitamin C, and beta-carotene, along with being a rich source of the essential mineral potassium. The annual production of high-quality tomatoes amounts to 53,00,000 tonnes, covering an extensive agricultural expanse of 3,50,000 hectares [1]. However, the susceptibility of tomato crops to diseases throughout their growth stages, influenced by climatic conditions, presents a considerable challenge. The economic repercussions of tomato leaf diseases are statistically substantial, causing yield losses ranging from 20% to 50%, as reported by the Indian Council of Agricultural Research (ICAR). These losses impact not only the quantity but also the quality of tomatoes, affecting market value and consumer satisfaction. Pathogens annually inflict damage on tomato crops, spreading diseases from leaves to fruits. Timely identification of plant leaf diseases is imperative to ensure adequate nourishment in both quantity and quality of the fruits. Conventional approaches to detecting plant leaf diseases are slow-going, prompting a need for more efficient methodologies. Farmers often resort to chemical interventions such as fungicides and bactericides, incurring increased production costs. This extensive use of agrochemicals poses economic and environmental concerns, particularly affecting the livelihoods of small and marginal farmers dependent on tomato cultivation. A shortage of professional guidance results in farmers resorting to conventional disease prevention methods, leading to over or underuse of pesticides and resulting in crop damage. This motivates the proposed methodology, aiming to effectively recognize and sort diseases affecting tomato crops. A total of 16,665 images depicting tomato leaf diseases, categorized into ten disease types, were taken from the publicly available Plant Village Dataset for this research. To reduce yield losses due to tomato disease which is causing economic burden, robust deep learning models needs to be developed. With that motivation the work has been initiated. Where various researchers are developing more complex models for achieving higher accuracy which is found to suffer the problem overfitting, proposed work is focused on development of a much simpler model with substantial accuracy. The novelty lies in the simple architecture of the model achieving significant accuracy. Also this article is also connected to SDG (Sustainable Development Goal) 12: Eco-friendly consumption and manufacturing practices designed to reduce pesticides and minimized waste. It is helpful for society and environment also. The structure of the article is as follows: related work is discussed in section 2, and the methodology and experiment setup is described in section 3,analyzation of the results is discussed section 4, focusing on the model's performance and interpretability, and finally, Section 5 wraps up the study and outlines possible areas for further research.

LITERATURE SURVEY

Tomato leaf disease detection has witnessed significant advancements through the employing Convolutional Neural Networks (CNNs) in recent research endeavors [5, 6, 8, 11]. Agarwal *et al*. (2020) introduced a customized CNN that demonstrated an impressive accuracy of 98.70%, leveraging a dataset comprising 18,160 images with a resolution of 128x128 pixels [5]. Building on this, Trivedi *et al*. (2021) presented another customized CNN, achieving a commendable accuracy of 98.49%,containing 3000 images at an increased resolution of 256×256 pixels.[6]. The research carried out by Sakkarvarthi *et al*. (2022) utilized a Custom-built CNN, achieving an accuracy of 88.17%, although specific details on batch size and epochs were not provided [8]. In the pursuit of efficient disease detection, Tarek *et al*. (2022) explored the application of MobileNetV3, achieving an outstanding accuracy of 99.81% using a dataset containing 16,004 images of 227 by 227 pixels in resolution[11]. Chen *et al*. (2022) suggested a customed AlexNet architecture, obtaining an accuracy of 98% with a dataset containing 22,930 images at a resolution of 64x64 pixels [9]. Saeed *et al*. (2023) incorporated the Inception V3 model, achieving an accuracy of 99.22% with 5225 images at a resolution of 229x229 pixels [14]. Further diversifying the landscape of CNN models, Ibañez *et al*. (2023) designed a customized CNN with an exceptional accuracy of 99.64%, utilizing with 13,500 images having a resolution of 112×112 pixels[8]. Ulutas *et al*. (2023) explored the ensemble CNN approach, achieving a high accuracy of 99.60% with a collection of data about 18,160 images of 255 by 255 pixels [13]. Sanida *et al*. (2023) introduced a hybrid CNN model, obtaining an accuracy of 99.17% with a collection of data about 18,160 images of 256 by 256 pixels [17].

Additionally, Islam *et al*. (2023) employed DenseNet 121, achieving an accuracy of 99.31% with 18,160 images at a resolution of 64x64 pixels [15]. The integration of novel approaches continued with Alzahrani *et al*. (2023) utilizing Transformer Learning (ViT), achieving an accuracy of 99.64% with a collection of data about 11,000 images of 72x72 pixels [20]. Umar *et al*. (2023) presented an improved YOLOv7 model with an accuracy of 98.80%, although detailed information on resolution, batch size, and epochs was not provided [19]. Attallah *et al*. (2023) applied KNN to achieve an impressive accuracy of 99.92% with a collection of data about 16,011 images of 224 by 224 pixels [14]. Baser *et al*. (2023) proposed a customized architecture of a convolutional model named "TomConv," achieving an accuracy of 98.19% with a collection of data about 16,000 images, 150 by 150 pixels [12].

Parvez *et al*. (2023) designed another customized CNN, achieving an accuracy of 98.39% with 6296 images at a resolution of 256x256 pixels [7]. In parallel, Paul S. G *et al*. (2023) developed a Real-time task-oriented convolutional network, achieving an accuracy of 95% with a collection of data about 32,535 images of 224 by 224 pixels [18]. Our proposed methodology contributes to this evolving landscape, employing a customized CNN with an accuracy of 97% on a dataset of 16,667 images, each with a resolution of 100x100 pixels. Another custom CNN model with an image size of 128x128, trained on 10 disease classes over 300 epochs with 3000 images, achieved a lower accuracy of 88.17% is discussed in Sakkarvarthi *et al*. 2022[8]. This lower performance might be due to differences in model architecture, dataset diversity, or hyperparameters. The article Wajid *et al*. 2024[24] used a custom CNN model for classifying 10 plant disease classes with an image size of 150x150 and a large dataset of 30,000 images, achieving an accuracy of 96%. However, the study highlights a common limitation of CNNs: they require large datasets to achieve high performance, which is often challenging to gather in applied settings. This research employed an improved YOLOv7 and CNN model to classify 7 disease classes, gain a high performance of 98.8% on 8,337 images in the article YILDIZ *et al*. 2024[25]. Despite its effectiveness, the study noted a limitation in existing algorithms' ability to handle images with multiple diseases, which poses a challenge for accurate multi-disease identification in complex real-world scenarios. Irmak *et al*. 2024[26] presented a custom CNN model which was used to classify 10 disease classes on a dataset of 14,828 images, achieving 97% accuracy. However, the model showed limitations in detecting a wide variety of plant species and diseases and severity of diseases indicating a need for further refinement to improve the model's performance. While many studies showcase impressive accuracy, variations in image resolution, dataset sizes, and model architectures make direct comparisons challenging. Additionally, some methodologies lack robustness when applied to diverse datasets, raising questions about their generalizability. Further investigations are warranted to address these limitations and enhance the reliability and applicability of CNN-based approaches in real-world tomato crop scenarios.

INTRODUCED METHODOLOGY

Figure 1 presents a schematic overview of our proposed system design. The intended system configuration has three stages: dataset preparation, model creation, and performance measurement. Throughout the development phase, data was acquired, refined, and utilized for training different models. Finally, to predict leaf disease in tomatoes, a special convolutional neural network model has been created. At the end, different types of measurements are done on our model to evaluate the performance.

Dataset

The "tomato disease multiple sources" dataset has been used for carrying out the experiments, consisting of visual data of tomato leaf diseases e. In this dataset, the images were taken in both lab and natural environments. Besides that, this image dataset was compiled using different sources.

Data Set Preparation

In the pre-processing step of the method, every image is scaled to 200 by 200 pixels and cropped to eliminate any undesirable backgrounds. Following pre-processing, the dataset is divided into two parts: 80% of the collected dataset is used for training purposes, and left is used for testing purposes.

Training and Testing

80% of the data is utilized for model training or fine-tuning, while 20% is set aside for evaluation. And also, 10% of the dataset is used for validation. For the suggested architecture, training was carried out over 100, 200, 300,400, 500 epochs using the Adaptive Moment Estimation (ADAM) Equation optimization function, and a base learning rate of 0.0001 was applied at the start.

Introduced CNN Model

The architecture of the proposed CNN incorporates both Convolutional layer and MaxPooling2D layers, ReLU for the activation function, a SoftMax layer for multiclass classification problems, fully connected layers, and the Adam optimizer. Convolutional layers make use of 3x3 kernels, subsequently followed by max pooling with a 2x2 operation. ReLU introduces non-linearity in the activation function, while SoftMax scales outputs into probabilities. In the fully connected layers, connections are made to each neuron from preceding and following layers. With the Adam optimizer, the learning rate is adapted for each weight as training progresses. It uses the Sparse categorical cross-entropy as the loss function, which is effective when performing classification with sparsely represented labels. These are discussed in detail below.

Explanations of each element of the Proposed CNN Architecture

a. Input (Dataset): The input consists of leaf images, which serve as the dataset utilized for both learning and assessment phases.
b. A Convolutional layer featuring32 filters and a 3x3 kernel size has been added as First Convolutional Layer. MaxPooling with a 2×2 window is applied afterward to compress spatial information. To mitigate overfitting, a dropout rate of 0.5 is introduced, randomly disabling 50% of the inputs during training.
c. A Convolutional layer containing 64 filters and a 3x3 kernel size has been added as Second Convolutional Layer, followed bya pooling layer configured with a 2×2 window size. Another Dropout layer has been added with a rate of 0.5.
d. A Convolutional layer containing 128 filters and a 3x3 kernel size has been added as Third Convolutional Layer, followed by a pooling layer configured with a 2×2 window size and a dropout rate of 0.5 is introduced, which randomly disables 50% of the inputs during training.
e. A Flatten layer has been inserted to compress the feature maps into a one-dimensional vector for the subsequent dense layers.
f. Three layers of dense neurons with 128,64 and 32 neurons respectively added having ReLU activation function for first two dense layers and Softmax activation function for third dense layers for providing the final probability distribution across classes.
g. The network's final output consists of classifications across several leaf disease categories

Convolution Layer

The first building block of neural network is Convolution layer. A convolutional layer contains a kernel, or filters, which are several feature detectors that are dragged across the entire image by the stride to identify the existence of a certain feature. In the proposed architecture there are four convolutional layers. The first block consists of two convolutional layers having 32 kernels each of size 3x3. The second block consists of one convolutional layer having 64 kernels of size 3x3 and last block consists of one convolutional layer having 128 kernels of size 3x3.

Max Pooling Layer

The pooling layer primarily serves to reduce the size of the output from the convolutional layer , thereby reducing the computational cost of the following layer. Max pooling is the most commonly used approach, where only the highest value in each kernel is retained after dividing the image into rectangular sub-regions defined by the kernel. In the suggested architecture, a 2x2 max pooling operation is applied after each convolutional block.

Activation Functions Used

The activation function enables or disables certain parts of a neuron's output based on a mathematical function. In the proposed model, each convolutional layer is followed by a RELU activation mechanism. The network gains non-

linearity from this activation function. The previous layer's generated output can be altered or removed by using non-linearity. This serves to restrict the amount of output generated. The suggestiveConv2D uses the Rectified Linear Unit (RELU) activation mechanism serves the following purposes:

$$g(z) = \max(0, z) \quad (1)$$

$$\frac{d}{dx} g(z) = \begin{cases} 1, & if z > 0 \\ 0, & otherwise \end{cases} \quad (2)$$

a. As seen in (1) and (2), simplicity in both function and derivative.
b. Decreased probability of a vanishing gradient.
c. A sparser representation is generated by ReLU. This occurs as a result of the gradient's zero producing a complete zero. [23]

In this article, due to the multiclass nature of disease detection, the output layer employs a softmax activation function. The network gains to scale numbers into probabilities from the SoftMax function. A vector (v) with the probabilities of every potential result is what a Softmax produces. For every potential outcome or class, the probabilities in vector v add up to one.

Densely Connected Layer

The structure of neurons in the fully-connected layer resembles that of conventional neural networks, with all neurons in this layer establishing direct connections with those in the preceding and succeeding layers. One significant disadvantage of the fully connected layer is that it involves a vast number of parameters, making the training process resource-intensive. Three dense layers using ReLU activation are implemented in the proposed model, with the final layer utilizing SoftMax for output classification.

Adam Optimizer

Moreover, the Adam optimizer is employed to minimize prediction errors and optimize node weights. Adam, short for Adaptive Moment Estimation, differs from SGD by adjusting the learning rate individually for each network weight instead of using a single learning rate for the entire training process. Due to its many benefits, deep learning projects might benefit greatly from using the Adam optimizer as their default optimization method. It is also a helpful benchmark. This optimization algorithm requires less tuning, utilizes less memory, and executes faster than previous algorithms. The RMSP algorithm and gradient descent with momentum are combined to create the Adam optimizer. Adam creates a better optimized gradient descent by combining the qualities of the two optimizers.

$$\omega_t = \omega(t-1) - \alpha * \left(\frac{\widehat{m_t}}{\sqrt{(\widehat{v_t})}+e}\right) \quad (3)$$

Here, Adam's optimizer formula is shown in Eq. (4). The aggregate of gradients at time t is represented by $\widehat{m_t}$ in the equations above. Conversely, $v_t$stands for the characteristic of algorithms that use Root Mean Square Propagation (RMSP). When mt and vt are first set to 0, they are typically more biased [22]. As a result, the biased problem was resolved by the Adam optimizer by computing the bias-corrected m̂t and v̂t. Ultimately, new weight ωtare computed by integrating the formula.

Error Function

The article employs the Sparse Categorical Cross-Entropy loss function. Sparse Categorical Cross-Entropy is a loss function utilized in machine learning for classification tasks, measuring the cross-entropy between predicted probabilities and true class labels, especially effective for scenarios with sparse labels. Equation (5) outlines the Sparse Categorical Cross-Entropy Loss function.

$$\text{Sparse Categorical Cross-Entropy} = -\frac{1}{N}\sum_{i=1}^{N}\sum_{c=1}^{c} y_{i,c} \cdot \log(\widehat{y_{i,c}}) \quad (4)$$

Here, N represents the number of samples, c is the number of classes,$y_{i,c}$is a binary indicator showing whether class c is the correct classification for sample i, $\widehat{y_{i,c}}$ is the predicted probability of sample i belonging to class c.

Experimental Setup

The experiment was conducted in Google Colab with default configuration of Intel(R) Xeon(R) CPU @ 2.20GHz with 2 vCPUs (virtual CPUs) and 13GB of RAM. NVIDIA Tesla K80 with 12GB of VRAM.

Evaluation Matrices

Evaluation metrics are used to determine how effective the statistical, machine learning, or deep learning model is. The suggested model in a study must be assessed using a range of assessment indicators. Evaluation metrics are essential to guaranteeing the performance of models. The equations of these metrics were calculated using Equations (5)– (12)

$$\text{Accuracy} = (\text{TP} + \text{TN})/\ (\text{TP} + \text{FP} + \text{TN} + \text{FN}) \quad (5)$$

$$\text{Precision} = \text{TP}\ /(\text{TP} + \text{FP}) \quad (6)$$

$$\text{Recall} = \text{TP}/(\text{TP} + \text{FN}) \quad (7)$$

$$\text{F1} - \text{Score} = 2\text{PR}/(\text{P} + \text{R}) \quad (8)$$

$$\text{Sensitivity} = \text{TP}/(\text{TP} + \text{FP}) \quad (9)$$

$$\text{Specificity} = \text{TN}/(\text{FP} + \text{TN}) \quad (10)$$

$$\text{Matthews Correlation Coefficient(MCC)} = (\text{TPxTN}) - (\text{FPxFN})/\ (\sqrt{(\text{TP} + \text{FP})}\ \text{x}\ \sqrt{(\text{TP} + \text{FN})}\ \text{x}\ \sqrt{(\text{TN} + \text{FP})}\ \text{x}\ \sqrt{(\text{TN} + \text{FN})}\ ) \quad (11)$$

$$\text{Negative Predictive value} = (\text{TN})/(\text{TN} + \text{FN}) \quad (12)$$

RESULTS AND DISCUSSIONS

Results of hyper parameter optimization

The hyper parameters optimized manually shown the following values of hyper parameters produces best possible performance of the model.

Training and Validation Accuracy and Loss graph with respect to Epochs

The overall accuracy and loss of model trained with various number of epochs has been shown here. From the table 2 we got, the training process started at epoch 100 with a relatively high accuracy of 0. 9463. The accuracy continued to improve, reaching 0.9544 at epoch 200 and remaining relatively stable. At epoch 400, there was a further increase in accuracy to 0. 9628. The final epoch, 500, showed a slight improvement, reaching an accuracy of 0. 9631. This set of data suggests a consistent and successful training process with increasing accuracy. Whereas training process started at epoch 100 with a loss of 0. 2286. Over the next few epochs, there was a consistent decrease in loss, indicating that the model was improving. The loss decreased to 0.188 at epoch 200.Althoughthere was a slight increase in loss to 0.2081 at epoch 300, it was still lower than the initial loss. The lowest loss was achieved at epoch 400, with a value of 0. 1692. At the final epoch, 500, there was a slight increase in loss to 0.1752, but it remained relatively low. We may conclude that, the decreasing trend in loss values suggests that the training process was effective in reducing the error of the model. Lower loss values typically indicate that the predictions made by the model were progressively approaching the true target values.. The slight increase in loss towards the end of training (epochs 300 to 500) may indicate some fluctuations, but the overall trend is positive.

Analysis by Standard Deviation

The difference between training accuracy and validation accuracy is first obtained after every epoch, then a standard deviation of the all differences obtained after each epoch has been calculated using the following formula. Standard deviation of differences of training accuracy and validation accuracy obtained for each epoch has been obtained.

$$sd = \sqrt{\frac{1}{N-1}\sum_{i=0}^{n}(Training_Acc_i - Val_Acc_i)} \quad (13)$$

From the above Table No 3, we see that, at epoch 100, the training process began with an accuracy of 0.034425. As training progressed, accuracy greatly increased and by epoch 200, it had decreased to 0. 019461.As the training went on, accuracy increased even further, peaking at 0.016113 at epoch 300.However, the precision increased somewhat to 0.018971 at epoch 400.Accuracy dropped somewhat to 0.01804 for the 500th and final epoch. Hence the model trained with 300 epochs has been considered for further analysis. A confusion matrix was derived for the proposed model following 500 epochs of training.

Performance Assessment of Model

The performance indicator for evaluating of different disease are shown in tabular structure below in Table 3. The class-wise performance metrics showcase the proposed model's proficiency in accurately classifying different disease classes, with accuracy percentages ranging from 99% to 100% across various categories. Notably, diseases like Bacterial spot, **E**arly Blight, **L**ate Blight, and **S**pider Mites secures perfect accuracy scores of 100%, demonstrating the model's remarkable capability to accurately detect cases of these diseases. For other classes such as leaf mold, mosaic virus, and target spot, the model achieves accuracy percentages of 99%, demonstrating its high reliability in disease detection. However, the classification of healthy plant samples exhibits a slightly lower accuracy percentage of 99%, suggesting some challenges in distinguishing healthy instances from diseased ones. Despite this, the model maintains high precision, sensitivity, specificity, F1-score, negative predictive value (NPV), and Matthews correlation coefficient (MCC) percentages above 90% for most disease classes, underscoring its overall effectiveness and reliability in plant disease diagnosis.

A comparison between the proposed model and pre-trained models.

The comparative analysis reveals compelling insights into the results of the proposed model against several pre-trained models across various evaluation metrics. While traditional architectures like AlexNet, ResNet50, and VGG16 exhibit respectable accuracies ranging from 79.09% to 82.45%, they are outperformed by more modern architectures like DenseNet and MobileNetV1, which achieve higher accuracies of 90.52% and 92.89%, respectively. Nonetheless, the proposed model outperforms all pre-trained models, achieving a remarkable accuracy of 96.31%, which reflects its excellent classification performance.

Box PlotGraph of Model accuracy and error rate

Figure 9 states that, the training accuracy is very high (mostly close to 1.0), indicating the model has learned the data well, though there are a few outliers below 0.95, and one around 0.825. Validation accuracy is slightly lower, with most values around 0.95, showing decent generalization but some outliers nearly about 0.925. The disparity between training and validation accuracy reveals mild overfitting, leading the model to excel more on the training data than on unseen validation data. The training loss is low (around 0.1), reflecting high training accuracy, with a few outliers above 0.2 and one near 0.5, suggesting rare difficulties in fitting the data. Validation loss is higher (around 0.2), with outliers above 0.4, indicating inconsistent performance on the validation data, possibly due to overfitting or noise.

Prediction analysis of proposed model

The model accurately identified several cases of Yellow Leaf Curl Virus and Spider Mites, showing reliable performance when symptoms are clear. Despite high confidence (100%), the model made errors, such as misclassifying Early Blight as Septoria Leaf Spot and Bacterial Spot as Late Blight. This suggests overconfidence and difficulty distinguishing diseases with similar symptoms, especially between Early Blight vs. Septoria Leaf Spot and

Bacterial Spot vs. Late Blight as shown in Figure 10. However, this type of misclassification try to be solved by using the concept of Explainable AI (XAI)[27].

Comparison with existing works

The number of epochs and dataset size play pivotal roles in model training, influencing its capacity to discern intricate patterns and generalize effectively. Models trained on longer durations and larger, more diverse datasets, such as Saeed *et al*. (2023) with Inception V3 and Tarek *et al*. (2022) with pre-trained CNN model, tend to exhibit heightened robustness and generalization capabilities. Training a model with higher epochs can improve its learning from the data but risks overfitting, while when the number of epochs is very low, the model might not fully converge, resulting in suboptimal performance and insufficient learning from the dataset. Many studies, such as Baser *et al*.(2023), Parvez *et al*.(2023) and Paul S. G *et al*. (2023) demonstrate a trend of employing a relatively low number of training epochs. This suggests a preference for expedited training processes, possibly due to computational constraints or a belief in early convergence. With an optimum number of epochs, such as 500 in the proposed model, there's more opportunity for the model to iteratively refine its parameters, potentially leading to improved performance and better convergence towards an optimal solution.

CONCLUSION

This research involved the development of a convolutional neural network (CNN) model to identify healthy and diseased tomato leaves, along with identifying specific diseases. The proposed approach resulted in impressive accuracy levels of 96.31% in identifying tomato leaf diseases. However, limitations exist. The accuracy of the model could be compromised due to inconsistencies in lighting and image resolution. The next phase of work will be directed towards improving the model's robustness and generalizability by incorporating real-world field images and exploring data augmentation techniques. The failure case of prediction will be solved by Tools from Explainable AI, including Saliency visualizations and Grad-CAM (Gradient-weighted Class Activation Mapping). Additionally, the research can be extended to develop an App to reach the farmers. By continuously developing and refining AI-powered disease detection systems, we can allow farmers to effectively decide on matters related to their crop health management, leading to enhanced agricultural efficiency and long-term sustainability.

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Table 1: Layer Wise Description in the Proposed System

Layer	Kernel Size	Stride	Padding	Input Shape	Output Shape	Dropout
Conv2D	(3,3)	(1,1)	same	(200,200,3)	(198,198,32)	-
Conv2D	(3,3)	(1,1)	same	(198,198,32)	(196,196,32)	-
MaxPooling2D	(2,2)	(2,2)	valid	(196,196,32)	(98,98,32)	-
Dropout	-	-	-	(98,98,32)	(98,98,32)	0.5
Conv2D	(3,3)	(1,1)	same	(98,98,32)	(96,96,64)	-
MaxPooling2D	(2,2)	(2,2)	valid	(96,96,64)	(48,48,64)	-
Dropout	-	-	-	(48,48,64)	(48,48,64)	0.5
Conv2D	(3,3)	(1,1)	same	(48,48,64)	46,46,128	-
MaxPooling2D	(2,2)	(2,2)	valid	(48,48,64)	23,23,128	-
Dropout	-	-	-	23,23,128	2323128	0.5
Flatten	-	-	-	23,23,128	67712	-
Dense	-	-	-	67712	128	-
Dense	-	-	-	128	64	-
Dense (Softmax)	-	-	-	64	10	-

Table 2: Hyper parameter value in suggestive model

Hyper parameter	Value
Convolutional depth	3
Max pooling layer	3
Dropout rate	0.5
Node activation mechanism	ReLu
Optimization rate	0.001
Number of epoch	500
Batch size	64

Table 3: Result of different epoch

#Epochs	100	200	300	400	500
Accuracy	0.9463	0.9544	0.9538	0.9628	0.9631
Loss	0.2286	0.188	0.2081	0.1692	0.1752

Table 4: Result of Standard Deviation

Epoch	Standard Deviation
100	0.034425
200	0.019461
300	0.016113
400	0.018971
500	0.01804

Table 5: Evaluation metrics for the proposed model at epoch 500

Disease Name	ACC	PRE	SEN	SPE	F1-SCORE	NPV	MCC
Bacterial spot	1.00	0.99	0.99	1.00	0.99	1.00	0.99
Early Blight	1.00	0.98	0.99	1.00	0.98	1.00	0.98
Healthy	0.99	0.91	0.84	1.00	0.88	0.99	0.87
Late Blight	1.00	0.99	1.00	1.00	1.00	1.00	0.99
Leaf Mold	0.99	0.93	0.92	1.00	0.93	1.00	0.92
Mosaic Virus	0.99	0.96	0.98	0.99	0.97	1.00	0.97

Septorial Leaf Spot	0.99	0.93	0.96	0.99	0.94	1.00	0.94
Spider Mites	1.00	0.96	1.00	1.00	0.98	1.00	0.98
Target Spot	0.99	0.97	0.92	1.00	0.95	0.99	0.94
Yellow Leaf Curl	0.99	0.94	0.94	0.99	0.94	0.99	0.93

Table 6: Performance Comparison with Proposed Methodology

Model	ACC	PRE	SEN	SPE	F1-SCORE	NPV	MCC
Alex Net	82.45	0.82	0.79	0.98	0.78	0.98	0.78
DenseNet	90.52	0.90	0.90	0.99	0.89	0.99	0.89
MobileNetV1	92.89	0.92	0.91	0.99	0.91	0.99	0.91
Rest Net50	79.09	0.81	0.76	0.98	0.75	0.98	0.74
VGG16	80.59	0.77	0.76	0.98	0.76	0.98	0.74
Proposed Model	96.31	0.96	0.95	1.00	0.95	1.00	0.95

Table 7: Performance Comparison with Proposed Methodology

References	**Method**	**Disease Classes**	**Image Size**	**Batch Size**	**Epoch**	**Dataset**	**Performance (%)**
Sakkarvarthi *et al*. 2022[8]	Custom CNN Model	10	128 x 128	-	300	3000	88.17%
Tarek *et al*. 2022[11]	MobileNetV3	10	227 x 227	32	50	16,004	99.81%
Chen *et al*. 2022[9]	Modified AlexNet	10	64 x 64	128	75	22,930	98%
Saeed *et al*. 2023[14]	Inception V3 model,	3	229 x 229	32	25	5225	99.22%
Islam *et al*. 2023[15]	DenseNet 121	10	64 x64	-	50	18,160	99.31%
Baser *et al*. 2023[12]	Custom CNN Model	10	150 x 150	32	105	16,000	98.19%
Parvez *et al*. 2023[7]	Custom CNN Model	2	256 x 256	-	50	6296	98.39%
Paul S. G *et al*. 2023[18]	Custom CNN Model	11	224 x 224	32	100	32535	95%
Proposed Model	Custom CNN Model	**10**	**200 x200**	**64**	**500**	**16,667**	**96.31%**

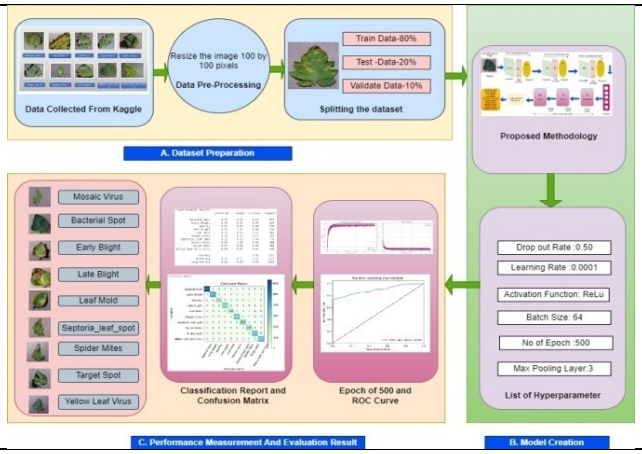


Fig 1: Work Flow diagram

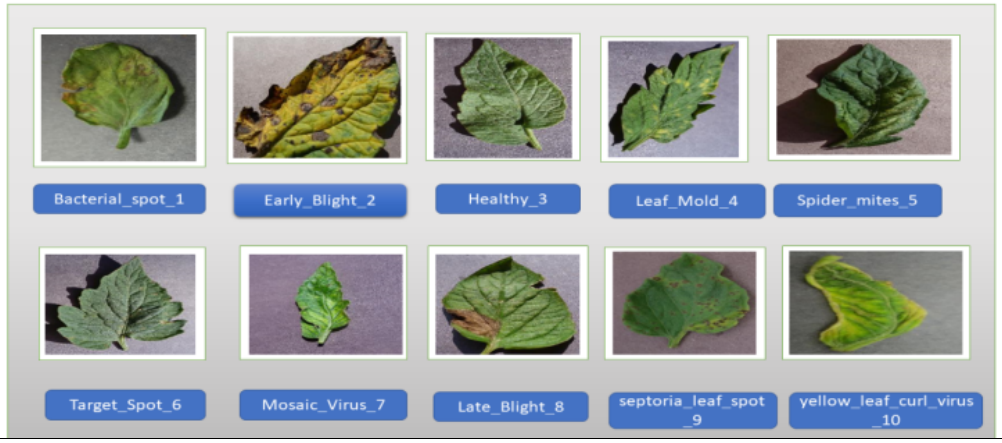


Fig. 2. Sample images of ten different diseases of Tomato Leaves.

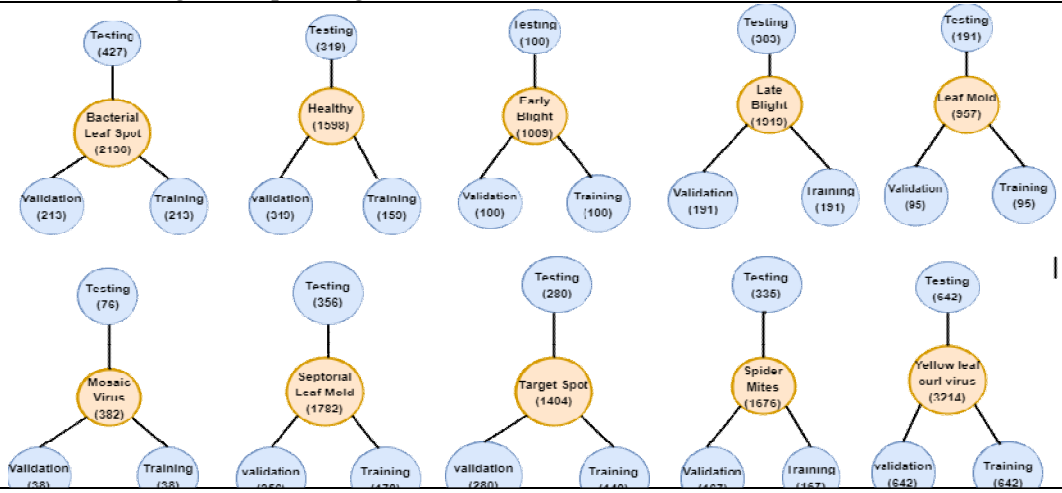


Fig. 3: Class wise images in training, testing and validation dataset

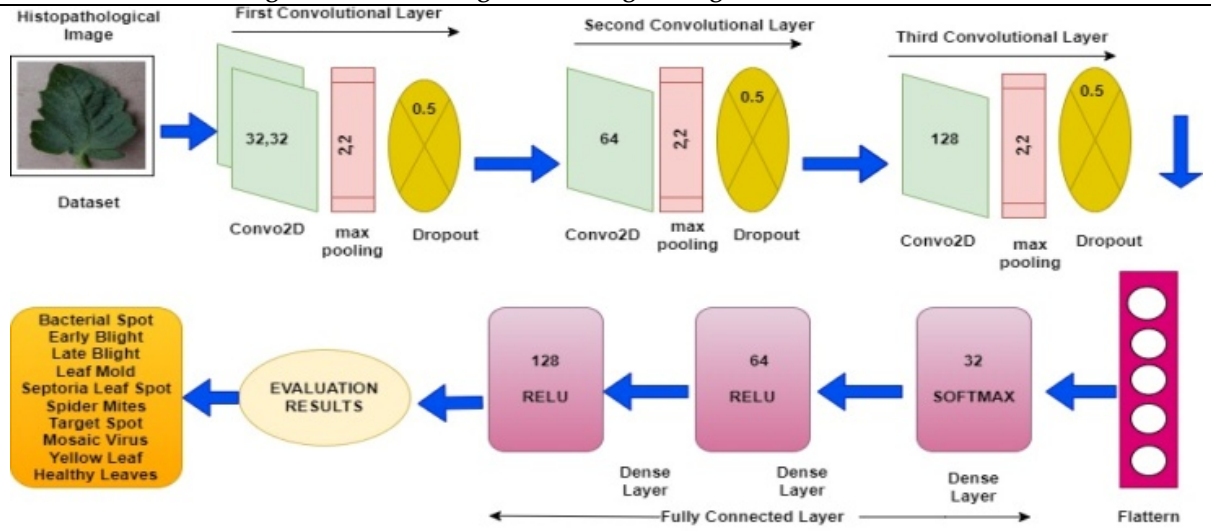


Fig 4: Proposed CNN Architecture

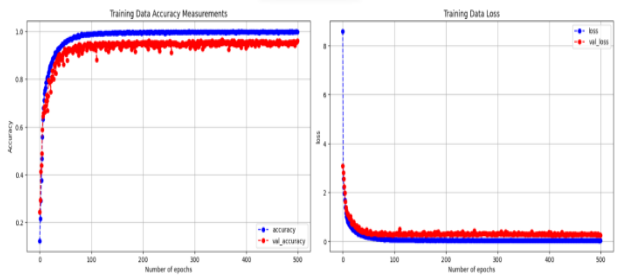


Fig 5. (a) Model accuracy on training dataand (b) Error rate of the proposed CNN model at500 epoch

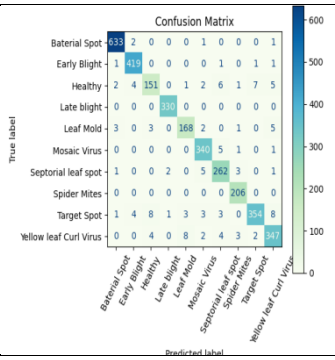


Fig. 6: Confusion matrix of the proposed model after training for 500 epochs

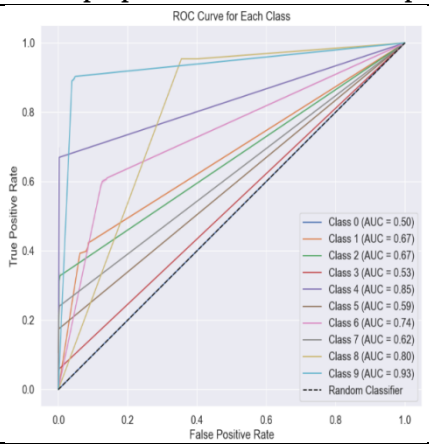


Figure 7: ROC Curve



Figure 8: Accuracy and Loss Plot



Figure 9: Prediction Analysis of Proposed Model

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RESEARCH ARTICLE

A Comprehensive Survey on Artificial Intelligence based Routing Techniques in 6G Empowered Wireless Communication Networks

R. Nagadevi[1]* and R. Beulah[2]

[1]Research Scholar, Department of Computer Science, PPG College of Arts and Science, Saravanampatti , (Affiliated to Bharathiar University),, Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, Department of Information Technology, PPG College of Arts and Science, Saravanampatti , (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
R. Nagadevi
Research Scholar,
Department of Computer Science,
PPG College of Arts and Science, Saravanampatti ,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: devivijay919phd@gmail.com

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ABSTRACT

The emergence of future communication systems is driven by a constant demand for higher transfer rates, lower latency, increased dependability, global connection, and extensive use. Both private and public sectors have shown significant interest in sixth-generation (6G) networks as they deploy fifth-generation (5G) technologies worldwide. 6G networks must offer extremely dependable and low-latency connection to accommodate the proliferation of smart devices as well as the Internet of Things (IoT). To improve the performance of a network, routing techniques are essential. The complex and ever-changing 6G scenarios will be too much for traditional routing methods to handle. A few instances of AI algorithms that have become essential for the present administration of massive and dynamic networks include Machine Learning (ML), Deep Learning (DL), along with Reinforcement Learning (RL).Nevertheless, several substantial issues must be resolved when implementing AI algorithms in 5G/6G networks. This paper aims to present a survey on various AI algorithms applied for routing in 5G/6G-empowered communication networks. Additionally, it discusses the benefits, limitations, and performance metrics to emphasize open research challenges that should be addressed in future studies.

Keywords: Future communication systems, IoT, 5G, 6G, Routing, AI, ML, DL and RL.

INTRODUCTION

The rate of growth of mobile data traffic is increasing at an exponential rate as the world becomes more interconnected. The International Telecommunication Union's Radio communication (ITU-R) sector reports that monthly mobile information traffic worldwide reached158 Exabytes (EB) in 2022, with predictions of 2194 EB in 2028 and 5016 EB in 2030 [1]. With an expected increase from 12.1 GB in 2022 to 257 GB in 2030, these numbers show that mobile data consumption is growing at an exponential rate among consumers [2]. Mobile data services are becoming increasingly popular across all demographics and geographies. The percentage of people using mobile services will reach 70% by 2025, with 60% of that total accessing the internet through mobile devices [3]. Some of the emerging technologies that are propelling this growth include the IoT, AI, blockchain, Virtual Reality (VR), three-dimensional video, and linked automobiles. As the need for mobile data services continues to rise, businesses throughout the world have begun implementing 5G technology. Nevertheless, it is clear that a technology overcoming previous 5G networks would be required to manage the increased data flow as the globe moves toward automation. While handling a huge increase in data traffic, the 6G network is expected to provide consumers with enhanced service [4]. Mobile wireless technology might be radically altered by the 6G network's extensive coverage, lightning-fast data rates, and negligible latency. By combining the digital, biological, and physical worlds, 6G networks will bring about a mobile network revolution [5]. This will pave the way for the development of novel applications for 5G and use cases that were before inconceivable. Furthermore, 6G networks will lay the framework for a plethora of applications including smart cities and driverless vehicles, which necessitate massive capacity, low latency, and global coverage.

Evolution of Telecommunication Technology: From 1G to 6G

Beginning in the early 1980s, the telecommunication age has seen great expansion in the subsequent decades, with notable developments in data rates, connection, and efficiency [6-7]. Figure 1 shows the development of telecommunication technology. Starting in the 1980s, the first generation of mobile wireless networks, commonly known as 1G, was mostly based on analog technology. Data transfer rates were slow as well as the sound quality was lacking. Second-generation (2G) cellular networks, which first appeared in the 1990s, made available services such as Short Message Service (SMS) as well as simple email capabilities. Among others are the Global System for Mobile Communications (GSM) and Code Division Multiple Access (CDMA). Mobile networks in the 2000s upgraded to third generation (3G), which permitted faster data transfers, internet surfing, and multimedia messaging capabilities. A couple examples are Enhanced Data Rates for GSM Evolution (EDGE), CDMA2000, and WCDMA. Online multiplayer games, conference calls via video, and High-Definition (HD) video streaming were made possible by fourth-generation (4G) networks in the 2010s, such as Long-Term Evolution (LTE) as well as Worldwide Interoperability for Microwave Access (WiMAX). These networks offered better coverage and higher data rates. The IoT, smart cities, as well as the fourth industrial revolution are expected to be set up by the 5G networks, which were released in 2018. With AI integrated into the network infrastructure at every level, 6G promises to be the next stage of mobile networks, bringing in unprecedented coverage, connection speeds, and latency. These networks will make use of cutting-edge technologies including quantum communication, AI, ML, ultra-massive Multiple-Input Multiple-Output (MIMO), and Terahertz (THz) communication.

Design of 6G Mobile Technology

The current terrestrial network is inadequate for 6G's global coverage, necessitating integration with non-terrestrial networks. Figure 2 shows the four levels of the cell-less architecture that will be used by 6G. These levels are space, air, terrestrial, as well as marine [8].The space tier will provide internet services for space missions, while the terrestrial tier will extend coverage using THz bands, but with higher path loss and congestion. IoT will expand into real-time monitoring via the Tactile Internet, allowing for real-time interaction with virtual items. Photonic solutions and Visible Light Communication (VLC) in the 400-800 THz range [9] will improve network performance, especially in aviation and maritime, outperforming Radio Frequency (RF) technologies.

Features of 6G Networks

6G networks are anticipated to greatly improve upon 5G technology, introducing several advanced features. They are projected to achieve data transfer speeds exceeding 10 Tbps, ensure extremely low latency, offer broader coverage, enhance user experiences with technologies like VR and AI, as well as deliver network and spectral efficiency that is tenfold higher than that of 5G networks. Further than 100 times the number of connections offered by previous 5G networks, they will provide excellent energy efficiency, which means longer battery life and better sustainability [10].By merging with other methods like the analytics of large data, cloud computing, as well as IoT, 6G networks offer great connectivity across diverse systems.4G, 5G, as well as 6G network performance comparisons are shown in Table 1 [11]. VR, machine-to-machine cooperation, the IoT, robotic surgery, and Unmanned Aerial Vehicles (UAVs) are all possible with 6G networks [12].They necessitate extensive connectivity, incredibly dependable and with little latency, optimized use of resources, higher energy efficiency, improved security and privacy, and worldwide coverage [13]. Although their widespread use may not occur until 2030, they may appear earlier in some areas.6G is currently being investigated by several companies, including China Mobile, ZTE, and Huawei [14]. The U.S. Federal Communications Commission (FCC) has just opened the "THz wave" to further standardized testing [15], and LG and Finland's University of Oulu are leading the research. Certainly, 6G is a quite recent idea, and it could be a while before its full promise is seen.

AI for 6G Networks

AI is expected to have a profound impact on 6G networks by improving management of networks and the user experience. Applications like VR and driverless vehicles rely on its ability to handle an influx of data and sensors, maximize processing power and bandwidth, and facilitate decision-making in real-time. Automated cybersecurity solutions powered by AI can monitor network traffic, identify unusual activities, and counteract any dangers. By detecting problems ahead of time and reducing the impact of interruptions, an AI-powered 6G network can improve reliability [16–17]. Algorithms powered by AI can study patterns of user behavior, optimize and manage network resources in real-time, and make consecutive judgments in unpredictable settings.

Convergence of AI, 6G and Wireless Communication

The three main services that 6G technology delivers are Massive Machine-Type Communication (mMTC), Ultra-Reliable Low Latency Communication (URLLC) as well as improved Mobile Broadband (eMBB) [18]. With respect to eMBB, URLLC, and mMTC, 5G stands apart from 6G technology powered by AI (refer to Table 2).

Applications of 6G Networks

The 6G network is expected to have numerous sectors as potential users, even if it is not yet available for commercial use [19].

Ultra-HD Video Streaming with VR: With the advent of 6G networks and AI, online gaming and ultra-HD video streaming will be possible without interruption, while real-time playback and personalized content recommendations will be much improved.

Healthcare Monitoring: Improved predictive analytics and individualized healthcare are possible outcomes of combining AI alongside the 6G revolution. Improving patient care through proactive, individualized healthcare delivery is made possible by the effective transmission of health data made possible by 6G networks' quick data transmission rates with minimal delay.

Remote Surgery: The 6G network and AI technology enable telesurgery, a surgical technique that has been transformed by robotics and wireless networking, to link physicians and patients over great distances. By facilitating the processing and analysis of data in real-time, this technology improves surgical practice, which in turn increases patient safety, increases access to care, and ultimately improves surgical outcomes.

Tactile Internet: The tactile internet, a novel form of interaction between humans and machines, stands to be greatly advanced through the incorporation of 6G technology. This would allow for real-time, high-quality transmission of tactile data, facilitating the creation of advanced haptic devices, remote control, as well as VR applications. This has the potential to greatly affect the healthcare, gaming, as well as industrial industries.

Industrial IoT (IIoT): 6G has the ability to revolutionize industrial tasks by providing real-time communication, better decision-making, and more security. It has the potential to pave the way for innovative IoT applications, such as remote management and predictive maintenance, which can increase efficiency and reduce expenses.

Mixed and Augmented Reality (MAR): The integration of actual and virtual environments, the transfer of higher resolution VR data, and the enablement of distant jobs, social engagement, as well as gaming are all ways in which 6G technology may improve MAR experiences. It is a primary application of 6G technology because of its real-time abilities and sophisticated security mechanisms, which can generate novel and engaging experiences.

AI and Robotics: With 6G's faster connection and greater capabilities, AI as well as robotics are expected to undergo significant changes. Improved speed and accuracy of AI algorithms, real-time communication for drones and autonomous robots, and the ability to operate and maintain industrial machinery remotely are all benefits.

Challenges for 6G Implementation

Difficulties that may arise when deploying 6G networks include [20]

- Technology innovation and standardization: Challenges associated with emerging methods includes THz connectivity, AI, ML, quantum communication, massive MIMO, as well as URLLC.
- Spectrum inadequacy: Propagation limits and current regulatory constraints make it difficult to find and allocate spectrum for 6G in the THz frequency band.
- Compatibility with existing networks: Difficulties in achieving seamless integration between diverse technologies and standards used by various networks.
- Regulation and policy: To meet the regulatory issues brought forth by emerging spectrums and technologies, new legislation and regulations are required.
- Global relationships and harmonization: There is a lot of pressure on businesses and governments to be the first to market with 6G, which calls for international cooperation and standards.
- Concerning privacy and security: There are new obstacles to overcome in the integration of various devices and systems, as well as in the transfer of sensitive data.
- Eco-friendly concerns: Environmental impacts of extracting raw resources for 6G infrastructure, leading to habitat destruction, pollution, and resource depletion.

Routing Challenges and Optimization in 6G Networks

The network routing method controls the process of routing, which entails finding a way for data to flow from one place to another. Distance vectors or connection measurements have formed the basis for numerous routing algorithms developed over the last several decades for use with various telecommunications systems. Nevertheless, 6G networks present numerous obstacles, such as:

- Dynamic and Complex topologies: Networks in which nodes are constantly joining and leaving, causing connection rates to change often [21]. Integration of autonomous vehicles, drones, and mobile devices adds complexity, requiring real-time adjustments to routing tables.
- High degree of heterogeneity: 6G networks will combine various network types and technologies, making difficult to seamless interaction and routing across them [22].
- Dense networks: The proliferation of IoT in 6G networks will increase device density, requiring robust routing solutions to maintain connections efficiently. Congestion, interference, and diverse Quality of Service (QoS) requests from different devices and applications pose additional challenges [23].

Inefficient routing and resource consumption are common results of using conventional routing strategies in dynamic, heterogeneous networks. Congestion results from the high computational complexity and inflexibility of

traditional shortest path techniques, which rely on static measures such as hop count or delay. On the other hand, by assessing intricate network conditions, AI-driven routing techniques especially those rooted in ML and DL offer adaptive, data-driven solutions. Benefits, drawbacks, performance measures, and major future research issues related to AI-based routing systems for 5G and 6G networks are all covered in this study. The following sections have been prepared: The second section provides a summary of the AI-based routing strategies used in 5G/6G wireless networks, and the third section concludes up the study and makes some suggestions for improvements in the 6G era.

Survey on Different Routing Techniques in 5g/6g-Empowered Wireless Communication Networks

For 5G and beyond, Jothikumar *et al*. [24] introduced an Optimal Cluster-Based Routing (Optimal-CBR) method to extend the life of Wireless Sensor Networks (WSNs) based on the IoT. The K-means approach and a multi-hop method were employed by the Optimal-CBR technique to group the nodes and route the chains. The grouping phase finished the data transfer when two-thirds of the node had perished. Then came the connection stage. The nodes were ordered by highest energy, with the default being the node nearest the centroid, to start the process of choosing Cluster Heads (CHs). The CH sent the BS later packets its members had received. During the chaining phase, the remaining nodes went on to build a chain utilizing multi-hop routing in spite of losing two-thirds of the nodes and lacking energy for grouping. The BS was reached by the packets continuing this process. Using 6G-enabled UAVs, Deb *et al*. [25] created a delay-aware Q-Learning (QL)-based routing method named XiA. XiA sends user packets to close by Access Points (APs). A framework was created to let UAVs and their several stakeholders engage while they exited the communication zone, hence facilitating connectivity with Low-Earth-Orbit (LEO) satellites. Incentive functions were applied using the user's information urgency as well as transferring links between the UAV players. They also evaluated the effectiveness of dynamically altering parameters to rectify prior decision-making errors using the QL method. Dogra *et al*. [26] suggested a 5G balanced energy-efficient routing method depending on smart MIMO to improve the Quality of Experience (QoE) for data transport in clusters of IoT equipment. This design simulated the processes for choosing the CH and producing intra- as well as inter-clustering for multi-hop routing. The network started with a vertical architecture into intra- and inter-clusters to spread the energy consumption among the CHs. Grouping and a multi-hop design were used to link the two data transmission ends next. Duhayyim *et al*. [27]] proposed EDACRP-6G, a new approach for best-path recognition and energy-effective cluster-based data gathering in their work on 6G-enabled UAVs. This approach was able to select the perfect set of CHs using an Artificial Fish Swarm-based Route Planning (AFSRP) scheme and an Improved Red Deer Algorithm-based Clustering (IRDAC) scheme. Arya *et al*. [28] investigated a DL-based routing system's performance to guarantee effective transmission of data in 5G WSNs. Initially, nodes belonging to certain clusters were rewarded to divide the network using the RL approach. Manta Ray Foraging Optimization (MRFO), a data-transfer-optimal method, chose the CH. The next stage was to create a more efficient routing technique allowing improved data transfer along the chosen path based on Deep Belief Networks (DBNs). Marinho *et al*. [29] presented CAIN, an AI method for 6G networks, as a routing protocol meant to enhance coverage regions that is both smart and energy-aware. Based on their connectivity, a CAIN would take out a series of communications to nodes farther away from an AP, trying to extend the range of the network. The CAIN will find a neighboring node with an AP connection to send the message when a node lacks an AP connection. Should there be no link to a nearby AP, the node would also choose an adjacent node to transmit the message.

DNN, Federated Learning (FL), as well as RL among other AI techniques enhanced this CAIN. De Lucia *et al*. [30] tackled the energy depletion issue in UAV swarms and proposed a novel routing method called ENSING to save energy and connection in 6G systems. By selecting the next-hop UAV forwarder based on energy limits, this technique ensures the selection of only suitable UAVs with adequate energy supplies for packet forwarding. Haseeb *et al*. [31] suggested an efficient and trusted autonomous vehicle routing algorithm for 6G networks to ensure maximum QoS and data coverage. Initially, a simulated annealing algorithm executed a routing task, optimizing energy between IoT-based vehicles. Then, because connected vehicles could reliably interact with network edges in a session-oriented way, a risk-aware security model emerged. This stopped uncertainties in the autonomous network. A Deep Q-Network-based Load-balancing Routing Algorithm (DQNLLRA) for the LEO satellites was created by Dong *et al*. [32] by modeling a satellite routing operation as a Markov Decision Process (MDP). Using iterative

learning and data from nearby nodes in the satellite network, the system found the best decision model taking into account aspects like latency, bandwidth, as well as queue usage. For feasible transmission in 5G/6G WSNs, Gururaj *et al*. [33] proposed a Collaborative Energy-Efficient Routing Protocol (CEERP). It all started with data collection at the sink node. After classifying the nodes in the network into groups according to their remaining energy, the RL chose the CH to facilitate better data transport. Using the Multi-Objective Improved Seagull Algorithm (MOISA) together with the CEERP helped to significantly improve the performance of the network. Naguib *et al*. [34] presented a novel paradigm for efficient control of virtual reality transmission of data in a 6G-enabled VR Software Defined Network (SDN) for End-to-End (E2E) VR interactions between users. An optimization problem was simulated with regard to the requirements of the VR slice in order to reach the greatest practical data rates in the framework of limited E2E latency and available resources throughout all layers. Tilwari *et al*. [35] designed the Multi-Criteria-Aware Integrated Decision-Making (MCAIDeM) routing protocol to ensure that end-to-end users in IoT communication may rely on a consistent and efficient path leading to 6G systems. Combining the leftover energy, waiting time, mobility, and link dependability of relay nodes, a single routing algorithm was developed to select a consistent and reasonably priced path. For judgments with several criteria, they applied an AHP (Analytic Hierarchy Process) system. This method selects acceptable relay nodes during path selection by calculating suitable weights depending on the node's value for parameters. Tshakwanda *et al*. [36] proposed a Speed-optimized LSTM (SP-LSTM) method combining predictive analytics with dynamic routing to increase resource economy and optimize network efficacy. This two-layered system combines SP-LSTM networks in RL for predictive modeling and routing improvement. SP-LSTM estimates network congestion for proactive measures, whereas RL enhances routing based on these predictions. Aligning with the dynamic aspects of 6G networks, this system is driven by continuous learning and adaptation to accomplish the objectives of extremely low latency, dependability, and heterogeneity management. The above-discussed routing techniques for 5G and 6G communication networks are evaluated in terms of their advantages, drawbacks, and performance metrics in Table 3.

CONCLUSION

A future communication system that is both reliable and efficient is in great demand. Future networks will operate in an even more complicated and ever-changing environment, making intelligent routing design all the more important for network performance. AI-empowered 6G networks are a promising solution to address these challenges. This paper surveys ML and DL algorithms developed for routing optimization in 5G/6G networks. As well, the advantages, limitations, and performance metrics of these algorithms are summarized in a tabular form to identify challenges. Therefore, DL algorithms will be crucial to the intelligence of 6G networks in the future, according to this study. Continuous innovation in DL and other AI techniques will enable 6G technology to meet the needs of individuals, organizations, and industries effectively. Therefore, future work will focus on developing advanced DL algorithms that facilitate robust communication in 6G networks.

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Table 1.Analysis of 4G, 5G, and 6G Networks

Performance indicators	4G	5G	6G
Highest data transmission rate	1 Gbps	10 Gbps	1 Tbps
Maximizing energy efficiency	< 1000x related to 5G	1000x related to 4G	> 10x related to 5G
Low delay	100 ms	1 ms	0.1 ms
High spectral effectiveness	15 bps/Hz	30 bps/Hz	100 bps/Hz
The density of connections	2000 devices / km²	1 million devices / km²	> 10 million devices / km²
Coverage %	< 70%	80%	> 99%
Driverless car	No	Partial	Completely
Precise positioning	Meter level (50 m)	Meter level (20 m)	Centimeter level
E2E reliability	99.9%	99.999%	99.9999%
Haptic transmission	No	Partial	Completely
The receiver's sensitivity	~ -100dBm	~ -120dBm	~ -130dBm
Mobility support	350 km/h	500 km/h	≥ 1000 km/h
Satellite combination	No	No	Completely
Structure	MIMO	Massive MIMO	Smart surface
Extended reality	No	Partial	Completely
Service level	Video	VR	Tactile

AI	No	Partial	Completely
Elevated frequency range	6 GHz	90 GHz	10 THz
THz transmission	No	Limited	Extensively

Table 2: Evaluation of 5G and 6G Technologies enabled by AI on eMBB, URLLC, as well as Mmtc

Technology	eMBB	URLLC	mMTC
5G	It paves the way for faster data transfers for mobile users, so they can view Ultra HD videos.	Its trustworthy and low-latency connection is vital for applications like remote surgery and industrial automation.	It facilitates extensive connectivity, allowing effective communication among several IoT devices.
AI-empowered 6G	It employs AI to optimize spectrum utilization and intelligent resource allocation.	Through the use of AI-driven optimization of networks and predictive maintenance functionalities, it improves URLLC by reducing latency and increasing reliability.	It improves ways of communicating and allocating resources so that devices may communicate with each other and networks can be divided up more efficiently.

Table 3: Comparison of Different Routing Techniques in 5G and 6G-Empowered Communication Networks

Ref. No.	Techniques	Advantages	Drawbacks	Performance Metrics
[24]	Optimal-CBR	It makes the sensor nodes more efficient by decreasing their power consumption, which in turn increases the network's lifetime.	It neglects the security considerations associated with data exchange via a multihop routing method.	Mean residual energy of nodes (@ 1200 rounds) = 0.49;No. of live nodes (@ 1000 rounds) = 278;The CH's energy loss (@ 200 rounds) = 0.13J;Packet Delivery Ratio (PDR) = 0.9;E2E delay = 11ms
[25]	A delay-aware QL-based routing (XiA)	It reduces delay effectively.	Requires integration of multiple ground-level APs to enhance performance.	No. of hops = 6; Delay = 0.000081s
[26]	MIMO based 5G balanced energy efficient routing	It achieved lower delay.	The network coverage was remained low.	Network coverage area (@ 1000 rounds) = 50; Delay (@ 600 rounds) = 0.1s
[27]	EADCRP-6G	It extends the life of the network and decreases its energy usage.	Delay and throughput were not satisfactory.	For 100 UAVs Energy consumption = 159mJ;Network lifetime = 4490 rounds; Throughput = 0.63Mbps; Mean delay = 6.88s
[28]	MRFO and DBN-based routing	Its network throughput and lifetime were both high.	There was a lot of energy used.	For 1000 nodes Energy consumption = 0.656; Network lifetime = 31200; Throughput = 0.918
[29]	Energy-aware intelligent increasing coverage region routing (CAIN)	It increases the network coverage and reduces storage overhead efficiently.	The mean number of messages reaching the CHs was low.	For 100 devices Mean communication delay = 2e-09s; Mean communication distance = 0.32m;Mean No. of messages reaching the CHs = 1.6;Mean storage overhead = 7 KB;

[30]	ENSING	It achieves high energy efficiency and PDR.	It has a high delay.	Mean UAV's battery energy (@ 120m distance for 20 UAVs) = 17500mAh; PDR (@ 180 min) = 96%; Throughput (@ 180 min) = 2.6 Mbps; Delay (@ 180 min) = 200ms
[31]	Efficient and trusted autonomous vehicle routing	It reduces energy consumption and computational cost effectively.	Latency and overhead were high. Also, PDR was low.	For 500 nodes PDR = 88%;Nodes overhead = 12%;Energy consumption = 0.13J;Latency = 3.2s;Computational cost = 10%
[32]	DQNLLRA	It reduced a mean queue utilization and achieved a robust load-balancing ability.	For optimum routing decisions, factors like network reliability, congestion and energy efficiency must be considered.	Path delay for 100 test flows = 90;Path maximization queue utilization for 100 test flows = 0.76;Path mean queue utilization for 100 test flows = 0.31
[33]	CEERP	It increased network lifetime and energy efficiency.	Routing overhead was high. Also, it needs to incorporate optimization schemes with more sophisticated data transfer.	Throughput = 90%; Network lifetime = 95%; Packet permission = 85%; Transmission speed = 92% Routing overhead = 95%; Energy consumption = 90%;
[34]	Efficient VR data transfer control using DQN	Improve data transfer by balancing network resources.	It does not consider other network factors like latency, throughput, etc.	Achievable data rate (@ 4000 rounds) = 5 bps/Hz
[35]	MCAIDeM	It offered key insights for real-time systems using network factors.	In future heterogeneous 6G networks, selecting optimal routes requires ML algorithms.	Throughput for 100 nodes = 25 Kbps; Delay for 100 nodes = 2.7ms;Energy consumption for 100 nodes = 110mAh
[36]	SP-LSTM with RL	Effectively balanced prediction and real-time response, improving network performance.	In real-time networks, a single RL agent may switch between policies, causing unstable routing decisions.	Training accuracy = 0.9822; Prediction accuracy = 0.99998; Mean squared error = 1.04132e-0.7.

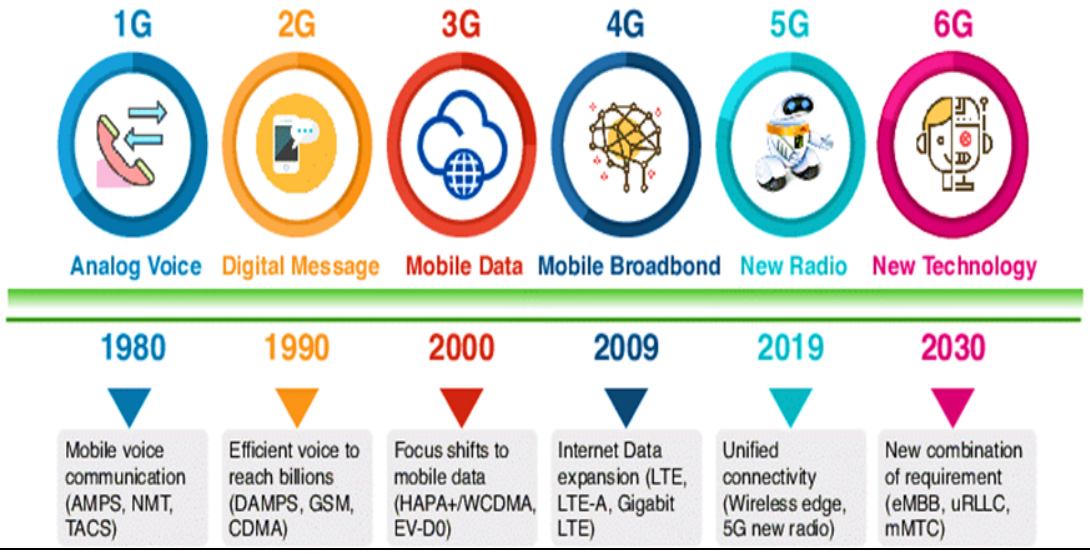


Figure 1: Evolution of Telecommunication Technologies

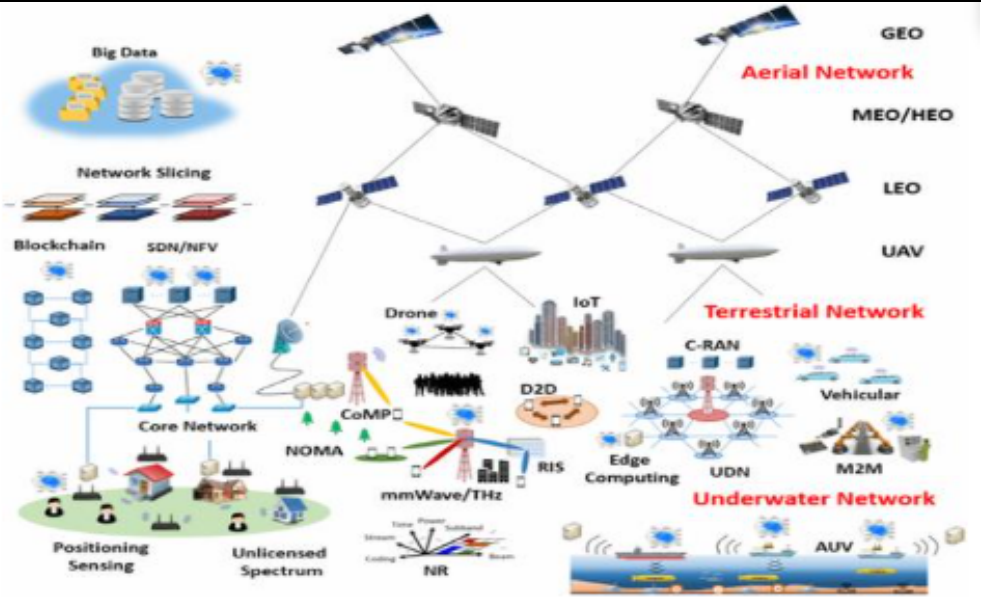


Figure 2: Design of 6G Mobile Technology

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RESEARCH ARTICLE

A Study on the Role of Job Portals in Streamlining the Recruitment Process: A Practical Perspective

Arjun[1]* and C.Sathish Kumar[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Assistant Professor (SG), School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Arjun
Second Year MBA Student, School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai, Tamil Nadu, India.

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ABSTRACT

This project report explores the role of job portals in streamlining the recruitment process at Durus Consulting Private Limited, a company specializing in providing tailored HR and business consulting solutions. As recruitment is a core function of the firm, the study investigates how online job portals such as Naukri, LinkedIn, and Indeed are utilized to meet the company's hiring needs and how these platforms enhance efficiency and outcomes.The findings reveal several positive impacts of job portals on Durus Consulting's recruitment operations. These platforms have enabled the firm to widen its talent pool, reduce time-to-hire, and improve candidate tracking through automation and advanced filters. Recruiters at Durus benefit from instant access to thousands of candidate profiles, enabling them to match specific job requirements more precisely. Additionally, job seekers gain improved visibility and access to opportunities within the firm, aligning well with its dynamic staffing needs.

Keywords: Automation, Healthcare, HR Analytics, Recruitment Process Outsourcing, Technology.

INTRODUCTION

The Human Resource (HR) consultancy industry is critical in shaping workforce strategies and supporting organizational development across various sectors. This industry encompasses firms that offer specialized services in recruitment, staffing, employee training, performance management, organizational development, payroll outsourcing, and strategic HR planning. Globalization, technological advancements, and the dynamic nature of work have significantly increased the demand for professional HR consulting services, especially among small and mid-sized enterprises (SMEs) and multinational corporations.

In India, the HR consultancy industry has witnessed steady growth over the last decade, fuelled by the expanding corporate landscape and a growing awareness of the importance of structured HR practices. Cities like Bengaluru, Mumbai, Delhi, Hyderabad, and Chennai have become hubs for HR consulting firms, catering to industries such as IT, manufacturing, retail, healthcare, BFSI, and more. The sector is segmented into various categories: executive search, recruitment process outsourcing (RPO), HR analytics, and talent management services. HR consultancies offer a cost-effective solution for companies looking to streamline their human capital operations without maintaining large internal HR departments. These firms bring industry-specific expertise, access to large talent databases, and strategic insights that help organizations improve employee engagement, reduce turnover, and enhance productivity.

However, the industry faces several challenges, including intense competition, pricing pressures, and the need to adapt to emerging technologies like AI and data analytics continuously. The rise of freelance platforms and internal HR tech solutions has created new market dynamics. Despite these challenges, the HR consultancy sector continues to grow, shifting towards offering end-to-end human capital solutions and becoming strategic partners in business transformation. In conclusion, HR consultancies are vital to the evolving business environment, offering scalable and flexible solutions to manage people effectively and align HR practices with broader organizational goals.

Need for the Study

The need for this study stems from the increasing significance of job portals in the recruitment process, particularly from the perspectives of both recruiters and job seekers. Platforms like Naukri, FoundIt (formerly Monster), and LinkedIn have transformed recruitment by providing recruiters access to a vast pool of candidates, while offering job seekers various opportunities. However, as these portals evolve, there is a growing need to explore how the features of these platforms influence candidate satisfaction and how they align with recruiters' needs. Understanding the impact of these portal features on candidate satisfaction is crucial for optimizing the recruitment process. As organizations strive to attract the best talent, the efficiency and effectiveness of job portals play a key role in successfully matching candidates with the correct positions. Job seekers prioritize different aspects of job portals, such as ease of use, interface design, responsiveness, and transparency in the hiring process. By examining these factors, recruiters can better tailor their strategies to meet candidates' Expectations. Additionally, the recruitment landscape is continually shifting, with job portals constantly enhancing their features to cater to both recruiters' and candidates' needs. Assessing how these features affect candidate satisfaction will provide valuable insights for improving recruitment outcomes. A satisfied candidate is more likely to actively engage in the hiring process, leading to more successful placements for recruiters and organizations.

This study aims to:

- Examine how job portal features influence candidate engagement, response time, and overall satisfaction.
- Evaluate the effectiveness of these portals in meeting the needs of both domestic and international recruitment.
- Investigate the evolution of job portal features and how they align with modern candidates' expectations.
- Understand the role of technology in shaping recruitment practices and enhancing candidate satisfaction.
- By conducting this research, companies will gain a deeper understanding of the factors contributing to a positive recruitment experience, enabling them to refine their hiring strategies. Moreover, job portal providers can use this feedback to enhance their platforms, ensuring they stay relevant and effective in meeting the demands of the ever-evolving recruitment industry.

Objectives of the Study

Primary Objective:

- To examine the practical role of job portals in streamlining the recruitment process at Durus Consulting Private Limited.

Secondary Objectives:

- To identify the advantages and limitations of job portals in recruitment.
- To evaluate the effectiveness of job portals in sourcing, shortlisting, and hiring suitable candidates.
- To understand recruiters' challenges while using job portals for talent acquisition.
- To assess the impact of job portals on recruitment timelines and hiring costs.
- To explore how job portals help find culturally and professionally compatible candidates.
- To suggest recommendations for improving the use of job portals as part of a hybrid recruitment strategy.

Scope of the Study

- **Job Portals Analyzed:** The research will focus on three major job portals—Naukri, FoundIt (formerly Monster), and LinkedIn—commonly used for domestic and international recruitment. The study will evaluate each platform's features, functionalities, and effectiveness from the viewpoints of both recruiters and candidates.
- **Recruitment Process:** The study will examine various stages of the recruitment process facilitated by these portals, including candidate sourcing, job matching, communication tools, and application tracking systems. It will explore how these aspects contribute to the efficiency and success of the hiring process.
- **Candidate Satisfaction:** The primary focus will be on understanding candidate satisfaction. The research will assess factors such as ease of use, interface design, transparency in job listings, recruiter responsiveness, and the accuracy of job recommendations. It will explore how these factors influence the candidate experience and overall portal satisfaction.
- **Recruiter Experience:** The study will also consider the experience of recruiters using these portals, looking at how well the platforms support candidate sourcing, time-to-hire, communication with candidates, and the tools available for filtering, screening, and shortlisting applicants.
- **Technological Advancements:** The research will investigate how technological innovations, such as AI-driven job matching, chatbots, automated responses, and personalized job recommendations, are incorporated into these portals and how they affect the experiences of both recruiters and candidates.
- **Domestic vs. International Recruitment:** The study will examine how job portals cater to domestic and international recruitment needs, analyzing their ability to address the unique challenges of recruiting for roles in various geographic locations and industry sectors.
- **Impact on Recruitment Outcomes:** The research will assess how the features of these portals correlate with recruitment outcomes, such as the quality of candidates, time-to-hire, and success in placements, providing a clearer picture of their effectiveness in achieving hiring goals.
- **Recommendations for Enhancement:** Based on the findings, the study will offer recommendations for improving the features and functionalities of job portals. The aim is to enhance the user experience for both recruiters and candidates, streamline the recruitment process, and enhance overall hiring outcomes.

Limitations of the Study

- **Geographic Scope:** The study primarily focuses on job portals like Naukri, FoundIt, and LinkedIn, which may be more prevalent in specific geographic regions, such as India and the United States. As a result, the findings may not fully apply to the other areas with different recruitment trends and practices.
- **Platform Variability:** Job portals constantly evolve, with features and functionalities subject to change. The study may be limited because the research is based on specific versions of these portals, and future updates or modifications to these platforms may not be captured.
- **Bias in Participant Feedback:** The study relies on feedback from both candidates and recruiters, which personal biases and individual experiences may influence. These subjective opinions could affect the objectivity and generalizability of the findings.
- **Sample Size:** The study may be constrained by the number of participants involved, meaning the sample size may not be large enough to represent the broader population of job seekers and recruiters using these portals. This could limit the robustness of the conclusions drawn.

- **Availability of Data:** The study depends on publicly accessible data and user-reported experiences, which may not capture all the features or provide a comprehensive view of the portals. Some internal or proprietary data from job portal providers may be unavailable for analysis.
- **Feature Coverage:** While the study examines key features of the job portals, it may not be able to explore every detail or the full range of advanced tools offered by these platforms, which could affect the comprehensiveness of the analysis.
- **Recruitment Context:** The study focuses on general recruitment practices but may not fully address the specific needs of particular industries or highly specialized roles, where job portal features may have a different impact.
- **Time Constraints:** The research process may be limited by time constraints, which could restrict the extent of data collection, analysis, and exploration of certain portal features or recruitment outcomes.
- **Impact of Evolving Technologies:** The fast-paced development of recruitment technologies, such as AI-driven tools and automation, could affect the study's ability to account for all relevant technological advancements and their influence on candidate satisfaction and recruitment effectiveness.

RESEARCH METHODOLOGY

Research Design

This study adopts a **descriptive research design** to explore and analyse the role of job portals in enhancing the recruitment process. Descriptive research is appropriate as it helps capture current recruitment practices, portal features, and user experiences without altering variables.

Research Approach

The research follows a **mixed-method approach**, combining both qualitative and quantitative data:

- **Qualitative** insights were drawn from observations and user comments.
- **Quantitative** data were collected through structured surveys administered to the general public.

Data Collection Methods

- **Primary Data**
  Collected using a structured questionnaire distributed to a general audience. Questions focused on perceptions, usability, satisfaction, and effectiveness of job portals such as Naukri, LinkedIn, and FoundIt.
- **Secondary Data**
  Obtained knowledge of academic journals, company reports, job portal whitepapers, and HR industry studies related to digital recruitment.

Sampling Method

A **convenience sampling technique** was used, targeting individuals from the general publicwith experience using job portals for job search or hiring purposes.

Sample Size

The study was conducted with **123 respondents**, collected through an online questionnaire circulated among the general public.

Research Instrument

A **structured questionnaire** was designed, comprising **closed-ended (Likert scale, multiple-choice)** and **open-ended questions**. These evaluated user satisfaction, job portal efficiency, feature preferences, and common challenges encountered.

Limitations of the Methodology

- The study relies on self-reported data, which may include biases.
- Since the sampling was based on convenience and limited to 123 individuals, the findings may not be fully generalizable across industries.
- The dynamic nature of job portals may result in platform features evolving beyond the scope of this research.

DATA ANALYSIS FINDINGS AND INTERPRETATIONS

CORRELATION ANALYSIS

Correlation Analysis

- Most correlations were weak and non-significant, implying low interdependence among the variables.
- One significant correlation at 0.05 level: Clarity of job descriptions vs. time-saving ($r = -0.181$, $p < .05$).
- One significant correlation at 0.01 level: Authenticity of listings vs. Overall satisfaction ($r = 0.275$, $p = .002$).

Inference

The Pearson correlation values among most items are **weak and not statistically significant**, except:

- A **negative correlation** between job description clarity and time-saving ($r = -0.181$, $p < .05$)
- A **positive correlation** between listing authenticity and overall satisfaction ($r = 0.275$, $p < .01$)

Inference:

The weak correlations suggest that respondents perceive each aspect of job portals as relatively independent. The only meaningful positive correlation implies that trust in job listings contributes significantly to overall satisfaction. Conversely, clarity of descriptions may not necessarily equate to efficiency.

FINDINGS

Positive User Perception of Job Portals:

Most users experienced portal-based recruitment as effortless to operate and search through, with the added benefit of time savings relative to conventional hiring approaches. The users rated the portal's ease of use with the highest mean score of 3.86.

Moderate Candidate Satisfaction:

Candidates demonstrated slightly lower overall satisfaction toward job portals with an average score of 3.53, indicating that portals succeed at handling functional elements but lack completely satisfying experiences.

Trust and Authenticity:

Job seekers' trust in authentic job listings directly influenced their satisfaction levels. Users moderately trusted job listings but presented doubts about outdated or fake profiles.

Challenges Identified

- High volume of irrelevant applications due to generic postings.
- The evaluation process for assessing soft skills and cultural compatibility remains challenging when completed electronically.
- The excessive use of algorithms in matching operations might result in valid candidates being ignored.
- Underrepresentation of candidates from tier-2 cities and rural areas.

Weak Internal Consistency:

According to its Cronbach's Alpha value (-0.106), the surveyed user experiences have low connectivity between factors, possibly representing separate dimensions instead of one "portal satisfaction" composite.

Multiple Recruitment Dimensions
Factor analysis showed five potential dimensions within user experience, but overlapping constructs made it hard to distinguish the separate constructs, including usability, trust, convenience, relevance, and satisfaction.

Employment Status Influence:
Students who also worked as freelancers demonstrated higher optimism regarding increased hiring opportunities from portals than unemployed workers and those retired from work.

Suggestions and Recommendations
Improve Candidate Matching Algorithms:
AI filters running on portals need improvements so candidates are matched based on their expertise, in addition to eliminating unneeded applications.

Integrate Soft Skills Assessment Tools:
Portal users must utilize video interviews, psychometric assessments, and soft skill evaluation plugins because these tools help better determine cultural compatibility and communication abilities.

Regular Database Cleaning:
Resumes and profile verifications must have stronger checks that remove outdated or fake applications to increase trustworthiness and authenticity.

Tier-2 and Rural Outreach:
The company should implement special initiatives such as mobile-accessible websites and local language support to attract candidates outside metropolitan areas to the employment platforms.

Personalized User Experiences:
A recommendation system improvement will occur through customized job suggestions that use candidate history and preferences to boost user satisfaction and engagement.

Hybrid Recruitment Approach:
Durus Consulting and other organizations should integrate job portal usage with established sourcing approaches, such as employee referrals, college recruiting, and specialized hiring of senior talent through headhunting techniques.

Continuous Feedback Loops:
Every job portal should include post-transaction candidate and recruiter feedback that helps the system evolve its functionalities and response time.

Enhance Mobile App Functionality:
The mobile-first approach demands portal developers enhance the application design and implement higher-speed functions and offline application capabilities.

CONCLUSION

Through job portals, organizations can enhance their recruitment operations by extending their reach to new candidates and achieving faster recruitment results. The job search portals Naukri, LinkedIn, and FoundIt provide users with ease of use, real-time updates, and wide-ranging candidate accessibility. Applications through portals face problems with unqualified candidates. At the same time, evaluating soft skills remains difficult, and rural job seekers struggle to connect with opportunities, demonstrating that job portals work better with additional recruitment

strategies. Durus Consulting Private Limited and similar organizations must combine their digital tool capabilities with personalized recruitment methods to enhance hiring quality and experience. Job portals reach their full potential when recruiters and platform providers dedicate efforts toward establishing trust among users and personalized features that promote inclusivity. The evolution of technology requires recruitment strategies to update their approaches to maintain effectiveness in finding top candidates.

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Table 1: Correlations

		Job portals are easy to use and navigate	I find relevant job opportunities based on my profile	Job descriptions on portals are clear and informative	I receive timely updates or notifications
Job portals are easy to use and navigate	Pearson Correlation	1	.104	.049	.083
	Sig. (2-tailed)		.252	.589	.362
	N	123	123	123	123
I find relevant job opportunities based on my profile	Pearson Correlation	.104	1	.094	-.101
	Sig. (2-tailed)	.252		.302	.266
	N	123	123	123	123
Job descriptions on portals are clear and informative	Pearson Correlation	.049	.094	1	-.107
	Sig. (2-tailed)	.589	.302		.239
	N	123	123	123	123
I receive timely updates or notifications	Pearson Correlation	.083	-.101	-.107	1
	Sig. (2-tailed)	.362	.266	.239	
	N	123	123	123	123
Job portals save time compared to traditional methods	Pearson Correlation	.043	-.062	-.181*	-.027
	Sig. (2-tailed)	.634	.493	.046	.769
	N	123	123	123	123
I trust the authenticity of job listings	Pearson Correlation	-.063	.089	-.114	.067
	Sig. (2-tailed)	.490	.327	.209	.463

	N	123	123	123	123
Recommendations match my skills	Pearson Correlation	.144	-.096	-.008	-.007
	Sig. (2-tailed)	.112	.289	.929	.942
	N	123	123	123	123
Applying for jobs is easy via portals	Pearson Correlation	-.085	.143	-.090	-.150
	Sig. (2-tailed)	.347	.115	.322	.099
	N	123	123	123	123
Job portals improve my chances of getting hired	Pearson Correlation	-.129	-.002	.086	-.089
	Sig. (2-tailed)	.154	.985	.346	.329
	N	123	123	123	123
Overall satisfaction with job portals	Pearson Correlation	-.023	.122	-.137	-.025
	Sig. (2-tailed)	.798	.180	.131	.787
	N	123	123	123	123

Table 2: Correlations

		Job portals save time compared to traditional methods	I trust the authenticity of job listings	Recommendations match my skills	Applying for jobs is easy via portals
Job portals are easy to use and navigate	Pearson Correlation	.043	-.063	.144	-.085
	Sig. (2-tailed)	.634	.490	.112	.347
	N	123	123	123	123
I find relevant job opportunities based on my profile	Pearson Correlation	-.062	.089	-.096	.143
	Sig. (2-tailed)	.493	.327	.289	.115
	N	123	123	123	123
Job descriptions on portals are clear and informative	Pearson Correlation	-.181*	-.114	-.008	-.090
	Sig. (2-tailed)	.046	.209	.929	.322
	N	123	123	123	123
I receive timely updates or notifications	Pearson Correlation	-.027	.067	-.007	-.150
	Sig. (2-tailed)	.769	.463	.942	.099
	N	123	123	123	123
Job portals save time compared to traditional methods	Pearson Correlation	1	-.032	.035	-.118
	Sig. (2-tailed)		.729	.703	.192
	N	123	123	123	123
I trust the authenticity of job listings	Pearson Correlation	-.032	1	-.069	.028
	Sig. (2-tailed)	.729		.448	.755
	N	123	123	123	123
Recommendations match my skills	Pearson Correlation	.035	-.069	1	-.002
	Sig. (2-tailed)	.703	.448		.985
	N	123	123	123	123
Applying for jobs is easy via portals	Pearson Correlation	-.118	.028	-.002	1

	Sig. (2-tailed)	.192	.755	.985	
	N	123	123	123	123
Job portals improve my chances of getting hired	Pearson Correlation	-.041	-.119	.015	-.078
	Sig. (2-tailed)	.655	.189	.865	.391
	N	123	123	123	123
Overall satisfaction with job portals	Pearson Correlation	.026	.275**	-.039	.165
	Sig. (2-tailed)	.774	.002	.669	.068
	N	123	123	123	123

Table 3: Correlations

		Job portals improve my chances of getting hired	Overall satisfaction with job portals
Job portals are easy to use and navigate	Pearson Correlation	-.129	-.023
	Sig. (2-tailed)	.154	.798
	N	123	123
I find relevant job opportunities based on my profile	Pearson Correlation	-.002	.122
	Sig. (2-tailed)	.985	.180
	N	123	123
Job descriptions on portals are clear and informative	Pearson Correlation	.086	-.137
	Sig. (2-tailed)	.346	.131
	N	123	123
I receive timely updates or notifications	Pearson Correlation	-.089	-.025
	Sig. (2-tailed)	.329	.787
	N	123	123
Job portals save time compared to traditional methods	Pearson Correlation	-.041	.026
	Sig. (2-tailed)	.655	.774
	N	123	123
I trust the authenticity of job listings	Pearson Correlation	-.119	.275**
	Sig. (2-tailed)	.189	.002
	N	123	123
Recommendations match my skills	Pearson Correlation	.015	-.039
	Sig. (2-tailed)	.865	.669
	N	123	123
Applying for jobs is easy via portals	Pearson Correlation	-.078	.165
	Sig. (2-tailed)	.391	.068
	N	123	123
Job portals improve my chances of getting hired	Pearson Correlation	1	-.006

	Sig. (2-tailed)		.951
	N	123	123
Overall satisfaction with job portals	Pearson Correlation	-.006	1
	Sig. (2-tailed)	.951	
	N	123	123

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

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RESEARCH ARTICLE

An Optimized Machine Learning Model for Predicting Biogas Production from Anaerobic Digestion: A Literature Survey

R. Anitha[1]* and K. Santhi[2]

[1]Assistant Professor, Department of Computer Science, Dr. N.G.P Arts and Science College, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Professor, Department of Computer Science, Dr. N.G.P Arts and Science College, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
R. Anitha
Assistant Professor,
Department of Computer Science,
Dr. N.G.P Arts and Science College,
(Affiliated to Bharathiar University),
Coimbatore, Tamil Nadu, India.
E.Mail: anithangparts@gmail.com

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ABSTRACT

Anaerobic digestion(AD) based biogas generation offers a sustainable option to convert waste into energy, but it is still extremely challenging to adequately model its nonlinear, multi-factorial processes. Research on biogas generation, a promising renewable energy source made from organic waste, has increased as a result of the global trend towards sustainable energy options. Predicting biogas yield accurately is essential for streamlining production procedures and enhancing operational effectiveness. This paper includes a thorough evaluation of the literature on the application of optimized machine learning models for biogas output prediction. Various ML models like ensemble learning approaches, DT, RF, SVM, ANN, gradient boosting machines (GBM), and hybridized frameworks integrating evolutionary algorithms and deep learning architectures are compared and analysed with respect to prediction accuracy, resilience, and dataset adaptation. Methods for feature selection, data preparation, and model optimization strategies including hybrid models and hyperparameter adjusting are all given special consideration. The lack of consistent datasets and the requirement for interpretable models are two of the major issues and research gaps highlighted in the survey. Lastly, future research avenues that focus on combining real-time prediction systems, explainable AI, and sophisticated optimization techniques are presented. This review is a fundamental resource for researchers and practitioners aiming to enhance biogas production through intelligent data-driven models.

Keywords: Anaerobic digestion; Machine learning; Artificial Intelligence; Metaheuristic algorithms; Deep learning

INTRODUCTION

An increasing demand for renewable energy bases and the need to manage organic waste sustainably, has catalyzed significant interest in biogas production through AD processes.Biogas, mostly consisting of methane (CH_4) and carbon dioxide (CO_2), is produced through the microbial decomposition of organic materials in anaerobic environments and acts as a feasible substitute for fossil fuels(Karellas *et al*.,2010). Despite its environmental and economic benefits, the efficiency of biogas production is highly dependent on numerous factors such as substrate type, pH, loading rate, retention duration, temperature, and microbial community dynamics, complicate the forecast and optimization of biogas yield (Holm-Nielsen *et al*., 2009).Traditional mathematical and kinetic modeling approaches are useful but, often fall short in capturing the nonlinear, multivariate, and dynamic nature of AD systems (Batstone *et al*., 2002). In recent years, the advent of MLtechniques has offered an alternative for modeling complex bioprocesses. ML algorithms may discern trends from past data without necessitating deep mechanical comprehension, facilitating precise predictions of biogas yield across diverse operational situations. Commonly used models include ANN, SVM, DT, RF, and ensemble learning frameworks (Almomani2020; Wu 2021).Predictive performance and model reliability are further improved by optimizing ML models using strategies including feature selection, hyperparameter tweaking, and hybrid modelling. Furthermore, recent research has investigated combining DL architectures like CNN and LSTM and evolutionary algorithms like GA, PSO to represent temporal relationships and enhance generalizability(Ling 2024; Zaied 2020). However, benchmarking and real-world implementation are limited by the absence of uniformity in data gathering, preprocessing, and model evaluation.

The most recent research on predicting thebiogas production from AD is presented in this literature survey, which also addresses the difficulties and future directions. The contributions of this study are

- The concepts of AD systems, data preprocessing methods, optimization techniques and evaluation measures are elaborated.
- It offers a comprehensive overview of the present state-of-the-art in optimized ML approaches for biogas prediction are listed.
- The various challenges and issues involved in creating an AD system using ML approaches are presented.
- The research gap is noted, and recommendations are made for further study to enhance the incorporation of intelligible ML-based solutions for biogas production systems.

The paper is structured as, Section II delivers the concepts of AD systems, data preprocessing methods and evaluation measures. A thorough summary of the most recent ML techniques for biogas prediction is provided in Section III.Section IV shows the challenges and issues related to AD system using ML approaches. The conclusion and future directions for improving biogas production are presented in Section V.

Anaerobic Digestion System Preliminaries

This section describes about the basics of AD system, data preprocessing methods, optimization techniques and evaluation metrics. AD is a complex, multistage sustainable biological process that converts organic waste into biogas and nutrient-rich digestate. This process happens in engineered digesters under controlled environmental and operational conditions and serves bothenergy recovery as well as garbage treatment. AD is a proven method for producing renewable energy and treating organic waste.The process consists of breaking down the biodegradable material with the help of microorganisms in the absence of oxygen, that produces biogas and digestate. The efficiency and stability of an AD system depend on several preliminary factors, including feedstock characteristics, inoculum selection, process parameters, and system design. The efficiency and stability of AD systems are ruled by several interdependent operational parameters like pH, temperature, alkalinity, loading amount and retention duration. The other parameters like substrate characteristics, substrate pretreatment, microbial community dynamics etc. Understanding these fundamentals is essential for designing effective AD systems for waste management and renewable energy production. Figure 1 shows the process of anaerobic digestion.

The data preprocessing methods for biogas prediction consists of

Data cleaning: Missing value imputation, KNN, regression-based imputation, interpolation, outliner detection and removal processes.
Feature engineering: Involves feature selection and feature extraction. Removal of irrelevant or reduction features. Derive new informative features like substrate volatility, temperature fluctuation index. Aggregate features like pH and temperature variance.
Data transformation: Involves min-max normalization and standardization
Data encoding: Perform one-hot encoding for nominal variables like substrate types.
Time series formatting: For dynamic models that lay features, rolling windows features, and resampling.
Dimensionality reduction: Applied when too many features like principal component analysis and use of autoencoders for nonlinear feature compression.
Data balancing: If classification is involved, conditions like fault detection in AD systems (Ling *et al*., 2024).

Optimization strategies in ML for Biogas Prediction

Optimizing ML models for biogas prediction involves fine-tuning hyperparameters, selecting relevant features, and enhancing model architecture to improve accuracy, robustness, and generalization. Due to the nonlinear and multivariate nature of AD processes, conventional parameter tuning may not yield optimal results. Instead, optimization techniques like evolutionary algorithms, Bayesian approaches are employed to automate and refine ML performance. The key optimization strategies used in biogas modeling are given below

- Hyperparameter optimization influences the learning behaviour of ML algorithms, including CNN architecture, tree depth in decision trees, and learning rate in GBMs. Manual tuning or grid search is computationally expensive and suboptimal in high-dimensional search spaces. Hence, advanced optimization techniques like grid search and random search are commonly used for small search spaces. While random search samples combinations randomly, grid search assesses combinations methodically, providing greater efficiency in huge spaces (Bergstra and Bengio, 2012).A probabilistic model-based optimization technique called Bayesian optimization builds a surrogate function to represent the objective function and chooses hyperparameters that are expected to enhance performance. It has been effectively used in tuning ANN and SVR models for biogas yield prediction (Ge *et al*., 2024).Cross validation-based tuning is involved in most optimization frameworks embed k-fold cross-validation to ensure that tuned parameters generalize across unseen data.
- Feature selection and dimensionality optimization involves high-dimensional data from biogas systems (e.g., real-time sensor inputs) may include irrelevant or redundant features. Feature selection improves learning efficiency and avoids overfitting. Feature selection methods like wrapper method uses the model performance (e.g., R^2, RMSE) as feedback for selecting feature subsets. Recursive feature exclusionis a wrapper technique in ANN optimization (Wang *et al*., 2021).Embedded methods like random forests and Lasso regression perform internal feature selection during model training, offering built-in dimensionality control. Evolutionary algorithms like GA, PSO, and ACO(Beltramo *et al*., 2019) are widely applied to simultaneously select features and optimize model parameters. Zaied *et al*., (2020) came up with a PSO-ANN hybrid outperformed standalone ANN models for methane prediction from co-digestion experiments.
- Model structure optimization consists of a deep learning and ANN-based models with structural parameters like hidden layer count, neurons count, initiation functions, and dropout rates. Neuroevolution approach uses GA to evolve ANN structures and connection weights, adapting model architecture dynamically based on performance (Stanley *et al*., 2002).
- Ensemble and hybrid optimization approaches consists of combining multiple models (ensembles) or integrating ML with optimization algorithms to show superior performance inAD process. It uses stacked generalization to train multiple base learners like ANN, RF and uses a meta-learner to combine their outputs, reducing bias and variance. Other hybrid models like GA-ANN, Bayesian and random forest. These hybridized models often yield better predictive accuracy and robustness, particularly in handling heterogeneous and sparse datasets from biogas production systems.

Evaluating the performance of ML models in predicting biogas production is critical to ensure accuracy, reliability, and generalizability of the predictions. Since biogas yield forecasting is typically formulated as a regression task (predicting continuous output such as methane volume or biogas production rate), the choice of evaluation metrics focuses on error quantification, model stability, and comparative performance. The various evaluation metrics involved are RMSE is used in regression tasks due to its sensitivity to huge mistakes. Without taking into account the direction of the errors, MAE calculates the average size of forecast errors. Compared to RMSE, it is less susceptible to outliers. The percentage of the dependent variable's variance that can be predicted from the independent variables is shown by the coefficient of determination, or R2 score. MAPE expresses prediction accuracy percentage, allowing for better interpretability across datasets with different scales.NSE is frequently used in hydrology and is being utilized more and more in biogas modeling to evaluate the model's predictive accuracy in relation to the mean of observed data. Cross validation error is for small or imbalanced datasets, k-fold cross-validation helps evaluate generalization by dividing data into k subsets, using each subset for validation once(Gupta *et al*., 2023).

Machine learning approaches for Biogas Prediction

This section discusses ML based approaches for biogas prediction. The findings, experimental outcomes, and different methodologies are tabulated. The comparison of ML algorithms for biogas prediction is shown in Table 1.

Challenges and Issues in AD System

ML based biogas generation forecasting in AD systems holds promise for significantly improving operational effectiveness, optimizing energy production, and cutting expenses.However, in order to increase the consistency and usefulness of AI models in AD, a number of factors need be taken into account. The key challenges are listed below

1. Data related challenges like limited or noisy data, high variability in input feedstock, imbalanced datasets and lack of standardized data.
2. Model related challenges like overfitting, black-box nature of ML models, trade-off between accuracy and computational cost and difficulty in capturing the temporal dynamics.
3. Process related challenges like nonlinearity and dynamic behaviour of AD processes, uncertainty in biological systems, lay effects and delayed responses.
4. Practical implementation issues like scalability of models, real-time prediction limitations and integration with existing systems.
5. Domain specific challenges like lack of universal input features and validation with real-world data.

It is evident from the literature that the co-digestion of cow manure and mango flesh to create useful biogas has not been thoroughly studied. The incomplete utilization of the substrate in the production of biogas is another problem that has been noted. The majority of the literature lacks information on digestion process optimization. It is necessary to investigate the impact of variables such as temperature, pressure, pH, C/N fraction, and retention period. The following is a list of the research gaps identified in this study

i. Most ML models are purely data-driven and lack integration with biochemical/kinetic models. There is a limited integration of domain knowledge with ML models.
ii. Many models assume static conditions, but biogas production is highly dynamic due to feedstock variability, microbial shifts, and process instabilities. Handling dynamic and non-stationary biogas data is challenging.
iii. Complex models like deep learning, ensemble methods lack interpretability, limiting industrial adoption. The trust in ML predictions and other hybrid prediction models to examine the biogas production
iv. Most models are trained on specific feedstocks (e.g., cow manure) or lab-scale data but fail when applied to new substrates or full-scale plants. There is a need for a generalizability across different feedstock and scales.
v. Most models use only operational data (e.g., pH, temperature) but ignore microbial community data, spectroscopy, or imaging. There is a limited use of multimodal data fusion.

In order to optimize real-time predictive models, integrate dynamic and decentralized biogas plants, forecast emissions, and address the lack of standard data for feedstock inconsistency, the application of ML techniques must be justified. Effectively biogas production will profit from addressing in these research gaps.

CONCLUSION

This literature survey critically examined the development and optimization of ML models for predicting biogas production from AD processes. The review highlights the increasing shift from conventional kinetic and statistical models to data-driven approaches that can capture the complex, nonlinear relationships between operational parameters and biogas yield. A wide range of ML models like ANN, Support Vector Regression (SVR), RF, and recently, deep learning architectures like LSTM have demonstrated superior prediction performance. The integration of optimization techniques such as GA, PSO, and Bayesian methods further enhances model accuracy and robustness. Though, several critical research gaps continue, particularly in the areas of dataset availability, interpretability, model generalization, and real-time deployment. Moreover, there is a lack of standardized practices in data preprocessing, feature engineering, and evaluation methodologies, which impedes model comparison and benchmarking. The future research should focus on building hybrid mechanistic ML models that are scalable, interpretable, and real-time ML frameworks supported by high-quality, full-scale operational datasets. Furthermore, the integration of ML models with process optimization and control systems will be essential for developing smart, adaptive anaerobic digesters capable of maximizing biogas output under varying operational conditions In conclusion, ML offers a powerful toolkit for enhancing the efficiency and sustainability of biogas production. Realizing its full potential requires interdisciplinary collaboration, standardized protocols, and a shift toward explainable, robust, and deployment-ready solutions.

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Table 1 Comparison of ML algorithms for Biogas prediction

Reference	ML Model	Input Features	Optimization Method	Evaluation Metrics	Key Findings
Yildirim and Ozkaya (2023)	RF, ANN, KNN, SVR, and XGBoost	raw material variability, temperature fluctuations and pH	Manual tuning	R^2, RMSE	RF achieved best prediction, with R^2 = 0.9242
Sun *et al*., (2024)	ANN	Biomass, pH, solids, load amount, retention duration, temperature, and reactor volume	COA,MVO,LCA,ERWCA,SFS,TLBO	R^2, RMSEand MAE	R^2 = 0.9314 and 0.9302, RMSE = 0.1969 and 0.24925, and MAE = 0.1307 and 0.19591.
Shen *et al*., (2024)	LSTM, CNN-LSTM, and DA-LSTM	Time-series operational data	Manual tuning	R^2, RMSE	DA-LSTM R^2 = 0.87 and RMSE=1048.14
Imamoglu(2024)	RF, ANN, DT,	microalgae processes	GA	R^2	R^2=0.991

Zarabian (2024)	DNN	Homogeneous	GA	-	-
Madhavan *et al.*,(2021)	ANN,	type of solid waste,reactor configuration,temperature, OLR, HRT, pH,C/N ratio and total solids (TS) %	Manual tuning	R^2	R^2= 0.553

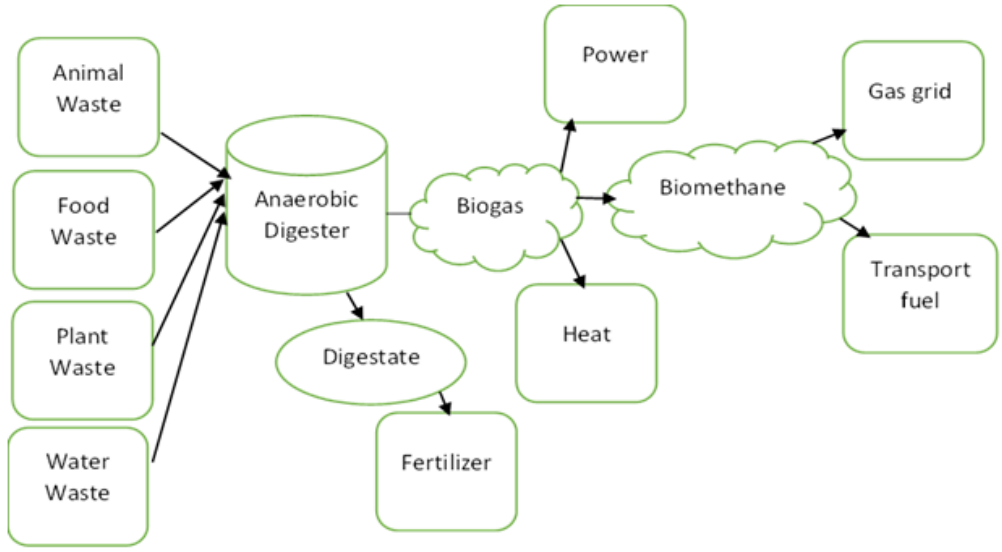


Figure 1: Process of Anaerobic Digestion

RESEARCH ARTICLE

Explainable AI for Interpretable Diagnosis of Plant Diseases from Leaf Images

Shaheen Layaq[1]* and Arpitha Pakalapati[2]

[1]Lecturer, Department of Computer Science, Singareni Collieries Women's Degree and PG College, Kothagudem, (Affiliated to Kakatiya University), Telangana, India.
[2]Lecturer in Commerce, TGSWRDC (W) Vikarabad, (Affiliated to Osmania University, Hyderabad), Telangana, India.

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***Address for Correspondence**
Shaheen Layaq
Lecturer, Department of Computer Science,
Singareni Collieries Women's Degree and PG College, Kothagudem,
(Affiliated to Kakatiya University), Telangana, India.
E.Mail: mdshaheen1905@gmail.com

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ABSTRACT

Plant diseases are rising a complex challenges across ecosystem, society and agriculture. Plant diseases occur in many types mainly caused by viruses, bacteria, fungi, a biotic factor, or nematodes. If plant is affected with disease it has to undergo diagnosing. Plant disease can be overcome by prevention, monitoring, and early detection. Artificial Intelligence and its components are playing an important role in early detection and prevention. As it is most important issue many researchers are working on it. But, most of the earlier methods lack in accuracy and clear visuals. This paper discussed about different plant diseases, its earlier work, plant disease datasets or repository, about preprocessing, Convolutional Neural Network model, performance evaluation and plant Disease visualization using Explainable Artificial Intelligence. By using Deep learning Convolutional Neural Network method for classification and Explainable Artificial Intelligence for visualization the performance, accuracy and visualization improves.

Keywords: Plant Diseases, Leaf images, Artificial Intelligence, Explainable AI, CNN

INTRODUCTION

If a plant not able to perform its maximum level due to living (fungi, bacteria, viruses, viroids, phytoplasmas, nematodes, and parasitic plants)or nonliving(environmental or physiological stress) factors then it is affected by plant disease. The following Table 1 shows different types of plant diseases, factor, their causes and symptoms examples. By understanding the different types of plant diseases, factor, their causes and symptoms examples it helps in accurate diagnosis of plant disease. The Figure 1 shows the diagram of healthy leaf with spotless and a diseased leaf with spots, color faded and malformed tissues. The plant disease effects ecosystem, society and

agriculture in many ways. The Table 2 gives a detail list of impact of plant disease. Impact of plant disease can be overcome by prevention, monitoring, and early detection. Artificial Intelligence and its components like machine learning (ML), deep learning (DL) and Convolutional Neural Network (CNN) model are playing an important role in early detection and prevention. As it is most important issue many researchers are working on it. The detailed framework of this paper can be explained using Figure 2.

Literature Survey

As plant disease are creating a great impact on society many researchers are working on it for early detection and prevention. The following Table 3 presents the earlier work related to plant disease with its technology used, advantages and disadvantages.

Steps for Early Detection of Plant Disease

For early detection of plant disease, the following steps shown in Figure 3 extraction, preprocessing, CNN classification, performance evaluation and visualization using XAI has to be followed. The detailed explanation of each step is given below.

Extraction of Plant Diseases Repository or Data set

There are many plant disease data sets which can be used in identification of plant disease. The following are the most popular plant disease dataset with their description.

Plant Village Dataset: It is basically used for classification and hold PyTorch/TensorFlow integration. It has about 50,000 leaf images, disease classes of about 39approximately and healthy samples. It is access through GitHub repository and Mendeley.

Plant Doc Dataset: It is mostly used for classification and object identification. It consists of 2.6K images, 13 species and 17 diseases. It is access through GitHub repository.

DiaMOS Plant: It is basically used for field diagnosis. It consists of 3.5Kimages and 4 diseases on pear. It is access through Zenodo DOI repository.

Rice Leaf Diseases (UCI): It is used for rice leaf classification. It consists of 120 images and 3 diseases classes. It is access through UCI Machine Learning Repository.

Paddy Doctor: it is used for paddy disease classification. It consists of16.2K images and 13 paddy classes. It is access through ArXiv/Zenodo.

Preprocessing and CNN Model for Plant Disease Dataset

A CNN model is one type of DLmodel which used to process and examine visual data. CNNs mainly used for classification, facial identification and object detection. CNN model [12] consists of five core components such as Input layer, convolutional layers, activation functions, pooling layers and fully connected layers**.** The convolutional layers, activation functions and pooling layers steps comes under preprocessing. The details of each layer are explained below.

Input layer: This layer extracts the data set from the mentioned above datasets which will be in the form of raw dataset.

Convolutional Layers: After extracting the required dataset, the wanted features are been retrieved by using this layer. It is one of the preprocessing steps. This layer uses filters for extraction of required features from raw dataset. By extracting features the task of debugging, interpreting and defining the structure improves.

Activation Functions: The activation functions help to learn nonlinear relationships, allows back propagations, selective preprocessing, improves computational speed and stable gradients are maintained. The CNN model uses different activation functions likeReLU, PReLU, ELU, Sigmoid, Tanh, Softmax, Swish and GELU. The most commonly used activation function among them is Rectified Linear Unit (ReLU). It's also one of the preprocessing tasks.

Pooling Layers: The height and width of the extracted feature are reduced. The pooling layer uses down sampling techniques for reduction. By this over fitting of data is reduced and in turn the complexity is reduced. It's also one of the preprocessing tasks.

Fully Connected Layers: After extracting and performing various preprocessing the final classification is done to produce the output.

Performance Evaluation: The performance evaluation methods are used to evaluate the accuracy and effectiveness of the model. The ML and DL supports following performance evaluation methods for classification models which are shown in Table 4.

Visualization using XAI

Explainable AI (XAI) is used by deep learning CNN model to obtain more clarity and understanding of the problem. XAI helps to understand the plant disease more clearly by producing different and clear images which helps to interpret it without any confusion. The following Figure 4 shows original and XAI (Segmented Image Heat Map) images.

CONCLUSION AND FUTURE WORK

Plant diseases are raising a complex challenge across ecosystem, society and agriculture. Plant diseases occur in many types mainly caused by viruses, bacteria, fungi, a biotic factor, or nematodes. If plant is affected with disease it has to undergo diagnosing. Plant disease can be overcome by prevention, monitoring, and early detection. Artificial Intelligence and its components are playing a important role in early detection and prevention. As it is most important issue many researchers are working on it. In this paper discussion about different plant diseases, its impact, its earlier work, plant disease datasets or repository, about preprocessing, CNN model, performance evaluation and plant Disease visualization using XAI was done. By using Deep learning CNN method for classification and XAI for visualization the performance, accuracy and visualization improves. In future work a particular dataset of plant disease has to be considered and implementation has to be done to check the accuracy.

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Table: 1.Disease Type, FactorCauses and SymptomsExamples of Plant Diseases

Disease Type	Factor	Causes	Symptoms Example
Leaf spot	Living factor	Fungi/Bacteria	Spots with halos
Powdery mildew	Living factor	Fungi	White dusty coating
Downy mildew	Living factor	Oomycetes (fungi-like)	Fuzzy underside growth
Rust	Living factor	Fungi	Orange pustules
Smut	Living factor	Fungi	Black spore masses
Wilt	Living factor	Fungi	Vascular browning and death
Blight	Living factor	Fungi	Rapid tissue collapse
Bacterial spots	Living factor	Bacteria	Water-soaked lesions
Fire blight	Living factor	Bacteria	Necrosis and ooze
Crown gall	Living factor	Bacteria	Tumorous growths
Viral mosaic	Living factor	Virus	Mottled, stunted leaves
Nematode spots	Living factor	Nematodes	Angular lesions
Abiotic injury	Non living factor	Environment/chemicals	Sunscald, nutrient deficits

Table 2. Impact of Plant Disease.

Impacts	Description
Ecosystem Impacts	• Loss of biodiversity and changes in natural habitats • Changes in forest species composition • Disruption of underground forest communication networks
Society Impacts	• Challenges to human health and food security • Community-level economic challenges • Problems linked to changes in culture and demographics
Agricultural and Economic Impacts	• Substantial declines in yield and monetary losses • Rising production costs and growing resistance issues • Limitations on trade and disturbances in the market • Decline of export industries • Susceptibility of monoculture systems

Table 3. Literature Survey of Plant Disease.

S.No	Paper Title	Technology	Advantages	Disadvantages
1	Plant diseases and pests' detection based on deep learning: a review. (2021) [1].	DL/CNN	Classification, detection, and segmentation were done using DL and CNN methods.	Limited real world data was considered.
2	Convolutional Neural Networks in Detection of Plant Leaf Diseases: A Review. (2022) [2].	CNN	Analyzed leaf disease detection using CNN papersits challenges and future work.	Hyperparameters and datasets need to be defined in a better way.

3	A comprehensive review on Plant Leaf Disease detection using Deep learning. (2023) [3].	CNN, DCNN, ViT, YOLO, RSNSR-LDD	Discussed on multi-model performance measures using accuracy, precision and recall.	No peer-review, less focus on practical implementation and field-data.
4	A Review of CNN-Based Techniques for Accurate Plant Disease Detection. (2023) [4].	CNN	Wide CNN surveyconsidered pretrained methods and datasets.	Only for specific purpose.
5	A systematic review of deep learning applications for rice disease diagnosis. (2024) [5].	preprocessing, trends	Rice plant was considered and preprocessing techniques were applied.	Only applicable for rice crop.
6	Enhancing plant disease detection through deep learning: a Depthwise CNN with squeeze and excitation. (2024) [6].	CNN	In deep CNN methods were considered with high accuracy.	Computational cost was not explained in deep.

Table 4. Performance Evaluation for Plant Disease

Performance Evaluation Methods	Formula
Accuracy	$\frac{(TP + TN)}{N}$
Precision	$\frac{TP}{(TP + FP)}$
Recall (Sensitivity)	$\frac{TP}{(TP + FN)}$
F1-Score	$2 \times \frac{Precision * Recall}{Precision + Recall}$
Confusion Matrix	2×2 or N×N matrix (TP, TN, FP, FN)



Figure 1: Healthy Leaf and Diseased Leaf

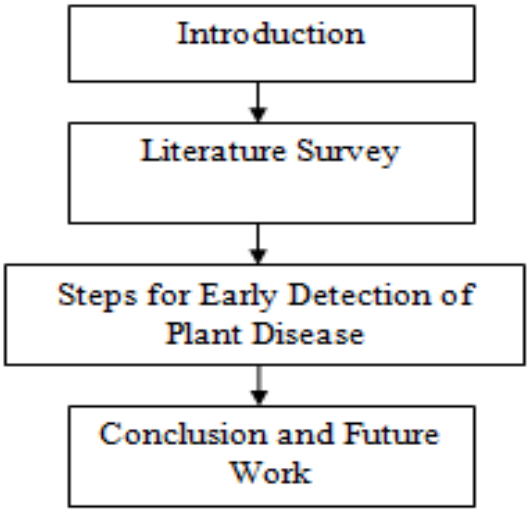


Figure 2: The Detailed Framework

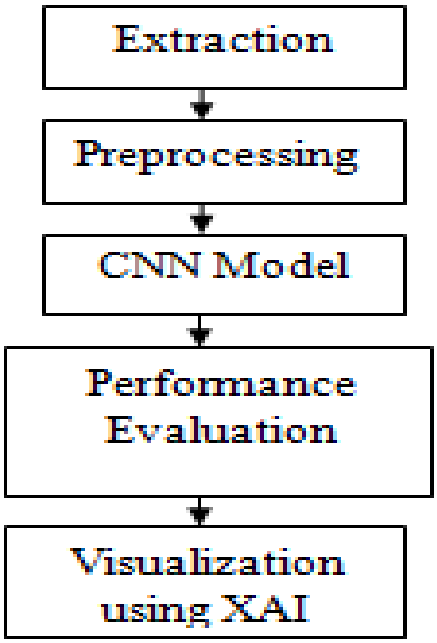


Figure 3: The Detailed Steps for Early Detection of Plant Disease

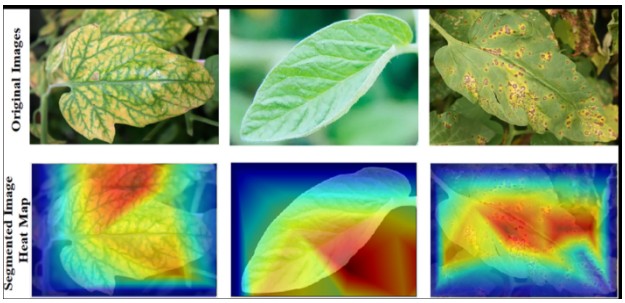


Figure 4: The Original and XAI (Segmented Image Heat Map) Images of Leaf

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RESEARCH ARTICLE

Optimizing Trash Collection and Transportation Routes using EGAAV with EGACW

Seema .S*

Assistant Professor, Department of Computer Science, Centre for Computer Science and Information Technology, Calicut University Campus, University of Calicut, Kerala, India.

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***Address for Correspondence**
Seema .S
Assistant Professor,
Department of Computer Science,
Centre for Computer Science and Information Technology,
Calicut University Campus,
University of Calicut, Kerala, India.
E.Mail: seema.karuvath@gmail.com.

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ABSTRACT

Effective waste management is a critical challenge faced by urban environments worldwide. The rapid increase in population and urbanization has led to a significant rise in waste generation, putting immense pressure on existing waste collection and disposal systems. Traditional methods of waste management often suffer from inefficiencies in route planning, inconsistent waste categorization, and suboptimal recycling practices, leading to increased operational costs and environmental impact. Garbage collection, as the first step in the waste management process, plays a crucial role in ensuring that waste is efficiently transported from residential, commercial, and industrial areas to processing or disposal sites. The efficiency of this process is heavily dependent on optimized collection routes, which minimize travel time and fuel consumption while maximizing the amount of waste collected. However, planning these routes manually can be a complex and time-consuming task, especially in densely populated urban areas with variable waste generation patterns. The EGAAV (Enhanced Genetic Algorithm with Adaptive Variable Neighborhood Search) algorithm, combined with the EGACW (Enhanced Genetic Algorithm with Clarke and Wright's Savings Algorithm) approach, is employed to calculate the most efficient collection routes. The algorithm efficiently determines the shortest and most effective routes by considering multiple factors, such as pickup locations and vehicle capacities. This optimization leads to a significant reduction in travel time and distance, enhancing operational efficiency and reducing fuel consumption. During the collection process, images of the collected waste are captured for further analysis, providing a dataset for subsequent phases.

Keywords: Optimized collection routes, Genetic Algorithm, Pickup locations.

INTRODUCTION

Ecosystems and human health are gravely threatened by the increasing volume and complexity of trash produced by modern economies. Roughly 5% of the world's greenhouse gas emissions come from the breakdown of organic waste, which contributes to the roughly 11.2 billion tons of solid trash collected annually. Every year, garbage collectors throughout the world gather almost 11.2 billion tons of solid waste. The most rapidly expanding waste stream in both developed and emerging nations is that of electrical and electronic devices, which often include complex and innovative dangerous substances. India produces more solid waste than any other country, third only to the US and China. The processes of garbage collection, transportation, treatment, and disposal present significant challenges. Solid Waste Management (SWM) is becoming a major development priority in India. Microbial decomposition, weather, trash characteristics, and land-filling processes all contribute to the formation of harmful gasses and lactates, according to many studies. The Indian government does not have the resources to acquire more land for landfills since all of the available locations have been utilized. Finding other landfill locations is also difficult. As a whole, urban areas produced 2.01 billion tons of municipal solid waste in 2016, or 0.74 kg daily per person. Rapid urbanization and population expansion are expected to cause a 70% rise in annual waste generation from 2016 levels to 3.40 billion tons in 2050. A study found that only around two-thirds to a third of the municipal trash that is collected really gets processed and disposed of. Composting relies on properly segregating waste, which is a challenge in India due to the prevalence of open-air rubbish dumps. In addition to reducing emissions of greenhouse gases and leachate, segregation can ease the load of transporting garbage. Separating waste at the source allows for the reuse of individual components in a variety of production processes, which in turn provides economic value. Recovering and using a higher proportion of recyclable trash is made easier by garbage segregation at the source. The dry garbage does not include recyclable items such as plastic bags, Mylar or laminated metalized plastic, shredded paper, or textiles. Paper, in contrast to metals, plastics, and glass, can be recycled and broken down naturally. As of now, "seven million tons of paper are utilized in India for packaging, with just 33% recovered and recycled." Paper bags and newsprint are two products made from recycled newspapers, textbooks, magazines, and the like. The production process uses less energy when plastic is reused and recycled.

RELATED WORKS

Ali, B. *et al*. (2023) to optimize the vehicle route for garbage collection, the suggested approach evaluates critical waste variables such as toxicity, volume, time duration of the waste, and distance between the bins. When compared to algorithms that optimize routes based on a single parameter, the suggested technique cuts the overall distance travelled by the garbage pickup truck by 14%, according to the simulation findings. Alwabli, A. S. (2021) the optimization of Vehicle Routing Problems (VRPs) was a prevalent challenge for most firms that want to maximize their resource use, according to this research study. Wherever the vehicle routing problem arises, the study's findings can inform the employment of various algorithms to devise optimal routes for cars. Based on its dependability and optimal solutions, the tabu search algorithm stands out as the top choice among the other algorithms in the research for optimizing vehicle routes.

Francisco, F. *et al*. (2024) several important conclusions have been drawn from the study's results. The first step was to determine that, with the exception of the problem of paywalls preventing access to real-time traffic data, it was viable to model the operational road network of University of the Philippines Diliman (UPD) Campus. The precision of real-time route analysis might be affected by this restriction. The second takeaway from the research was that one-way pathways were now preventing the achievement of time-efficient routing for garbage collection. For the sake of efficiency and speed, trash trucks should not be subject to the same regulations that other vehicles were when it comes to one-way streets. A more time- and resource-efficient garbage collecting process can be possible as a result of this modification.

Hu, Y. *et al*. (2024)the author used an enhanced ant colony-hybrid frog-jumping algorithm to address the path-planning issue for urban home garbage collection, taking capacity limits for both single- and multi-model trucks into account. To evaluate and contrast these methods, the author employed numerous viewpoints. Idrissi, A. *et al*. (2024)smart bins and Vehicle Routing Problem (VRP) tools together have the power to revolutionize trash management by cutting down on fuel usage, emissions of greenhouse gases, and related societal costs; all while increasing efficiency and sustainability. Improvements in garbage collection and transportation operations were seen as a consequence of optimization, which successfully met the needs of stakeholders regarding volume and time restrictions. Kamarudin, S. F., and Jamaian, S. S. (2023) when deciding on the most efficient method of trash transportation, distance and optimal routing play crucial roles. This research used the Ant Colony Algorithm to find a better way to move garbage from homes in the Marang area. It was suggested that more trash trucks be added to the Marang region's fleet in order to facilitate more efficient waste collection in future studies. It would be ideal if the actual state of the roads could be anticipated as well, allowing for more effective garbage transportation.

Kangah, J. *et al*. (2022) finally, the author have created and implemented a hybrid algorithm that combines Genetic Algorithm (GA)'s mutation and crossover parameters with Particle Swarm Optimization (PSO). Data testing and comparisons with other models in use led to the conclusion that the model was suitable. Zoomlion Ghana Limited can utilize it to address their issue, since the error estimation reveals it works equally well. When compared to other hybrid models that were already available, the findings from this one were clearly superior and more ideal. Lavigne, C. *et al*. (2021) with the use of a mixed integer linear programming model, this study lays out a strategy for optimizing garbage collection routes that take advantage of many depots, homogenous, capacitated trucks, intermediate stops at various processing facilities, and gatherings at each site. Policymakers can examine the effects of policy changes on expenses and the necessary trash collection feet by using the model to simulate different garbage collection situations.

Nurprihatin, F., and Lestari, A. (2020) by using the closest neighbor algorithm technique, the proposed model has effectively eliminated any garbage that would otherwise go untransported from each hamlet in Caking District. A positive effect on the whole process was guaranteed by any reduction in waste. Attaining the global optimum solution was the goal of the identified path. The number of nodes has a positive and exponentially increasing relationship with the running time. Peña Morales, D. *et al*. (2024) For the purpose of optimizing the routing issue of garbage collection utilizing electric refuse vehicles, this research presents a new, very realistic problem formulation. Within the limitations of the available battery and load capacity, the objective was to discover the paths that minimize the total travelling distance required to collect all bins. This issue takes into account the features of actual situations that were represented by data gathered from open data sources, data collecting firms, and mapping technologies. Pitakaso, R. *et al*. (2024) Overall, the He-DWS model was a big step forward in using AI for disaster waste management, but there's still a lot of room for improvement and adaptability due to these restrictions. To make the model more versatile in its deployment across different settings, future studies should work to diversify datasets, make the model more resistant to environmental variables, and maximize computing efficiency. More flexible and broadly applicable waste management methods will be possible if these problems were resolved.

Rabbani, M., and Sadri, S. (2017) Modern communities incur additional expenses due to the fact that garbage collection operations were seen as a crucial practical concern. Because garbage was certain to accumulate, it was the responsibility of local governments and commercial groups to coordinate the most efficient routes for trash trucks to take from residents' homes to landfills. Variable traffic rates and garbage volume collected from each node were two useful criteria for route optimization. Eliminating non-value-added operations was crucial for improving system efficiency, according to lean principles in the service industry. It was presumed that the time spent on load-unload procedures does not provide value to the garbage collecting process. Rahman, A., and Maryono, M. (2020) the tools, such as ArcGIS, can be used to optimize garbage transportation in Pati Regency. One of the tools included in ArcGis software, Network Analyst, can be used to assess a transportation network from Temporary Trash Shelter (TTS) to Landfill using spatial analysis. Networks connecting TTS and Landfill can be more realistically modelled using ArcGis Network Analyst, taking into account predefined speed limitations and traffic situations.

Rahmandoust, A. *et al*. (2023)The sky-high prices of creating basic commodities like metals, glass, paper, and plastic have long made trash recycling an attractive alternative. Unfortunately, the massive environmental contamination catastrophe was likely to occur if nonbiodegradable materials were returned using procedures that were not suitable. Consequently, governments should take a number of steps to bolster contractors engaged in municipal garbage collection and recycling. Ramdhani, M. *et al*. (2018) the findings obtained from the model of vehicle assignment difficulties on establishing a new route indicate that thirteen out of the thirty-seven truck compactors held by the Surabaya City Council were minimal requirements. Additionally, there must be at least five cycles each departure time and no more than two cycles every destination route. The daily operations of transferring garbage to all temporary disposal sites supplied by the Surabaya City Council include calculating the total number of truck compactors and their departure timetables. The number of vehicles required to transport goods from current locations was sensitive to changes in demand. The greater the demand, the greater the fleet size needed to carry the trash. The demand determines how often the vehicle serves the temporary dumping site.

Rashid, T. *et al*. (2023) Using CVRP to its full potential, this case study optimizes SWM's impact on the environment. This lawsuit centered on solid waste management (SWM) in the Islamabad Capital Territory (ICT), which was overseen by the Capital Development Authority (CDA) and operated by MCI. The cost consistently and gradually decreases with each iteration of the algorithm used to optimize the routes. Importantly, the algorithm guarantees that there was no way to reduce costs any more. The program found the best pathways after 1200 iterations, and further improvement of the routes was not possible. This discovery demonstrates how well the method improves SWM efficiency. Rekabi, S. *et al*. (2024) these authors research develops a new mixed-integer bi-objective mathematical model for building efficient SW networks. SW has a major effect on people's daily life. Human health and ecological balance were more vulnerable to this environmental hazard now than they were in the past. Therefore, this research proposes a new integrated multi-level model for a comprehensive SWM system that includes three levels: Virtual Reality (VR), disassembly science, and the distribution of RCs to depot centers.

Silva, A. *et al*. (2023) increasing the execution time improved the distance travelled, as shown in this paper. Since long execution times might impede real-world applications in dynamic contexts, it was crucial to emphasize that these durations were short in these authors evaluation. To rephrase, research into garbage collection that takes dynamic route planning into account necessitates lightning-fast algorithms for route planning and vehicles for trash service. Because of this, there were cases when the execution duration was less than 20 seconds. Subburaj, S. *et al*. (2023) the aforementioned system has offered thorough methodologies and an outline for a state-of-the-art vision for resolving traffic congestion. It takes mundane, unplanned tasks like garbage collection and transforms them into a coordinated, well-planned operation that could save time, energy, and chaos by harnessing the power of the rapidly developing IoT in cities and mobile computing.

Thi, N., and Phuong, l. (2022) the bulk of Municipal Solid Waste (MSW) originates in households, hence understanding their trash production rate and physical composition was important for effective waste management. In addition to outlining specifics for garbage collection, transportation, and disposal, this lays the groundwork for figuring out other crucial parts of the MSW system as a whole. One month of sampling can have been insufficient to capture certain seasonal differences, as stated in the study's limitations. Tirkolaee, E. *et al*. (2023) In order to find the best routes for garbage collection and the minimum number of trucks needed, this research developed an efficient system. In order to create a sustainable Periodic Capacitated Arc Routing Problem (PCARP) that can accomplish several objectives across multiple trips, a new Mixed-Integer Linear Programming (MILP) model was created. Maximizing total employment opportunities while simultaneously minimizing total expense, total pollution output, and total workload variation was the major objective of the challenge.

Existing methodology

Wu, H.*et al*. the paper built a model for waste collection and transportation with the minimized total comprehensive costs including routes costs and GHG emissions costs. An improved genetic algorithm, local search hybrid algorithm (LSHA), is designed to solve the proposed PCGVRP model. Furthermore, the effectively of LSHA is proved by a

contrast experiment using data from classic CVRP database. After that, the applicability and validity of the proposed PCGVRP model are confirmed by setting different numerical parameters.

Dijkstra's Algorithm

Dijkstra's Algorithm is used to find out shortest path from a source node to all other reachable nodes. It is one of the fundamental shortest path algorithms. The tentative distances are maintained for the nodes to find out the shortest path (Dijkstra 1959). This algorithm visits each node in order to find out the shortest-path and its distances. It will terminate the search when it reaches all target nodes. Thus it finds out the shortest path by minimizing the distance.

Vehicle routing problems

Requests for transportation services are defined in the General Vehicle Routing Problem (GVRP) by a nonempty set of locations that a single vehicle must visit in a specific order, time windows in which these locations must be visited, and the revenue that will be gained upon completion of the service. Moreover, some attributes might be defined that limit the likelihood of allocating transportation requests to certain vehicles owing to limitations in compatibility and capacity. You may load or unload shipments with several descriptive features at each of these locations. Not every transportation request has to be allocated to a vehicle, unlike many other well-known routing challenges. That is the question that needs answering before a transportation request can be fulfilled. The transportation needs may be met by drawing from a fleet of diverse vehicles. The vehicles might vary in capacity, journey duration, and cost depending on the distance traveled. Cargoes may be transported by using a portion of the vehicle's capacity. Each vehicle is assigned a starting point from whence it becomes accessible at a certain time and with a predetermined load, rather than supposing that it becomes available at a central depot. Along with not having to return to a central depot, each vehicle has a certain end destination that they must reach by a certain time with a given amount of cargo. From their starting point to their ultimate destination, each vehicle may be required to visit certain spots in a certain order. You have a limited amount of time to visit each area. The car will have to wait until the time frame starts if it gets to one of these spots earlier. From its starting point to its final destination, a vehicle's tour encompasses all the locations it must visit in the correct order, as well as all the locations associated with the transportation requests assigned to it. In order for a tour to be viable, compatibility requirements must hold for all orders allocated to the tour, and capacity constraints must hold at each point in the tour time frame. The goal is to identify separate, workable trips that maximise profit, where profit is defined as the sum of all transportation requests fulfilled minus the sum of all operational expenses. The Vehicle Routing Problem (VRP) primarily aims to find the best possible path for vehicles to take from a depot, meet all client demands, and then return to the depot. Capacity is a major factor for most VRPs. Based on this, capacitated VRP (CVRP) is the name given to the generalization of VRP. CVRP is described as the challenge of finding the best routes for trucks to take, given certain limitations, from one or more depots to meet all of a customer's demand. If the author goes back to the original concept, CVRP is the challenge of determining the best collection or delivery route to meet consumer demand. According on the problem's objectives, the aim may be to reduce the number of cars, trip time, or cost. Also, there are a lot of cars waiting in the depot to meet client demand, and they all have to go back after the operations are over. There is a set capacity for every vehicle.

A complete graph G = (V, E), where V = {0,1,...,n} is the vertex set and E is the edge set.
Vertices i = 1,2,...,n correspond to the customers. Vertex 0 corresponds to the depot and n is number of customers.
Each customer i has non-negative demand di that must be delivered to the depot.
A set of vehicles H = {1,2,...,h}, where every vehicle has capacity C, is available at the depot and must return to depot after finishing their deliveries.
A non-negative cost cij is associated with each edge (i,j) 2 E and represents distance from vertex i to vertex j for all i – j.
Decision variables
$x_{ijk} = \{1, \quad if vehicle k passed route from i to j \; 0, \quad otherwise$ ---------- (4.12)
$y_{ik} = \{1, \; if vehicle k passed route from i to j \; 0, \; otherwise$ ---------- (4.13)
Minimize

$\sum_{k=1}^{h} \sum_{i=0}^{n} \sum_{j=0}^{n} c_{ij} x_{ijk}$---------- (4.14)

Subject to,

$\sum_{i=0}^{n} x_{i0k} - \sum_{j=0}^{n} x_{ojk} = 0, \forall k = 1, \dots, h$ ---------- (4.15)

$\sum_{i=1}^{n} d_i y_{ik} \leq C, \forall k = 1,2, \dots, h$ ---------- (4.16)

In basic CVRP, it is assumed that all variables are deterministic. But many distribution challenges in the actual world include non-fix data. Consequently, the development of the CVRP model has been the focus of a great deal of CVRP-related literature. Most studies have focused on developing CVRP models that can handle uncertain and stochastic data. Stochastic CVRP allows for the analysis and prediction of CVRP with uncertain data using probabilistic assumptions. More recently, fuzzy variables were used to handle ambiguous data in an effort to enhance CVRP. Uncertain demand and time frames are estimated using fuzzy variables. As a non-crisp border theory with membership degree components with fuzzy set theory. A number of theories on fuzzy event measurement have been put up with regard to the use of fuzzy variables in CVRP with uncertain data. These ideas include credibility theory, possibility and necessity theory, and others.

Proposed methodology

This research focuses on optimizing trash collection and transportation routes using the EGAAV in conjunction with the EGACW algorithm. These algorithms are employed to strategically plan and optimize garbage collection routes, considering variables such as pickup locations and vehicle capacity. The objective is to minimize both travel time and distance covered by garbage collection vehicles, thereby enhancing operational efficiency and reducing environmental impact. Throughout the collection process, images of the gathered trash are systematically captured for subsequent processing and analysis.

Optimizing Trash Collection and Transportation Routes using EGAAV with EGACW

Preprocessing and Augmentation

At preprocessing stage, performed the basic steps such as Resizing, Denoise, Smoothing and Segmentation after the above all the images set in similar size. After preprocessing, Image Augmentation performed for better projection of the dataset. It generates more images with the process flipping, shearing, width and height shifting, zooming and rotating and rescaling so on. Preprocessed Image (Figure: 1). Augmented Image (Figure: 2)

EGAAV algorithm

By using a box-type filter based on integral pictures, the EGAAV approach has decreased the operating time required to detect and define interest spots. The definition of the integral image pixel S at (h, v) is given for each pair of coordinates (i, j) in the input picture as

$S = \sum_{i=0}^{i \leq h} \sum_{j=0}^{j \leq v} I(i, j)$ ---------- (4.1)

This equation can get the total number of pixels within a rectangular section of the input picture with just three additions and four memory accesses by using the integral image and the calculation in Equation (4.1). As a result, many integral-picture-based box-type filters are used to calculate HWRs and approximate the convolutions of the Gaussian second-order partial derivatives.

The SSR step involves building a scale space using the response layers obtained at different scales, which are then divided into several groups called octaves.

$det\left(H\left(x, y, \sigma^i\right)\right) = \left|\frac{D_{xx}(x,y,\sigma^i) w D_{x,y}(x,y,\sigma^i)}{w D_{xy}(x,y,\sigma^i) D_{yy}(x,y,\sigma^i)}\right|$ ---- (4.2)

The horizontal, vertical, and diagonal convolutions of the Gaussian second-order partial derivatives that are approximated using the box-type filters based on the integral image are denoted as D_{xx}, D_{yy}, and D_{xy}, respectively. Points of interest are identified in the LOC stage using the response layers. This equation compare the "X"-marked

pixel P in the middle layer to its neighbors and the 3 neighborhoods in the top and bottom layers that share the same position. The localized interest point is obtained by applying an interpolation procedure to the maximum-determined pixel P.

The OA and DE steps are responsible for creating the points of interest's descriptions. The OA process begins with obtaining horizontal and vertical HWRs for all of the pixels that are part of a circular neighborhood around the point of interest. Afterwards, the major orientation is sought for by using the Gaussian-weighted HWRs of the pixels inside the orientation window of size p/3. To ensure that the descriptor vector remains stable as the image is rotated, the DE phase involves squarely around the point of interest in accordance with the main orientation that was specified. The response layer requires 4 bytes to hold a pixel point since the determinant value in Equation (4.3) is a real integer. Therefore, the memory needed for a five-octave scale space can be expressed as

$$\left\{4.\left(\frac{N}{2}\right).\left(\frac{M}{2}\right)+\sum_{k=2}^{5} \quad 2.\left(\frac{N}{2^2}\right).\left(\frac{M}{2^k}\right)\right\} \text{--------- (4.3)}$$

$$.4\ bytes = \frac{N.M.597}{128} Bytes \text{ ---------- (4.4)}$$

Notably, the first octave generates four response layers, but the subsequent octaves produce just two. According to this study's analysis of Equation (4.4), building the scale space for an input picture with dimensions 640 by 480 requires 1.37 MB of memory. The memory consumed for the scale space is therefore a major aspect that makes implementing the SURF method challenging. It is well understood that there is a spatial correlation between neighboring pixels in a picture. In this case, it would be easy to find a large number of non-maximum points by comparing them to the neighborhood and nearby pixel points in the next response layer. This fascinating phenomenon demonstrates that the local maximum detection approach makes several unnecessary comparisons to the bottom layer, and therefore the SSR step is unnecessary even without the box type filterings applied to the pixel points in the bottom layer. To begin improving the EGAAV algorithm's performance, it is prudent to study the computing demands of its four individual phases. It is also readily apparent why Han's technique, which eliminates the need to repeat computations during the OA step's orientation search, fails to provide a discernible improvement in speed. A large chunk of the total computational burden is accounted for by the HWR process in both the OA and DE steps, as can be shown from this study. This indicates that the HWR process's computational burden greatly affects the operating speed. It suggests a point feature registration procedure that remains invariant when pictures are rotated or translated, and it is based on invariant feature technology. Locate the boundaries of scale space. In other words, the initial picture is transformed using the Gaussian convolution kernel to get the representation sequence in multi-scale space, where the calculation formula is for

$$L(x,y,\sigma) = G(x,y,\sigma) * I(x,y) \text{ ---------- (4.5)}$$

A scale-variable Gaussian function $G(x,y,\sigma)$ is defined as$G(x,y,\sigma) * I(x,y)$, where (x,y) is the gray value of the image and σ is a Gaussian scale factor. Lastly, in order to extract features, the spatial extremum is taken from these sequences. Find the feature points and sort them. The local extremism point (x_0, y_0, σ_0) is where the Taylor formula is developed, and the derivative of the expansion formula is 0. To get the original function's extremum formula, this equation take the coordinate location of the extreme point (∂X) and plug it into the scale space function.

$$D(x) = D + \frac{1}{2}\frac{\partial D^T}{\partial X}x \text{ ---------- (4.6)}$$

In order to reject the extreme point of the class, the interpolation center is moved to the nearby point if the offset in either direction is more than 1/2. Lastly, the stability and anti-noise capabilities are improved by removing unstable edge response spots using the Hessian matrix. Directions are given feature points. To ensure that each feature point is rotationally invariant, the primary direction must be chosen after the feature points have been found. One way to determine the primary orientation of feature points is. The feature point's gradient modulus value is represented by$m(x,y)$, its gradient direction is denoted by$\theta(x,y)$, and the feature point's location on the scale is represented

by$L(x, y)$, the Gaussian image of the feature point. To compensate for the feature points' lack of stability due to affine invariance, each gradient histogram should weight the sample points' gradient amplitudes; the weights of the sample points are then determined by their gradient modulus values and the Gaussian weights. A crucial point identifier is built. At the end of the three-step computation process, every identified feature point has three messages:(x, y),σ,andθ.Specifically, location, size, and orientation. Since the feature point scale affects the feature point descriptor, it is necessary to generate the feature point descriptor in the matching scale's Gaussian pyramid space. To begin, the area around the feature point is partitioned into sub-blocks of dimensions BP × BP, and the size of the edges of each sub-block is M_F pixels. Feature point descriptors are built in the following way: Initially, with the feature point in the center, the image's rotation angle near the feature point is θ in the direction of the feature point's main axis. Next, using the feature point in the middle, choose an image block of dimensions m_FBp × ρBp. Next, split the interval into BP × BP subblocks using the gradient histogram. Finally, determine the seed point by adding up the values of all pixels in each subblock in eight directions. A feature vector of 128 dimensions is created. As an added step in building feature point descriptors, it is necessary to apply a Gaussian weight to all the pixels surrounding the feature point and to normalize all the pixels within a two-fold range to eliminate the impact of lighting and other variables.

EGACW

In the first step, known as scale space extreme discovery, potential key points are located. To build a pyramid of blurry images with different levels of Gaussianity, it first convolves the image S_k using Gaussian filters $GF(x, y, k\sigma)$ at different scales$k\sigma$. In which

$S_k = S(x, y, k\sigma) = GF(x, y, k\sigma) * S_{k-1}$ ---------- (4.7)

Where for k = 1, Sk−1 = S0 = $I\ (x, y)$ is the input image, and

$GF(x, y, k\sigma) = \left(\frac{1}{2}\pi(k\sigma)^2\right) e^{-\frac{(x^2y^2)}{2(k\sigma)^2}}$ ---------- (4.8)

The specified value of k is used to organize the convolved images per octave, with a predetermined number of photos per octave. Frames in pyramids of Gaussian blurred pictures are denoted in this work by their octave and scale, $G[0,0]$. This allows us to define the Gaussian pyramid in the following way

$G[0, 0] = I(x, y): theoriginalframe$ ---------- (4.9)

$G[0, 1] = GF(x, y, \sigma) * G[0,0]$ ---------- (4.10)

$G[m, k] = GF(x, y, k\sigma) * G[m - k - 1] fromm, k \geq 0$ ------- (4.11)

After then, the blurred pictures of the DoG can be calculated by this study.

$DOG[m, k] = G[m, k + 1] - G[m, k]$ ---------- (4.12)

This research could use DoG to find the local maximum or minimum in a 3 × 3 frame that might be keypoint candidates by comparing the pixels at neighboring scales.

By using a low contrast and edge detection test, the keypoint localization procedure finds stable keypoints among the candidates by removing troublesome keypoints. The low contrast test rejects the unstable extreme position by definition.

$x = -\left(\frac{\partial^2 DoG^{-1}}{\partial x^2}\right)\left(\frac{\partial DoG}{\partial x}\right)$ ---------- (4.13)

Based on the Taylor expansion of DoG [m, k], equation can calculate a low contrast threshold.

$DoG(x) = DoG + \frac{1}{2}\left(\frac{\partial DoG^T}{\partial x}\right)x$ ---------- (4.14)

Edge detection is decided by

$\frac{Tr(H)^2}{Det(H)} < \frac{(r+1)^2}{r}$ ---------- (4.15)

The coefficient r is used to adjust the sharpness of the object's edges, and the traces and determinants of the 2×2 Hessian matrix are $Tr(H)^2$ and$Det(H)$, respectively.

$H = \left|DoG_{xx}\ DoG_{xy}\ DoG_{xy}\ DoG_{yy}\right|$ ---------- (4.16)

$Tr(H) = DoG_{xx} + DoG_{yy} = a + \beta$ ---------- (4.17)

When the candidate passes the aforementioned test and is thus confirmed to be a feature point, EGACW starts to determine its orientation. At the orientation assignment step, this section uses the directions of the local image gradients to assign one or more orientations to each keypoint, ensuring rotation invariance. To be more precise, EGACW substitutes the largest vector, the S-vector, for the orientation assignment. Using these methods ensures that the photo will remain in the exact same place, size, and orientation. Finally, for every keypoint, a partially invariant descriptor vector is generated at the local image detector step.

EGAAV with EGACW

The hybrid algorithm for finding optimized collection routes using the EGAAV with EGACW approach begins by initializing the algorithm and setting up the necessary data. The process starts with defining the pickup locations and existing trash collection points as nodes on a graph. To facilitate route optimization, the algorithm calculates the distance between all pairs of these nodes and creates a matrix to store these distances. Next, the algorithm initializes a list to track visited pickup locations and another to manage the routes assigned to each garbage collection vehicle. For each vehicle, the algorithm selects the nearest unvisited pickup location as the starting point and begins constructing its route by adding this location to the current route, updating the vehicle's position, and marking the location as visited. This process continues until the vehicle reaches its capacity or there are no more unvisited locations available. The total travel distance for each route is then computed. The algorithm repeats the process of selecting and visiting locations until all pickup points are covered. Once all routes are established, their efficiency is evaluated based on travel time and distance. To further optimize the routes, the EGAAV (Enhanced Genetic Algorithm with Adaptive Variable Neighborhood Search) with EGACW (Enhanced Genetic Algorithm for Collection and Waste management) algorithm is applied. This optimization step involves rearranging pickup locations to minimize overall travel time and distance. Algorithm 1: EGAAV with EGACW

RESULT AND ANALYSIS

An assessment parameter was used to assess the model's Training Accuracy, Validation Accuracy, Training loss, and Validation loss. Initially evaluated the accuracy of all 3 models with normal data and compared the result as well. In table: 1 and figure: 3 the given data, all methods—GA, GAAV, EGAAV, EGACW, and Hybrid—are associated with the same number of routes, which is 10. This uniformity suggests that regardless of the method employed, each method provides an equal number of routes. This consistency in route count could indicate that the methods are comparable in terms of their route generation capabilities, or it may imply that the routes are pre-defined and influenced by the choice of method. To gain deeper insights, further analysis would be required to assess other performance metrics or variations beyond just the number of routes. The table 2 and figure 4. data indicates that the

methods for finding the shortest path vary in effectiveness. The GA and GAAV methods each identified 1 shortest path, suggesting their limited capability in solving the shortest path problem compared to others. The EGAAV and EGACW methods each found 2 shortest paths, demonstrating a moderate improvement in performance over the previous methods. The Hybrid method outperformed all others, identifying 3 shortest paths, which indicates its superior ability to handle shortest path computations. This progression from fewer to more paths found suggests that the Hybrid method integrates features or optimizations that enhance its path finding performance. The table 3 and figure 5 time data for each method reveals significant differences in performance. The GA method took the longest time at 0.5 milliseconds, indicating it is the slowest among the methods. The GAAV method improved upon this with a reduced time of 0.15 milliseconds. Further optimization was observed in the EGAAV method, which took 0.045 milliseconds, and the EGACW method, which took 0.085 milliseconds. The Hybrid method demonstrated the fastest performance with an impressive time of 0.00267 milliseconds. This substantial decrease in time with the Hybrid method suggests it is highly efficient, likely due to advanced optimizations or algorithmic improvements that significantly reduce computational time.

CONCLUSION

This study centers on optimizing trash collection and transportation routes through the implementation of the EGAAV (Enhanced Genetic Algorithm with Adaptive Variable Neighborhood Search) and EGACW (Enhanced Genetic Algorithm with Clarke and Wright's Savings Algorithm) algorithms. These algorithms are instrumental in planning efficient collection routes for garbage collection vehicles, prioritizing factors such as pickup locations and vehicle capacity to minimize both travel time and distance. The integration of these algorithms aims to enhance operational efficiency in waste management, facilitating timely and resource-efficient collection processes. Concurrently, the phase involves the systematic capture of images depicting collected trash, laying the groundwork for subsequent phases focused on advanced image processing and waste categorization techniques. This approach sets the stage for developing smarter and more sustainable waste management practices, utilizing cutting-edge technologies to optimize route planning and improve environmental outcomes.

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Algorithm 3: EGAAV with EGACW

Input

- List of pickup locations.
- Vehicle capacity.
- Locations of existing trash collection points.

Steps

1. Initialize the algorithm.
2. Define pickup locations as nodes on a graph, including the location of existing trash collection points.
3. Calculate the distance between all pairs of pickup locations and collection points.
4. Create a matrix to store distances between locations.
5. Initialize a list to keep track of visited pickup locations.
6. Initialize a list of routes for each garbage collection vehicle.
7. For each garbage collection vehicle:
   o Select the nearest unvisited pickup location as the starting point.
   o Add it to the current route.
   o Update the vehicle's current location.
   o Mark the pickup location as visited.
   o Repeat the process until the vehicle's capacity is reached or no unvisited locations remain.
8. Calculate the total travel distance for each vehicle's route.
9. Repeat steps 7-8 until all pickup locations are visited.
10. Evaluate the efficiency of each route based on travel time and distance.
11. Optimize routes by rearranging pickup locations to minimize travel time and distance using the EGAAV with EGACW algorithm.
12. Output the optimized collection routes for each garbage collection vehicle.

End the algorithm.

Output

- Optimized collection routes.

Table 1: Number of routes

Methods	Number of Routes
GA	10
GAAV	10
EGAAV	10
EGACW	10
Hybrid	10

Table 2: Finding shortest path

Methods	Finding Shortest Path
GA	1
GAAV	1
EGAAV	2
EGACW	2
Hybrid	3

Table 3: Execution Time Comparison of Different Methods

Methods	Time (ms)
GA	0.5ms
GAAV	0.15ms
EGAAV	0.045ms
EGACW	0.085ms
Hybrid	0.00267ms

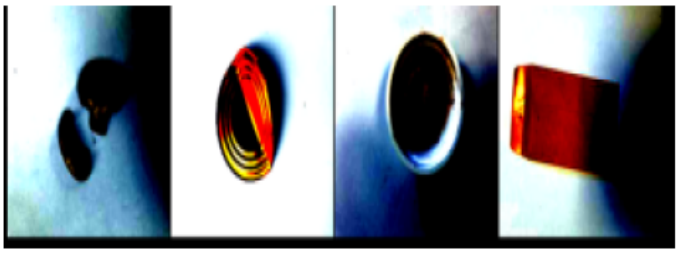


Figure 1: Preprocessed Image

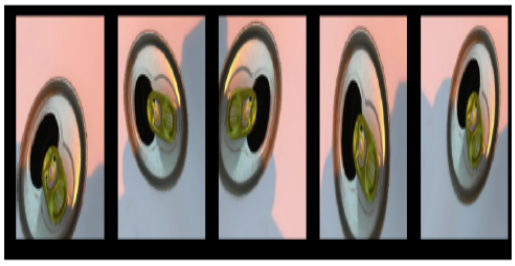


Figure 2: Augmented Image

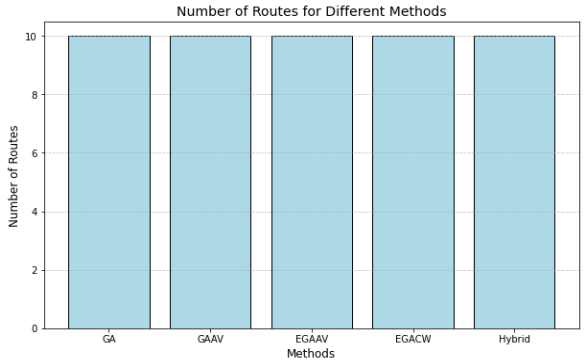


Figure 3: Number of routes

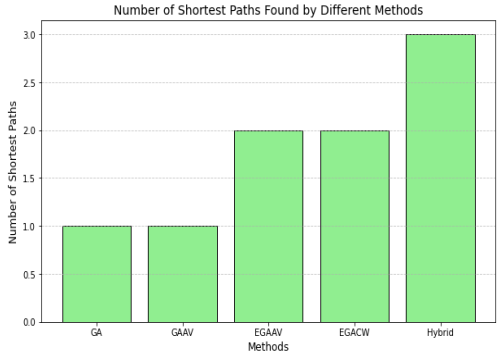


Figure 4: Finding shortest path comparison chart

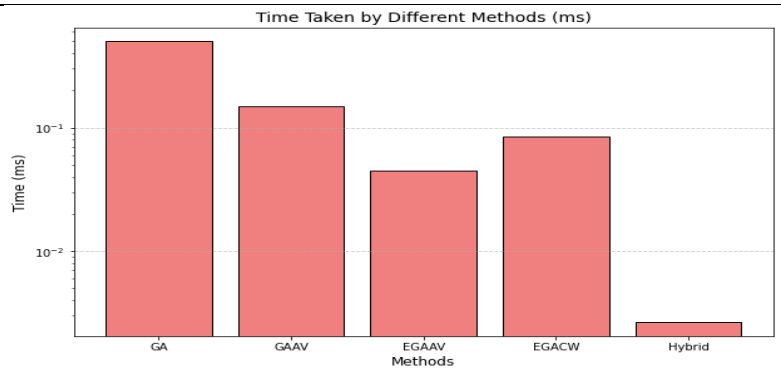


Figure 5: Execution Time Comparison chart

REVIEW ARTICLE

Review on Zagreb Index Energy

Sateesh Kumar T V[1]* and D Bhuvaneswari[2]

[1]Assistant Professor, Sri Ramachandra Faculty of Engineering and Technology, Sri Ramachandra Institute of Higher Education and Research, (Deemed to be University) Porur, Chennai, Tamil Nadu, India.
[2]Assistant Professor, Department of Mathematics, M.O.P. Vaishnav College for Women, Nungambakkam, (Affiliated to University of Madras), Chennai, Tamil Nadu, India.

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***Address for Correspondence**
Sateesh Kumar T V
Assistant Professor,
Sri Ramachandra Faculty of Engineering and Technology,
Sri Ramachandra Institute of Higher Education and Research (Deemed to be University),
Porur, Chennai, Tamil Nadu, India.
E.Mail: sateeshkumar@sriramachandra.edu.in

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ABSTRACT

The Zagreb indices are closely associated with connectivity of graph and Zagreb energy is addition of absolute Eigen values of matrix. In this article the extremal results about the Zagreb Indices and Energy with their theorems was discussed.

Keywords: Zagreb, energy, matrix.

INTRODUCTION

The Zagreb Indices and Zagreb Energy is one of the blooming topics in graph theory with more chemical related results. The First Zagreb Indices M_1 and Second Zagreb Indices M_2 [7] are defined as sum of square of degrees of all vertices.

$$M_1 = \sum_{v \in V} \deg(v)^2$$

$$M_2 = \sum_{u,v \in V} \deg(u)\deg(v)$$

The First Zagreb Matrix $Z^{(1)}$ and Second Zagreb Matrix $Z^{(2)}$ is defined as [7] $d_i + d_j$ and $d_i d_j$ when $i \neq j$, and 0 when $i = j$.

$$\left(Z^{(1)}\right) = \begin{cases} d_i + d_j & v_i, v_j \in E(G) \\ 0 & else \end{cases}$$

$$\left(Z^{(2)}\right) = \begin{cases} d_i d_j & v_i, v_j \in E(G) \\ 0 & else \end{cases}$$

The energy of the graph defined as The energy of the graph defined as

$E(G) = \sum_{i=1}^{n} |\lambda_i|$

The First Zagreb Energy $Z^{(1)}$E(G) and Second Zagreb Energy $Z^{(2)}E(G)$ is as the combination of matrix of absolute eigenvalues.

$$Z^{(1)}E(G) = \sum_{i=1}^{n} \left|\gamma_i^{(1)}\right|$$

$$Z^{(2)}E(G) = \sum_{i=1}^{n} \left|\gamma_i^{(2)}\right|$$

The Zagreb Indices and Energy were initiated by I Gutman et. al. [4] in 1970's, which has been useful for properties of molecules and connectivity between them. The purpose of Zagreb Indices are relations of degree of vertices and structures and the purpose of Zagreb Energy are spectral properties of graph.

The Zagreb Indices is useful to predict properties like melting points and boiling points correlated with molecular structure. The Zagreb Energy is to estimates electronic structure of molecule's.

In this review, we restricted our focus to the extremal results on Zagreb Indices and Energy for recent decades.

Extremal Results of Zagreb Indices

The paper [17] Upper Bounds of Zagreb Connection Indices(ZCI) of Tensorand Strong Product on Graphs investigates the ZCI upper bound (1stZCI, 2nd ZCI, and modified 1st ZCI) for the tensor and strong productof two graphs. The authors provide general results and formulas forcomputing these upper bounds, which are important in the discussion ofQSAR (quantitative structure-activity relationships) and QSPR (quantitativestructure-property relationships) in chemistry and materials science.The key findings include explicit expressions for the upper limits of the ZagrebConnection index in terms of the properties (degrees and connectionnumbers) of the original graphs. Degree-based TIs, such as the 1st and2nd Zagreb indices, have been used to predict result in molecular structures,including molar, absolute correlation, volume, acentric factor, andstandard enthalpy of formation.

The results [17] of the ZCI of strong and tensor product of graphs as

Tensor Product

Exact value of $ZC_1(P_3 \otimes P_3) = 40$;
Computed value of upper bound $ZC_1(P_3 \otimes P_3) \leq 136$;
Exact value of $ZC_2(P_3 \otimes P_3) = 4$;
Computed value of upper bound $ZC_2(P_3 \otimes P_3) \leq 128$;
Exact value of $ZC_1^*(P_3 \otimes P_3) = 20$;
Computed value of upper bound $ZC_1^*(P_3 \otimes P_3) \leq 52$.

Strong Product

Exact value of $ZC_1(P_3 \boxtimes P_3) = 136$;
Computed value of upper bound $ZC_1(P_3 \boxtimes P_3) \leq 169$;
Exact value of $ZC_2(P_3 \boxtimes P_3) = 156$;
Computed value of upper bound $ZC_2(P_3 \boxtimes P_3) \leq 264$;
Exact value of $ZC_1^*(P_3 \boxtimes P_3) = 112$;
Computed value of upper bound $ZC_1^*(P_3 \boxtimes P_3) \leq 152$.

The paper [17] obtains general results of upper limit for the ZCI, namely the 1st ZC_1, 2nd ZC_2, and modified 1st Zagreb connection index ZC_1^* , on the resultant graphs obtained by operating the strong and tensor product on 2 simple graphs. The paper [6] Comparing Zinc Oxide- and Zinc Silicate-Related Metal- Organic Networks via Connection-Based Zagreb Index discusses 2 distinct zinc-related metal-organic networks (MONs): zinc oxide (ZNOX(n)) and zinc silicate (ZNCL(n)). The authors compute various connection-related Zagreb index, such as the 1st ZCI, 2nd ZCI, modified 1st ZCI, modified 2nd, 3rd and 4th ZCI, to compare the properties of these two MONs as the number of layers (n) increases. The key findings indicate that the zinc silicate-related MON is good to the zinc oxide-related MON for all values of n.

Theorem 1 [6] LetH ≅ ZNOX(n)of dimensions n ≥ 3. Then, ZCI are
$ZC_1(H) = 1068n + 692$
$ZC_2(H) \cong 1376n + 896$
$ZC_1^*(H) \cong 672n + 432$
$ZC_2^*(H) \cong 1740n + 1124$
$ZC_3^*(H) \cong 1808n + 1184$
$ZC_4^*(H) \cong 10344n + 7224$
The comparison between the (ZNOX(n)) and (ZNCL(n)) metal-organic networks (MONs) based on the provided contexts: The zinc silicate-related MON (ZNCL(n)) has higher values and upper lines for all computed connection-based Zagreb indices (ZCIs) compared to zinc oxide-related MON (ZNOX(n)). This includes the 1st, 2nd, modified 1st, modified 2nd, modified 3rd, and modified 4th ZCI. The modified 4th ZCI (ZC_4^*) attains the highest values among all the ZCIs for both the zinc-related MONs. The modified 1st ZCI showed best correlation with the physico-chemical possessions of octane isomers compared to other Zagreb indices. Similarly, the novel $ZC4$ also showed best correlation for the increasing in both zinc-related MONs. Overall, the comparison shows that the MON (ZNCL(n)) is good than the MON (ZNOX(n)) for all values of the number of layers (n). The research article concludes that the zinc silicate-related MON exhibits superior properties compared to the zinc oxide-related MON based on the computed connection-based Zagreb indices. The paper [8] Computing Analysis for First Zagreb Connection Index and Coindex of Resultant Graphs, investigates the graphs obtained through tensor and strong product, symmetric difference, and disjunction operations. Authors derive upper limits for the 1st ZCI and co-index in terms of the index of the factor G. The paper includes an analysis of the obtained results using numerical values and graphical representations. The study contributes to the understanding of connection number-based topological indices and their applications in cheminformatics and molecular graph analysis.

Theorem 2 [8] Let G_1andG_2be connected and $\{C_3, C_4\}-$free graphs. Then, $ZC_1$and$\bar{Z}C_1$of the tensor product ($G1 \oslash G2$)are
$$ZC_1\left(\frac{G1}{G2}\right) = n_2ZC_1(G_1) + n_1ZC_1(G_2) + ZC_1(G_1)ZC_1(G_2) + 2AB + 2AZC_1(G_2) + 2BZC_1(G_1),$$
$$ZC_1(G_1 \oslash G_2) = 2\mu_2ZC_1(G_1) + 2\mu_1ZC_1(G_2) + 2\sum_{a_1a_2\notin E(G_1)}\sum_{b_1b_2\notin E(G_2)}\left[\tau_{G_1}(a_1)\tau_{G_2}(b_1) + \tau_{G_1}(a_2)\tau_{G_2}(b_2)\right]$$
The proof involve expanding the connection number of the tensor product and rearranging the terms to obtain the final expressions for the 1st Zagreb connection index and co-index. It conclude by providing the upper limits for the 1st and coindex ZCI of the tensor, strong, symmetric difference, and disjunction of graphs . The graphical and numerical analyses of the exact and upper limits for the 1st ZCI and co-index of the tensor and strong product are presented. The paper [16] Some Novel Results Involving Prototypical Computation of Zagreb Polynomials and Indices for SiO_4 Embedded in a Chain of Silicates focuses on computing based on valency invariants molecular for SiO_4 embedded in a chain of silicate. The authors used the atom-bond partitioning approach based on the valence of Si and O_2 atoms to derive exact formulas for various Zagreb polynomials and indices. The computed topological indices include the first, second, hyper, modified, enhanced, and redefined Zagreb (first, second, and third). The results demonstrate the dependence of these topological indexs on the specific polynomial structure parameters of the silicate chain. The findings provide insights into using topological indices to characterize the chemical and physical properties of silicate compounds. The basic unit of silicates is a SiO_4 tetrahedron, which can join with other SiO_4 tetrahedra to form a silicate chain of SC_q^p, where p and q are the chain of silicate numbers and total number of SiO_4 in 1 silicate chain, respectively. In the chain of silicates SC_q^p, 3 types of atom-bond partitions based on the valencies of the Si and O_2 atoms: (3~3), (3~6), and (6~6). The authors derived exact formulas for various Zagreb polynomials and indices for the silicate chain SC_q^p. The results demonstrate the dependence of that topological index on specific polynomial parametric structure of the silicate chain. The findings provide insights into using topological indices to characterize the chemical and physical properties of silicate compounds.
Theorem 3 [16] For $p > 1$, then hyper-Zagreb polynomial of $SC_q^p$is$(3p + 2)y^{36} + (3p^2 + 3p - 4)y^{81} + (3p^2 - 6p + 2)y^{144}$.

Theorem 4 [16] For $p > 1$, then modified Zagreb polynomial of $SC_p^p$is $(3p + 2)y^{\frac{1}{9}} + (3p^2 + 3p - 4)y^{\frac{1}{18}} + (3p^2 - 6p + 2)y^{\frac{1}{36}}$

Theorem 5 [16] Let odd $p$and $p < q$. Then 3rd redefined Zagreb polynomial of $SC_q^p$is $3(p + 1)y^{32} + (3pq + p + 2q - 5)y^2 + (3pq - 4p - 2q + 2)y^3$.

Theorem 6 [16] Let p be odd and $p < q$. Then 3rd redefined Zagreb polynomial of $SC_q^p$is $3(p + 1)y^{54} + (3pq + p + 2q - 5)y^{196} + (3pq - 4p - 2q + 2)y^{432}$.

The key points to take is detailed analysis of various based on valency invariant molecular, such as the first, second, hyper, modified, enhanced, and redefined Zagreb indices, for SiO_4 embedded in a silicates chain SC_q^p. Applied the atom-bond partitioning method related to atom valences and used spectral graph theory to fet the exact values of these topological indices. The results are particularly interesting when the silicate chain numbers $p \geq q$, as indicated by the authors. The paper provides a graphical results, which tells about dependence of the topological index on particular polynomial parametric structure. The findings of this study contribute to the understanding of the chemical characteristics and properties of SiO_4 embedded in silicate chains, which can be useful in various applications in theoretical chemistry and materials science. The paper [9] Laplacian energy and first Zagreb index of Laplacian integral graphs derived formulas for the Ehodnegry by Laplacian met and 1st Zagreb index of graphs realizing the Laplacian spectrum $S_{i,n}$. The authors provided lower and upper limits for the Laplacian energy of such graphs. Also discussed a conjecture that the set $S_{n,n}$ is not Laplacian realizable, and proved some related results. All the L-borderenergetic and L-equienergetic graphs realizing $S_{i,n}$ were listed. And found some graphs realizing $S_{i,n}$ with the same number of spanning trees.

Theorem 7 [5]Let $n \geq 2$and $1 \leq i \leq n$. Suppose that $S_{i,n}$ is Laplacian realizable. If $n \equiv 0\ mod\ 4\ or\ n \equiv 3\ mod\ 4$ then i is even, while if $n \equiv 1\ mod\ 4\ or\ n \equiv 2\ mod\ 4$, then i is odd.

Theorem 8 [5]Suppose $n > 2$

If $n \equiv 0\ mod\ 4$, then for $i = 1, 2, 3, \ldots, \frac{n-2}{2}$, $S_{2i,n}$ is Laplacian realizable

If $n \equiv 1\ mod\ 4$, then for $i = 1, 2, 3, \ldots, \frac{n-1}{2}$, $S_{2i-1,n}$ is Laplacian realizable

If $n \equiv 2\ mod\ 4$, then for $i = 1, 2, 3, \ldots, \frac{n}{2}$, $S_{2i-1,n}$ is Laplacian realizable

If $n \equiv 3\ mod\ 4$, then for $i = 1, 2, 3, \ldots, \frac{n-1}{2}$, $S_{2i,n}$ is Laplacian realizable

Theorem 9 [5]Let$G$ be a connected graph of n order with m edges and Δ maximum degree. Then
$LE(G) \geq 2(\Delta + 1 - \bar{a})$,
with equality if and only if G is isomorphic to $K_{1,n-1}$. Here a is the mean degree of the graph G

Theorem 10 [5]Let a graph G of order n and of size $m, m \geq \frac{n}{2}$. Then

$$LE(G) \leq 4m - 2\Delta - \frac{4m}{n} + 2.$$

Here the equality holds iff$G$ is isomorphic $K_{1,n-1}$ or G is isomorphic $K_{1,\Delta} \cup \overline{K}_{n-\Delta-1}, \frac{n}{2} \leq \Delta \leq n - 2$.

Theorem 11 [5]Suppose $n > 2$. If G is a graph of order n realizing $S_{i,n}$ for certain $1 \leq i \leq n$ then

$$M_1(G) = \frac{n(n+1)(n-1)}{3} - i(i-1)$$

This paper [5] concluded by deriving exact formulas for the Laplacian energy and the first Zagreb index of graphs whose matrices have the set $S_{i,n}$ as its eigenvalues. The authors find that the Laplacian energy of graphs realizing $S_{i,n}$ does not exceed the first Zagreb index of such graphs. All the L-borderenergetic and L-equienergetic graphs realizing $S_{i,n}$ were listed. The authors were unable to find a formula for the second Zagreb index of graphs realizing $S_{i,n}$ and

state this as an open problem. The authors discuss a conjecture stating that the set$S_{n,n}$ is not Laplacian realizable and provide some related results, including proving that if such graphs exist, they could not be the Cartesian product of 2 graphs. The paper [12] Expected value of first Zagreb connection index in random cyclooctatetraene chain, random polyphenyls chain, and random chain network derived expected values of the 1st ZCI for random cyclooctatetraene, polyphenyls, and chain networks with pentagons, hexagons and octagons,. Examined the mean and strong values of the Zagreb connection index among the different random chain types. The Zagreb connection indices provide insights into the connectivity characteristics of molecular graphs, with applications in areas like discovering drug, research on physico-chemical, and chemistry. Builds on previous work exploring values of other topological indices for random chain structures.

Theorem 12 [12]For $l \geq 2$, the expected value for the 1st Zagreb connection index of COC_l is
$E\ (ZC_1\ (\ (COC_l))\ _ = (8k_1 + 2k_2 + 76)l\ -\ 16k_1\ -\ 4k_2\ -\ 44$

Theorem 13 [12]For $l \geq 2$, the expected value for the 1st Zagreb connection index of PPC_l is
$E\ (ZC_1\ (\ (PPC_l))\ _ = (8k_1 + 2k_2 + 68)l\ -\ 16k_1\ -\ 4k_2\ -\ 44$

Theorem 14 [12]For $l \geq 2$, the expected value for the 1st Zagreb connection index of PG_l is
$E\ (ZC_1\ (\ (PG_l))\ _ = (6k_1 + 66)l\ -\ 12k_1\ -\ 48$

Theorem 15 [12] The average value for the 1st Zagreb connection index concerning the set G_l is given as
$ZC_l^{avg}(G_1) = \frac{157}{2}l - 49$
The key points to take away from his paper [10] are it calculated the expected values of the 1st Zagreb connection index in a random cyclooctatetraene, random polyphenyls, and random chain network with one, pentagon octagons, and hexagons respectively. It discussed the maximum chain and the minimum chain of the COC_l, PPC_l, and PG_l, respectively, concerning the expected values of these chains. It also discussed the average values considered for all the above random chains for unique chains. The paper [13] On the first Zagreb index of graphs with Self-Loops studies the first Zagreb index $M_1(GX)$ of a graph GX with self-loops is stated as the addition of squares of the vertex degrees. Authors derive upper limits for $M_1(GX)$ in phrase of the maximum and minimum degrees, the number of vertices and edges, and the spectral radius of the adjacency matrix. Lower bounds for $M_1(GX)$ are also provided, using the eigenvalues of the adjacency matrix. The results extend a existing literature on degree-based topological indices and the energy of graphs with self-loops.

Theorem 16(For Upper Bound) [13]Let $G_X$be a graph obtained from a simple graph G of order $n$and size $m$by attaching a self-loop at each vertex of $X \subseteq V(G)$with $|X| = \sigma$, Then
(Table: 1)

Theorem 17(For Lower Bound)[11] Let $G_X$be a graph obtained from a simple graph G of order $n$and size $m$by attaching a self-loop at each vertex of $X \subseteq V(G)$with $|X| = \sigma$. Then (Table: 2)
The paper [2] Extremal values of the first reformulated Zagreb index for molecular trees with application to isomers octane 1st reformulated Zagreb index is a topological index that will assist out to estimate the chemical or physical properties of molecules. Authors derived formulas to calculate the 1st reformulated Zagreb index of molecular trees and determined the extremal trees that reach the minimum and maximum values of this index. An application, the authors used the extremal graphs to develop models on regression to guess acentric factor and entropy of isomers octane. The maximum value of 1st reformulated for molecular trees of n order is bounded, and the conditions for attaining this maximum value are provided.

Theorem 18 [2]Let T be a molecular tree of order $n \geq 5$, then the maximum value of the first reformulated Zagreb index is

$$EM_1(T) \leq \begin{cases} 18n-64 & n \equiv 0(mod\ 3) \\ 18n-72 & n \equiv 1(mod\ 3) \\ 18n-54 & n \equiv 2(mod\ 3) \end{cases}$$

and the maximum first reformulated Zagreb index is achieved if, and only if, one of the following conditions is satisfied:

- T has exactly 1 edge of degree 1 and 1 edge of degree 16,
- 2 edges are of degree 4 and degree of 1 edge is 25,
- Degree of all the edges are either 9 or 36.

The proof says if T is a molecular tree with the maximum first reformulated Zagreb index, then T must satisfy one of the following three conditions

- All vertices of T have a degree of either 1 or 4
- Exactly 1 vertex of the graph with degree 2 and remaining are with degree 1 or 4
- Exactly 1 vertex of the graph with degree 3 and remaining are with degree 1 or 4

The proof considers different cases based on the order of the molecular tree (n) modulo 3, and derives the maximum value of EM1(T) for each case.

Theorem 19 [2]Let a molecular tree with n vertices such that $n \geq 5$ and$2 \leq \Delta \leq 4$, then the least value of the 1st reformulated Zagreb index is given as

$$EM_1(T) \leq \begin{cases} n-10 & for\ \Delta = 2, \quad \text{when T is a path} \\ 4n-2 & for\ \Delta = 2, \quad \text{when T contains 2 edges of degree 4} \\ 4n+20 & for\ \Delta = 2, \quad \text{when T contains 3 edges of degree 9} \end{cases}$$

Application to octane isomers in [2] says the acentric factor is a measure of molecules non-centricity, and as the acentric factor increases, down vapor pressure happens, occurs in higher boiling points. Entropy is a measure of the unavailability of a energy system to do work, and the maxi the disorder in a molecule, the maxi the entropy. The authors considered 18 octane isomers and their acentric factors, entropy measures, and the first reformulated Zagreb indices. The authors performed a linear regression and obtained equations with degree 1 that can say the acentric factor and entropy of isomer octane based on the 1st reformulated Zagreb index. The authors found that 2,2,3,3-tetramethyl butane (18th isomer) has the maxi reformulated Zagreb index with the mini acentric factor and entropy, and this implies that chemical trees with the maxi reformulated Zagreb index have the mini acentric factor and entropy measure. The paper [1]focuses on the Circumacenes family of organic molecules, which have potential applications in molecular electronics. The researchers calculate the 1st and 2nd Zagreb indices, as well as related generalized indices, to understand the structure-property relationships of these molecules. The Topological Indices Method (TIM) is used as an efficient and cost-effective approach to predict important thermodynamic properties like gap energy and electron affinity energy. The paper provides mathematical proofs for calculating the 1st and 2nd Zagreb indices for the Circumacenes family. The findings demonstrate the utility of topological indices in predicting the properties of complex organic molecules, which can aid in the development of molecular electronics.

Theorem 20[1]If A_i for $i \in \{1,2,\dots\}$ are simple molecular graphs of Circumacenes $\left(C_{\left(\frac{8i+16}{3}\right)}H_{\left(\frac{2i+22}{3}\right)}\right)$, then $M_1(A_i) = 62i + 94$.

Theorem 21[1] If A_i for $i \in \{1,2,\dots\}$ are simple molecular graphs of Circumacenes$\left(C_{\left(\frac{8i+16}{3}\right)}H_{\left(\frac{2i+22}{3}\right)}\right)$, then for $i \in \{1,2,\dots\}$

$HM(A_i) = 2276i + 3652$

$SC(A_i) = H_{-\frac{1}{2}}(A_i) = -(0.066i + 1.326)$

$H(A_i) = 2H_{-1}(A_i) = -2(0.108i + 0.702)$

Theorem 22[1]If A_i for $i \in \{1,2,\dots\}$ are simple molecular graphs of Circumacenes $\left(C_{\left(\frac{8i+16}{3}\right)}H_{\left(\frac{2i+22}{3}\right)}\right)$, then $M_2(A_i) = 3(29i + 66)$.

This paper [1] explores the thermodynamic properties of Circumacenes, a family of organic molecules, through the calculation of topological indices such as the 1st and 2nd Zagreb indices. The researchers demonstrate how these topological indices can be used to predict crucial thermodynamic properties like gap energy and electron affinity energy using an innovative method called the Topological Indices Method (TIM). The paper provides mathematical proofs for calculating the first and second-Zagreb indices for the Circumacenes family, highlighting their structure-property relationships. The findings show the utility of topological indices in predicting the properties of complex organic molecules, which can aid in the development of molecular electronics.

Extremal Results Of Zagreb Energy

The paper [18] Zagreb Energy of Weighted Graphs studied the concepts of 1st Zagreb and 2nd Zagreb energy for weighted graphs, where edge weights are positive definite matrices. The authors define the weighted first Zagreb and second Zagreb matrices, and derive the corresponding eigenvalues and energies. They then present upper and lower limits for the 1st Zagreb energy of $+ve$ definite matrix weighted graphs, and obtain some limts for number weighted and unweighted graphs as special cases. The paper also includes examples demonstrating the calculation of the first and second Zagreb energies for the methane (CH4) molecule. The example [18] considers the edge-weighted molecular graph of the methane (CH_4) molecule, where each edge has a bond length weight of 0.11 nanometers. The first Zagreb matrix $z_w^{(1)}$ for this graph is

$$z_w^{(1)} = \begin{bmatrix} 0 & 0.55 & 0.55 & 0.55 & 0.55 \\ 0.55 & 0 & 0 & 0 & 0 \\ 0.55 & 0 & 0 & 0 & 0 \\ 0.55 & 0 & 0 & 0 & 0 \\ 0.55 & 0 & 0 & 0 & 0 \end{bmatrix}$$

with eigenvalues -1.1 (once), 0 (3 times), and 1.1 (once). The first Zagreb energy of this weighted graph is calculated to be $Ez_w^{(1)} = 2.2$. The first Zagreb index W_1 for this graph is calculated to be $W_1 = 1.21$. The lower and upper limits of 1st Zagreb energy of this weighted G, are also provided.

It [18] conclude, For an unweighted graph G of order n (≥ 3), the first Zagreb energy is upper bounded as $E_{Z_w^{(1)}}(G) \leq \left|\lambda_{w,1}^{(1)}\right| + \sqrt{(nt-1)\left(2\mathcal{W}_1 - \left(\lambda_{w,1}^{(1)}\right)^2_{!}\right)}$, where $\lambda_{w,1}^{(1)}$ is largest 1st Zagreb eigenvalue and HM is hyper-Zagreb index. For an unweighted graph G of order n (≥ 3) with zero not being an eigenvalue of $Z_w^{(1)}(G)$, the first Zagreb energy is lower bounded as $E_{Z_w^{(1)}}(G) \geq \frac{2\sqrt{\lambda_{w,1}^{(1)}\lambda_{w,n}^{(1)}\sqrt{(2n)\mathcal{W}_1}}}{\lambda_{w,1}^{(1)}+\lambda_{w,n}^{(1)}}$, where $\lambda_{w,1}^{(1)}$ and $\lambda_{w,n}^{(1)}$ are the maximum and minimum of the absolute values of the first Zagreb eigenvalues, respectively. A matrix weighted graph G of order n (≥ 3), the second Zagreb energy satisfies the following bounds $\sqrt{2\mathcal{W}_2} \leq E_{Z_w^{(2)}} \leq \sqrt{2n\mathcal{W}_2}$ where $\mathcal{W}_2$ is 2nd Zagreb index of weighted graph. For an unweighted G of order n (≥ 3) with zero not being an eigenvalue of $Z^{(2)}(G)$, the second Zagreb energy is lower bounded as $E_{Z_w^{(2)}}(G) \geq \frac{2\sqrt{2nM_2^{(2)}\lambda_2^{(2)}\lambda_n^{(2)}}}{\lambda_2^{(2)}+\lambda_n^{(2)}}$, where $\lambda_2^{(2)}$ and $\lambda_n^{(2)}$ are the maximum and minimum of the absolute values of the 2nd Zagreb eigenvalues, respectively, and $M_2^{(2)}$ is the general Randić index with $\alpha = 2$.

The paper [11] On Zagreb energy and edge-Zagreb energy presents several results related to Zagreb and edge-Zagreb energy of graphs. The authors obtain upper and lower limits for the general extended energy of a graph, and apply these results to derive bounds for the Zagreb and edge-Zagreb energy. They also establish a relationship between the Zagreb and edge-Zagreb energy for graphs with minimum degree 2. Additionally, the paper provides a lower and upper limit for the spectral radius of the edge-Zagreb, and describes methods to construct Zagreb and edge-Zagreb equienergetic graphs.

Theorem 23[11] Let $f(d_i)$ and $g(d_i)$ be non-negative real numbers. If $F(d_i;\, d_j) = f(d_i) + f(d_j) + g(d_i).g(d_j)$. Then $\mathcal{E}_T(G) \leq (2f_m + g_m^2)\mathcal{E}(G)$

where $f_m = \max\{f(d_1); f(d_2); \ldots ; f(d_n)\}$ and $g_m = \max\{g(d_1); g(d_2); \ldots ; g(d_n)\}$. Equality attained iff $f(d_1) = f(d_2) = \cdots = f(d_n)$ and $g(d_1) = g(d_2) = \cdots = g(d_n)$.

Theorem 24[11] n vertices. Then

$$\mathcal{E}_T(G) \leq \sum_{i=1}^{n} \sqrt{\sum_{v_i v_j \in E(G)} \mathcal{F}^2(d_i, d_j)}$$

Theorem 25[11] Let graph G with n vertices with degree $\delta \geq 2$. Then

$$Z\mathcal{E}_2(G) \geq \sqrt{\frac{2}{n}} Z\mathcal{E}_1(G)$$

Theorem 26[11] Let graph G with order n, m edges whose minimum degree δ and maximum degree Δ. Then $\delta\,(2m - (n - 1 - \delta)\Delta - \delta) \leq \gamma_1(G) \leq \Delta(2m - (n - 1 - \Delta)\delta - \Delta)$, it hold iff$G$ is a regular.

Theorem 27[11] Consider G_1 be a r_1-regular of order $n_1$andG_2be r_2-regular graph of order n_2. Then the spectrum of Z_1 $(G_1 \vee G_2)$ consists of $2(r_1 + n_2)\lambda_i(G_1)$ and $2(r_2 + n_1)\lambda_j(G_2)$ for $i = 2, 3, \ldots ; n_1$and $j = 2, 3, \ldots; n_2$, and the two roots of the equation $(x - 2(r_1 + n_2)r_1)(x - 2(r_2 + n_1)r_2) - (n_1 + n_2 + r_1 + r_2)^2 n_1 n_2$. The results [11] are The authors obtained upper and lower limits for the general extended energy of a G, and applied these aggregate to derive bounds for the Zagreb and edge-Zagreb energy. They initiate a relationship between the Zagreb and edge-Zagreb energy for graphs with minimum degree 2. The paper provided a lower and upper limit for the spectral radius of the edge-Zagreb. The authors described methods to construct Zagreb and edge-Zagreb equienergetic graphs, and showed that such equienergetic graphs exist for orders $n \geq 9$.The paper [3] On Second Zagreb Energy of Graphs explores the mathematical properties and application potential of the second Zagreb energy (ZE_2). $ZE_2$is derived from the modified adjacency matrix with the 2nd Zagreb index (M_2). The authors investigate the role of ZE_2 in modeling isomer discrimination and physico-chemical. They objective to establish limits on the spectral radius and spread of the M_2-spectrum, and characterize extremal graphs.The ZE_2 is studied for various special graphs, including regular graphs, semi-regular graphs, and chain graphs. A matrix of divisor-type is proposed to approximate the M_2-spectrum and ZE_2 energy of chain graphs. The chemical significance of the second Zagreb energy in the following ways: The authors investigate the role of ZE_2 in modeling physico-chemical of molecules and isomer discrimination. The ZE_2 energy is found to correlate with entropy (S)and acentric factor (AF) of isomer octane. Linear regression models are developed to predict these properties using ZE_2 as a predictor variable. The ZE_2 model explains 90% and 99% of the data variance for entropy and acentric factor, respectively, with reduced errors. The models are shown to be reliable based on the F and SF values. The predictive ability of the ZE_2 model is further validated using a test set of nonane isomers, which shows a data variance of 88%. The discrimination potential of ZE_2 is compared to other well-known descriptors, and it is found to outperform them in isomer discrimination for octane, nonane, and decane isomers.

Theorem 28[3] For a graph G of order n with second zagreb index $M_2(G)$, we obtain

$$\frac{2M_2(G)}{n} \leq \nu_1 \leq \sqrt{\left(1 - \frac{1}{n}\right)\Upsilon_2}$$

whereΥ_2 is given. The right equality holds iff $G \cong \overline{K}_n$or $G \cong K_n$ and left equality occurs iff G is regular.
For lower bound, authors use the Rayleigh-Ritz principle to show that:

$$\nu 1(G) \geq \frac{e^t \Omega(G) e}{e^t\, e} = \frac{2M_2(G)}{n}$$

where e is the all-ones vector.
The upper bound is derived by using the fact that the largest eigenvalue of $\Omega(G)$ is bounded above by the expression given. In summary, Theorem provides tight bounds on the biggest eigenvalue of the 2nd Zagreb matrix $\Omega(G)$ in terms of the 2nd Zagreb index $M_2(G)$ and the parameter Υ_2 from Lemma, with the equalities characterizing the extremal graphs.

Theorem 29[3] Let graph G with $\Delta$and δ, respectively, we have

$\lambda_1\delta^2 \leq \nu_1 \leq \lambda_1\Delta^2$
where equalities occur iff$G$ is regular.
The left equality occurs iff G is a regular. Additionally, it states that: $\lambda_1 \leq \sqrt{2m - n + 1}$ where equality appears iff $G \cong S_n$ or $G \cong K_n$. Moreover, the right equality happensiff G ~= Sn or G ~= Kn.
In summary, Theorem 3 provides tight bounds on the biggest eigenvalue of the 2nd Zagreb matrix $\Omega(G)$ in terms of m edges and the maximum degree Δ, with the equalities characterizing the extremal graphs

Theorem 30[3] For a connected graph G having n (≥ 2) vertices, we have

$$\nu_1 - \nu_n \leq \frac{n}{n-1}\nu_1 + \sqrt{\frac{n-2}{n-1}\Upsilon_2 - \frac{n^2-2n}{(n-1)^2}\nu_1^2}$$

Equality appears iff$G$ is complete or complete bipartite.
From Lemma, we have $\Upsilon_2 - {\nu_1}^2 - {\nu_n}^2 = \Sigma({\nu_2}^2 + {\nu_3}^2 + \ldots + \nu(n-1)^2)$.
Using the Cauchy-Schwarz inequality, we get $\Upsilon_2 - {\nu_1}^2 - {\nu_n}^2 \geq \frac{(\nu^1+\nu_n)^2}{n-2}$.
Rearranging the terms, we obtain $(n-1){\nu_n}^2 + 2\nu_1\nu_n + (n-1){\nu_1}^2 - \Upsilon_2(n-2) \leq 0$.
Solving this inequality for ν_n, we get the desired upper bound on $\nu_1 - \nu_n$.
The equality in the upper limit holds iff$\nu_2 = \nu_3 = \ldots = \nu(n-1)$, which is true if and only if G is complete or complete bipartite. In summary, Theorem provides a tight upper bound on the difference between the largest and smallest eigenvalues of the second Zagreb matrix $\Omega(G)$, with the equality characterizing the complete and complete bipartite graphs.

Theorem 31[3] Let graph G of order $n \geq 3$ having no isolated vertices, we have

i. $ZE_2(G) = r^2\,\varepsilon(G)$, when G is r-regular. Particularly, $ZE_2(K_n) = 2(n-1)^3$ and $ZE_2(C_n) = 8\sum_{i=0}^{n-1}\left|\cos\left(\frac{2\pi i}{n}\right)\right|$ When G is connected $Gs(n, r, a_1, b_1)$, we have
$$ZE_2(G) = r^2\left(r + \frac{2(n-1)(r-b_1) - r(a_1-b_1)}{\sqrt{(a_1-b_1)^2 + 4(r-b_1)}}\right)$$
ii. $ZE_2(G) = \alpha\,\beta\,\varepsilon(G)$, when G is (α,β)-semiregular bipartite. Particularly, $ZE_2(K_{p,q}) = 2\,pq\sqrt{pq}$where $p + q = n$. Also $ZE_2(S_n) = 2\,(n-1)\sqrt{n-1}$

For ZE_2 of regular and semi-regular graphs $G_s(n, r, a_1, b_1)$ can be derived using the M_2-eigenvalues of these graph classes, as discussed in Lemma.

Theorem 32[3] Let $K \cong G(n_1, n_2, \ldots, n_h; m_1, m_2, \ldots, m_h)$be a chain graph of order n. Then

$$ZE_2(G) \geq 2\sqrt{\sum_{i=1}^{h}(h+1-i)m_i n_i(\alpha_i\beta_i)^2 + h(h-1)\prod_{i=1}^{h}\left[(m_i n_i)^{\frac{1}{h}}(\alpha_i\beta_i)^{\frac{2}{h}}\right]}$$

with equality occurs iff$G \cong K_{n_1,m_1}$ where $(n_1 + m_1 = n)$.
For the case $h = 1$, where $G \cong K_{n_1,m_1}$, the M_2-spectrum of G is known, and the equality in the theorem holds.
For the case $h \geq 2$, the graph G contains an induced subgraph P_4, and by Lemma, the second M_2-eigenvalue $\nu_2(G)$ is greater than 0. This implies that the inequality in the theorem holds strictly for $h \geq 2$. The proof constructs a divisor type matrix to approximate the M_2-spectrum andZE_2 of the chain graph G. Therefore, the lower bound on the ZE_2 energy of chain graphs and characterizes the extremal graph where the equality is attained. The paper [10] More on Second Zagreb Energy of Graphs investigated the 2nd Zagreb energy of a graph is stated as the sum of eigenvalues of the 2nd Zagreb matrix. The authors expressed the relationship between the 2nd Zagreb and the adjacency matrix of a G. They use this relationship to establish new upper limits for the 2nd Zagreb energy. Authors also evaluate the 2nd Zagreb energy of m-splitting and m-shadow graphs. The general results are(Table:3). The paper [9] Hub Zagreb Energy Of Graphs discussed the 1st hub Zagreb matrix and 2nd hub Zagreb matrix of a graph are defined. The first hub Zagreb energy is the combination of eigenvalues of the 1st hub Zagreb matrix. Bounds for the 1st hub Zagreb energy are established in hub and hyper-hub Zagreb index. The hydrocarbons hub Zagreb energy is calculated

Theorem 33 [9] Let a graph G with hyper-hub Zagreb index $HZ_2^*(G)$ Then $e^{-\sqrt{2HZ_2^*(G)}} \leq HZE_1(G) \leq e^{\sqrt{2HZ_2^*(G)}}$

Theorem 34 [9] Let a graph G with hyper-hub Zagreb index $HZ_2^*(G)$ Then $HZE_1(G) \geq \frac{1}{2HZ_2^*(G)}$

Theorem 35 [9]Let a graph G with $n \geq 2$vertices. Then
$HZ_1(G) \geq \frac{1}{n}\Psi(G)$, where $\psi(G) = \sum_{v \in V(G)} {d_h}_i^2(v)$
The paper [9] conclude first hub Zagreb energy is the combination of eigenvalues of the first hub Zagreb matrix of a graph. The 1st hub Zagreb energy can be bounded in the hub and hyper-hub Zagreb index. The hydrocarbons hub Zagreb energy can be calculated. The coefficient of characteristic polynomial of the first hub Zagreb matrix has specific properties, such as the constant term being 1 and the coefficient of the linear term being the trace of the first hub Zagreb matrix. The 1st hub Zagreb energy is related to the energy of the graph. Specifically, the first hub Zagreb energy is equal to the addition of the squares of the eigenvalues of the first hub Zagreb matrix. The second hub Zagreb energy is related to the energy of the graph in a similar way, with the second hub Zagreb energy being equal to the sum of the cubes of the eigenvalues of the 2nd hub Zagreb matrix. The 1st and 2nd hub Zagreb energies can be expressed in terms of the energy of the graph and the total number of vertices. The paper [14] Energy and spectral radius of Zagreb matrix of graph with applications the Z-matrix of a simple graph is a square symmetric matrix, where the ij^{th} entry is the addition of the degrees of vertices i^{th} and j^{th} if they are neighbour, and 0 else. The Zagreb eigenvalues are the Z-matrix eigenvalues, and the Zagreb energy is the total of the eigenvalues. Authors studied the variation in the Z-spectral radius during the deletion and rotation of edges, and provided bounds for the Z-spectral radius. They also investigated the relationship between the Zagreb energies of graphs obtained by identifying vertices or adding edges. The paper highlights the strong $+ve$ correlation (correlation coefficient of 0.9989) between the acentric factor of n-alkanes and the energy of the Z-matrix. Novel correlations between the refractive index of m-alkanes, density, and the spectral radius of the Z-matrix were also noted.

Theorem 36 [14]Let Γbe a graph and edge $e = uv$ in Γand let $d_u$and $d_v$be the degrees of uand v in the resultant graph $\Gamma - e$. Then $Z\mathcal{E}(\Gamma - e) \geq Z\mathcal{E}(\Gamma) - 2\left(\sqrt{d_u} + \sqrt{d_v} + d_u + d_v + 2\right)$ equality if and only if $e = uv$is an isolated edge in Γ.
The application discussed in the paper [14] are the importance of pi-electron energy in material science and chemistry, as it is important to explaining interactions of electron in the pi-orbitals of neighbor atoms in conjugated systems such as twice bonds and rings in aromatic. The total pi-electron energy can be obtained the Hückel molecular orbital (HMO) theory, which can provide crucial property of conjugated molecules. The correlation between the Zagreb energy and the energy of pi-electron of polyenes and compounds of vinyl, with a correlation coefficient of 0.9971. This suggests that the Zagreb energy can be used to predict the pi-electron energy of these compounds. The strong $+ve$ correlation (correlation coefficient of 0.9989) between the acentric factor of m-alkanes and the Zagreb energy, which can be used to predict the acentric factor of m-alkanes. The positive correlations between the density and refractive index of n-alkanes with the Zagreb spectral radius, with correlation coefficients of 0.9821 *and* 0.9693, respectively. This indicates that the Zagreb spectral radius can be used to predict the density and refractive index of n-alkanes. The paper [15] On Zagreb Energy of Certain Classes of Graphs discussed the 2nd Zagreb energy is always greater than the first Zagreb energy for all complete bipartite graphs of even order greater than or equal to 4, and for complete tripartite graphs. The two Zagreb energies coincide exclusively for the complete bipartite graph with equal partite sets if and only if the graph is of order 2. The paper provides a criterion leading to an infinite set of non-isomorphic Zagreb equi-energetic graphs for all $r > 1$ within partite graphs. The computations of the two Zagreb energies for graph operations like t-splitting graph and t-shadow graph are illustrated. Bounds on Zagreb energies for wheel graphs are discussed.

Theorem 37 [15] Let G be a k-regular of order r. Let $A(G), A[FZ(G)], A[SZ(G)]$ denote respectively the adjacency, first Zagreb and second Zagreb matrices of G. If λ is an eigenvalue of $A(G)$, then
1. $2k\lambda$will be an eigenvalue of $A[FZ(G])$;
2. $k^2\lambda$will be an eigenvalue of $A[SZ(G)]$.

Theorem 38 [15] Consider G to be a k-regular of order r. Then the first Zagreb spectrum of t-splitting graph of G are 0 with multiplicity $t-1, \frac{(t+1)\pm(\sqrt{t^3+5t^2+6t+1})}{2}$ each with multiplicity 1and the first Zagreb energy of t-splitting graph of G is given by $\sqrt{t^3+5t^2+6t+1}\mathcal{E}[FZ(G)]$

Theorem 39 [15] Consider G to be a k-regular of order r. Then the 2nd Zagreb spectrum of t-splitting graph of G are 0 with multiplicity $t-1, \frac{(t+1)[(t+1)\pm(\sqrt{t^2+6t+1})]}{2}$ each with multiplicity 1and the 2nd Zagreb energy of t-splitting graph is given by $\mathcal{E}[SZ(S^t(G))] = [(t+1)\sqrt{t^2+6t+1}]\mathcal{E}[SZ(G)]$.

CONCLUSION

This paper collected all the optimal results on the Zagreb Index energy of past decades and presents some Theorem in order to motivate readers to focus to results on this topic.

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Table. 1: Theorem 16

Graph G_X	Upper bound
$\Delta$and$\delta$of graph G_X.	$M_1(G_X) \leq \frac{4(m+\sigma)^2}{n} + \frac{n(\Delta-\delta)^2}{3}$
$\lambda_1(G_X)$ is the largest eigenvalue of $A(G_X)$	$M_1(G_X) \leq n(\lambda_1^2(G_X)+1) + 4(m+\sigma)$
$E(G_X)$ is the largest eigenvalue of $A(G_X)$	$M_1(G_X) \leq n(\lambda_1^2(G_X)+1)$
$E(G_X)$ is the energy of $A(G_X)$	$M_1(G_X) \leq n\big(E(G_X)\big)^2 - 2m(n-2) - \sigma(n-2\sigma-4) - n$
$\overline{G_X}$is also connected	$M_1(G_X) + M_1(\overline{G_X}) \leq n(n^2-1)+4$
$G_X$be obtained from a simple graph G	$M_1(G_X) + M_1(\overline{G_X}) \leq n^2(n+1)^2 + 4(m+\sigma)\big(2(m+\sigma) - n(n+1)\big)$
Gbe a connected $K_{\omega+1}$-free graph of n vertices and $m > 0$ edges, where $2 \leq \omega \leq n-1$	$M_1(G_X) \leq 8(m+n) + (2m-4)n - \frac{2mn}{\omega}$
Triangle-free graph	$M_1(G_X) \leq 8m + (m+4)n$

Table. 2: Theorem 17

Graph G_X	Lower bound
Regular G_X	$M_1(G_X) \geq \frac{4(m+\sigma)^2}{n}$ $M_1(G_X) + M_1(\overline{G_X}) \geq \frac{1}{n}[n^2(n+1)^2 + 4(m+\sigma)(2(m+\sigma) - n(n+1))]$
Maximum degree Δ	$M_1(G_X) \geq \frac{1}{n-1}[4(m+\sigma)(m+\sigma-\Delta) + n\Delta^2]$
Minimum and maximum degree δ andΔ	$M_1(G_X) \geq \frac{1}{n-2}[4(m+\sigma)(m+\sigma-\delta-\Delta) + (\delta+\Delta)^2] + \Delta^2 + \delta^2$
$\lambda_1(G_X)$ be the spectral radius	$M_1(G_X) \geq \lambda_1^2(G_X)$
λ_1 and $E(G_X)$ respectively be the spectral radius and energy	$M_1(G_X) \geq \frac{\lambda_1}{n^2}\left[\big(E(G_X)\big)^2 + \sigma^2\right]$
$\overline{G_X}$ be connected	$M_1(G_X) + M_1(\overline{G_X}) \geq \begin{cases} \frac{n(n+1)^2}{2} & odd\ n\ and\ even\ \frac{n+1}{2} \\ \frac{n(n+1)^2}{2} + 2 & odd\ n\ and\ oddd\ \frac{n+1}{2} \\ \frac{n}{2} + \frac{n(n+1)^2}{2} & even\ n \end{cases}$

Table. 3: Theorem 32

Properties	Second Zagreb
D be the diagonal matrix of order n	$\boldsymbol{Z} = DA(G)D.$
G possesses isolated vertex	$det(\boldsymbol{Z}) = det(A(G)) = 0$
G does not possess isolated vertices	$\det(z) = \prod_{i=1}^{n} d_i^2 \det\big(A(G)\big)$

metrix of graph G	$tr\,(Z) = 0$ $tr\,(Z^2) = 2\sum_{i\sim j} d_i^2 d_j^2$ $tr\,(Z^3) = 2\sum_{i\sim j}\left(d_i^2 d_j^2 \sum_{k\sim i,j} d_k^2\right)$ $tr\,(Z^4) = \sum_{i=j}\left(\sum_{i\sim j} d_i^2 d_j^2\right)^2 + \sum_{i\neq j}\left(d_i^2 d_j^2 \sum_{k\sim i,j} d_k^2\right)^2$
N vertex	$ZE_{(2)}(G) \leq \sqrt{2n\sum_{i\sim j} d_i^2 d_j^2}$
Regular graph	$ZE_{(2)}(G) = r^2 E(G)$
non isolated vertices	$ZE_{(2)}\left(Spl_m(G)\right) = (m+1)\sqrt{m^2+6m+1}ZE_{(2)}(G)$ $ZE_{(2)}\left(D_m(G)\right) = m^3 ZE_{(2)}(G)$

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REVIEW ARTICLE

Named Entity Recognition for Indic Languages using Deep Learning: A Review

Ravi Patel[1]* and Bhagirath Prajapati[2]

[1]Research Scholar, Faculty of Computer Engineering, C.V.M University, Vallabh Vidhyanagar, Gujarat, India.
[2]Head and Associate Professor, Computer Engineering Department, A. D Patel Institute of Technology, (Affiliated to C.V.M University), Vallabh Vidhyanagar, Gujarat, India.

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***Address for Correspondence**
Ravi Patel
Research Scholar,
Faculty of Computer Engineering,
C.V.M University, Vallabh Vidhyanagar,
Gujarat, India.
E.Mail: Patelravigs32@gmail.com

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ABSTRACT

Named entity recognition, often known as NER, is the process of recognizing and categorizing named entities that are existed in to the text. Entities in the text are the general classes like People, Organizations, and Months. In many applications of Natural Language processing (NLP) like Chatbot, Text Summarization, NER plays significant role to identify the Entities and classifying them accurately into the predefined classes. Recently published work shows that how NER has achieved higher F-1 score and Accuracy for English language which has large publically available datasets as well as developed NLP components like Word Embedding and POS Taggers. Named Entity recognition is challenging task for low resource languages like Indian Languages. In this paper, how different authors have improved the NER performances of different Indian Languages using different approaches are discussed. This paper also elaborates the Datasets for Indian languages, methodologies and number of alternative approaches that have been published so far, including rule-based methods, machine learning-based methods, and deep learning approaches for NER. At the end of this paper the future scope for improving the performances of Indian Language will be discussed.

Keywords: Named Entity Recognition (NER), Natural Language Processing (NLP), Deep Learning, Machine Learning, Pre Trained Embedding Model.

INTRODUCTION

Named Entity Recognition (NER) is an artificial intelligence based technique that uses machine learning, and deep learning approach that automatically recognizes Named Entities such as Person, Location, Organization, etc. from unstructured text [1]. Chabot, text summarization, and other applications rely heavily on NER as these entities play vital roles to process output. The NER operation can be carried out using various approaches. Most of the research work have been done on English language as it is one of the most popular language in western countries. In the era of Artificial Intelligence, demands for the development of the application in the native language is increasing greatly. Most of the research works for improving performance of AI based application have been done on English language as it is one of the most popular language in western countries. Even in India, English language is playing important role in all aspect. But the people of different Indian states prefer region language for any task. Different challenges of Indian Languages are making difficult to develop high performance NLP application. One of the major tedious task is NER for Indian Languages. The reason behind this study is to get the insights how different authors tried to improve the performance of the NER for any Indian languages. Here some of the challenges [2] are discussed:

Limited Resources: There are very limited resources i.e. Dataset available to train the model with Indian Languages. It is very tedious task to tag manually each word with the Entity manually as lack of predefined entity tagged dataset.

Morphological Richness: the structures and words of the sentences contain multiple level of information that makes this task very difficult for getting expected output.

Lack of NLP components for Indian Languages: POS taggers and Word Embedding are crucial components for NLP application. Part of speech tagger used to identify the verb, Noun etc. From the sentences and tagging them with the correct tags. Word Embedding is used to map words with the numerical vectors based on the context of the words and sentences. For English language high performance components are developed but for Indian Language very limited tools are available.

Language Complexity: Ambiguity is one of the major issue for Indian Languages. Some text can have different context for different sentence. For example "વિરાટએભારતમાટેવિરાટછે." Here first "વિરાટ" is the Proper Noun and the meaning of second "વિરાટ" is giant that is an adjective. Identification of such tags is the complex task for the Indian Language.

Any NLP application process will start with the input of the any unstructured data. After that, this text will move toward the pre-processing steps, such as eliminating stop words or noise removing process. NER systems are quite effective for the English language as for this language many POS tagger are already implemented, but for other language native POS taggers [3] and NER taggers are essential to identify important aspect of the text for the Indian language. POS is a crucial component while machine learning models are trained for NER and aids in determining a word's context. For English language, standard library like Stanford NER are available. Also Stemming and Lemmatization process will help to get the root word. Three major methods for implementing Named Entity Recognition are Machine Learning (ML), Rule-Based NER, and Hybrid NER methods. Currently more research are focusing on Deep Learning model for efficient NER in Indian languages [2]. This method work with neural networks for recognizing complex patterns and correlations within the dataset. This will help to enhance the accuracy and performance in entity identification across various linguistic structures.

Named Entity Recognition Approaches

Rule Based Approach

This method identifies a list of different rules for matching the entities and tagging the same with predefined entities tags. This will require a lot of effort to create linguistic lookup dictionaries for these kind of rules. The primary issue

with this strategy, is that entity may not be handle the ambiguity in named entities or those named entities that contain multiple words. This means for Low resource like Indian languages, this methods perform poorly for tagging entities. List Lookup Approach and Linguistic Approach are two different rule-based approaches for Named Entity Recognition [4]. Linguistic analysis extracts speech patterns by observing linguistic aspect and surrounding contextually identified entities. The drawbacks of these rule-based methods are that they are will not fitted to other languages or domains and need extensive experience and grammatical knowledge of the specific language or subject. This limitation can lead to misclassification or incorrect interpretations, ultimately affecting the accuracy of natural language processing tasks that rely on precise entity recognition. To address these challenges, advanced techniques such as machine learning and deep learning algorithms are being employed to enhance the robustness of entity recognition systems, allowing them to better understand context and resolve ambiguities in complex named entities [5].

Machine Learning Approach

In NER using Machine Learning systems, the Named Entity Recognition technique employs a supervised classification statistical model to classify the suitable NER tags for the text tokens. This models follows the statistical methods and supervised learning algorithms for classification of NER tags in predefined groups of entities. This method searches for patterns and relationships within the text so. This methods resolves the context ambiguities of similar kinds of words for tagging the correct entities.

Deep Learning Approach

The concept of neural networks in deep learning, contain the multiple processing layers to acquire knowledge of data representations with different levels of abstraction. It is the ability of representation learning and the semantic composition that is enabled by both the vector representation and neural processing that is the primary advantage of deep learning. This ability enables a machine to be fed raw data and automatically discover latent representations and processing that is required for classification or detection. The forward pass and the backward pass are the two types of passes that are typically used in artificial neural networks. The forward pass is responsible for computing a weighted sum of their inputs from the layer below it and then passing the result through a non-linear function. On the other hand, the backward pass is responsible for computing the gradient of an objective function with respect to the weights of a multilayer stack of modules with respect to the weights of the module stack [6].

Literature Survey on NER on Indian Language

As we discussed earlier, researchers have done significant work to improve the NER performance in Indian languages. To train the NER models, some researchers have used different available language specific dataset or have developed the new dataset from the publically available resources like news, Wikipedia, twitter data etc. In this research paper, the performances of different NER models on Indian languages from recently published works are described. Most of the works on Indic NER are done using the Deep Learning models. The F1 Score of the different NER models is described in the results in Table 1.

Deep Learning Based Indic NER Model

The NER model that uses deep learning method follow General Processing Pipeline as defined in Fig.1. In Indic NER Model, The inputs are any Indic Text data from various Indian Resources. Then it will be passed through the Preprocessing steps for different functions like annotation, POS etc. Researchers may choose the pre trained embedding models from various available language specific or multilingual language pre trained models. This will significantly improve the results of Indic NER. Researchers may choose different models for processing the text like rule based models, deep learning models like BiLSTM or Hybrid models. Based on the data set and application, single model can be replaced with multilayer and multiple models. The output of this models will process through the tag decoders like CRF or Softmax functions used to get the probabilities distributions for all the Entities tags. Higher probability tag will be considered as the final Named Entity for that token. This approach is more suitable for Named Entity Recognition on various Indian languages.

Indic Dataset Source

Data Source plays significant role for any NLP applications especially for Low resource languages. To train the model, researchers use publically available data source or combine the multiple data sources for getting the best performance. The Mukhyansh dataset includes more than 3.39 million article-headline pairs for Indian languages, including Hindi, Tamil, Kannada, Gujarati, Punjabi, Marathi, and Telugu [21]. For Multiple Indian Languages, Naamapadam is one of the largest dataset for Named Entity Recognition (NER).It contains the data of two language combination for almost 11 languages. The dataset contains more than 400k sentences from different indian languages like Bengali, Gujarati, Hindi, Kannada, Malayalam, Marathi, Punjabi, Tamil, Telugu, Assamese and Oriya [22]. L3Cube-MahaCorpus monolingual dataset is developed from different internet sources for application of sentiment analysis or NER on Marathi Language [23]. The CFILT-HiNER dataset is made from various government information web pages, and newspaper articles for Named Entity Recognition in Hindi language [24].

Pre-trained Embedding

In the context of natural language processing (NLP), embeddings refer to word or phrase representations that have been learned from a large corpus of text data before being applied in specialized tasks. These embeddings hold meaning in relation to one another and therefore, models are able to utilize this information without the need for extensive retraining for each individual task. With pre-trained embeddings, time and computation is saved, as models can tap into knowledge from large text corpuses that have been previously processed. This means that demanding training on smaller, task-oriented datasets is no longer necessary. Models perform better in sentiment analysis, machine translation, and text summarization when starting off with pre-trained embeddings that contain a wealth of linguistic information [25]. Typically, pre-trained text embeddings come from self-supervised models. A model like GPT or BERT can derive representations of a language through self-learning from vast datasets. Transfer learning is the method through which these embeddings generally improved on specific tasks. This shows the model improves performance by tailoring the previously learnt information to the particular needs of a new task. [26] Word Embeddings like Word2Vec and GloVe focus on individual words and their contexts, providing dense vector representations that capture semantic similarities. [27]. More recent advancements include contextualized representations such as ELMo and BERT, which take into account the context in which words appear, allowing for a more nuanced understanding of meaning. Pre-trained embeddings are essential to recent natural language processing (NLP) because they offer fundamental knowledge that improves the efficacy and efficiency of a range of language processing activities. They are essential tools for creating intelligent language models because of their capacity to capture intricate semantic links. For Indian languages, Indic FastText is one of the most popular word embedding for NLP application.

Deep Learning Model

The implementation of several deep learning architectures, including Long Short-Term Memory (LSTM) networks, Convolutional Neural Networks (CNNs), and Transformers, has drastically changed the Named Entity Recognition (NER) landscape. The ability of LSTM networks to capture the sequential dependencies seen in languages is essential for interpreting context in Indian languages, which frequently have complex grammatical structures. The ability of CNNs to extract local features from input sequences, on the other hand, allows them to identify patterns and hierarchies in data. This method is especially useful for NER tasks that involve named entities embedded in lengthy texts. More recently, Transformer-based models like BERT have shown impressive effectiveness by utilizing self-attention processes, which enable context-aware representations of entities regardless of their sentence position. [28] In order to improve NER performance in the complex setting of Indian languages and increase its applicability across multiple areas, these developments represent a varied methodological framework [29].

Tag Decoder

A tag decoder is the last step in Named Entity Recognition (NER), which converts context-dependent representations of a sentence's words into a set of entity tags that specify the kind of entity to which each word belongs (e.g., PERSON, LOCATION, ORGANISATION). In basically, it is the component of the model that "decodes" the classified data into labels that may be used. Usually, a machine learning methods have been used by the tag decoder,

frequently consisting of: The Softmax layer [30] of a multilayer perceptron combines word representations to forecast each word's most likely tag. Conditional Random Fields (CRF)[31] are a common option for tag decoding since they can frequently achieve higher accuracy and take into account the dependencies between tags. They are often used in deep learning NER models.

CONCLUSION AND FUTURE WORK

For Indian Language, Named Entity Recognition is one of the complex process for any natural language processing applications. Due to many challenges of Indian languages, existing approach shows the poor results compared to the English Language. Accuracy is one of the important performance measure to understand the performance of the Indian NER system. Due to lack of POS Taggers and embedding system for Indian languages, very few research work have been done especially for Gujarati, Sindhi, Marathi etc.. Recent researches for Indian Named entity recognition suggest that Deep learning approach will significantly improve the results of NER on Indian languages. For Deep learning approach, multilayer or single layer BiLSTM model will achieve good results as these models process through both directions of the Indian language. Also adding CRFs layer for decoding the tags from the predefined entities class, will significantly improve the accuracy as well as F1 Score for the NER system.

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Table 1: Indic NER using Different approach and performances

Sr No	Reference	Language of NER	Model	Data	Performance
1	Litake *et al*. [7]	Marathi	BiLSTM	25,000 sentences from L3Cube-MahaNER	F1 Score : 80.4 Accuracy : 94.99
2	Nandigam *et al*.[8]	Kannada	BiLSTM – CRF	6530 Kannada-English code mix tweets.	F1 Score : 96
3	Ali *et al*. [9]	Sindhi	Bi-LSTM-CRF	SiNER : contain 1338 news articles	F1 score : 89.16
4	Ahmad *et al*. [10]	Punjabi	RNN	Sanmukhi Corpus : 318,275 tokens	F1 Score : 85
5	Laishram *et al*. [11]	Manipuri	Bi-LSTM + Skip Gram Embedding	3504 words from "The Sangai Express" Manipuri news paper	F1 Score: 94.98
6	Pathak *et al*. [12]	Assamese	BERT based Multilingual Representations for Indian Languages (MuRIL)	AsNER : 24,040 sentences from the translated speech of the Prime Minister published by Press Information Bureau, Govt. of India. Text from Assamese Wiki source and Wikipedia	F1 Score: 80.69
7	Dalai *et al*. [13]	Odia	Word Embedding-charCNN-BiLSTM-CRF	OdNER contains 48,000 sentences having 6,71,354 tokens	F1 Score : 88.78
8	Annie Rajan and AmbujaSalgaonkar [14]	Konkani	CRF	1000 Konkani sentences from Technology Development for Indian Languages (TDIL)	F1 Score : 73
9	M. Z. Haque *et al*. [15]	Bangla	BiLSTM + FastText Embedding	B-NER : 22,144 Bangla sentences from Bangla newspapers and Bangla Wikipedia.	F1 Score : 76
10	Sanjib Narzary *et al*. [16]	BODO	LSTM+Char Embedding	1,50,000 raw bank data from the IFSC Code Dataset repository which is provided freely by Razorpay	F1 Score : 99.35
11	R. Rajimol and V. S. Anoop [17]	Malayalam	BiLSTM	204833 entity tagged words from CUSAT	F1 Score : 95.26
12	Sharma *et al*. [18]	Hindi	MuRIL + CRF	ICON 2013 Hindi	F1 Score :

					85.77
13	Rajesh Mundotiya *et al*. [9]	Bhojpuri	LSTM-CNN-CRF	228,373 tokens for Bhojpuri language	F1 Score : 61.41
14	Gorla *et al*. [20]	Telugu	BERT embeddings + BiLSTM layer	767,603 tokens	F1 Score : 95.37

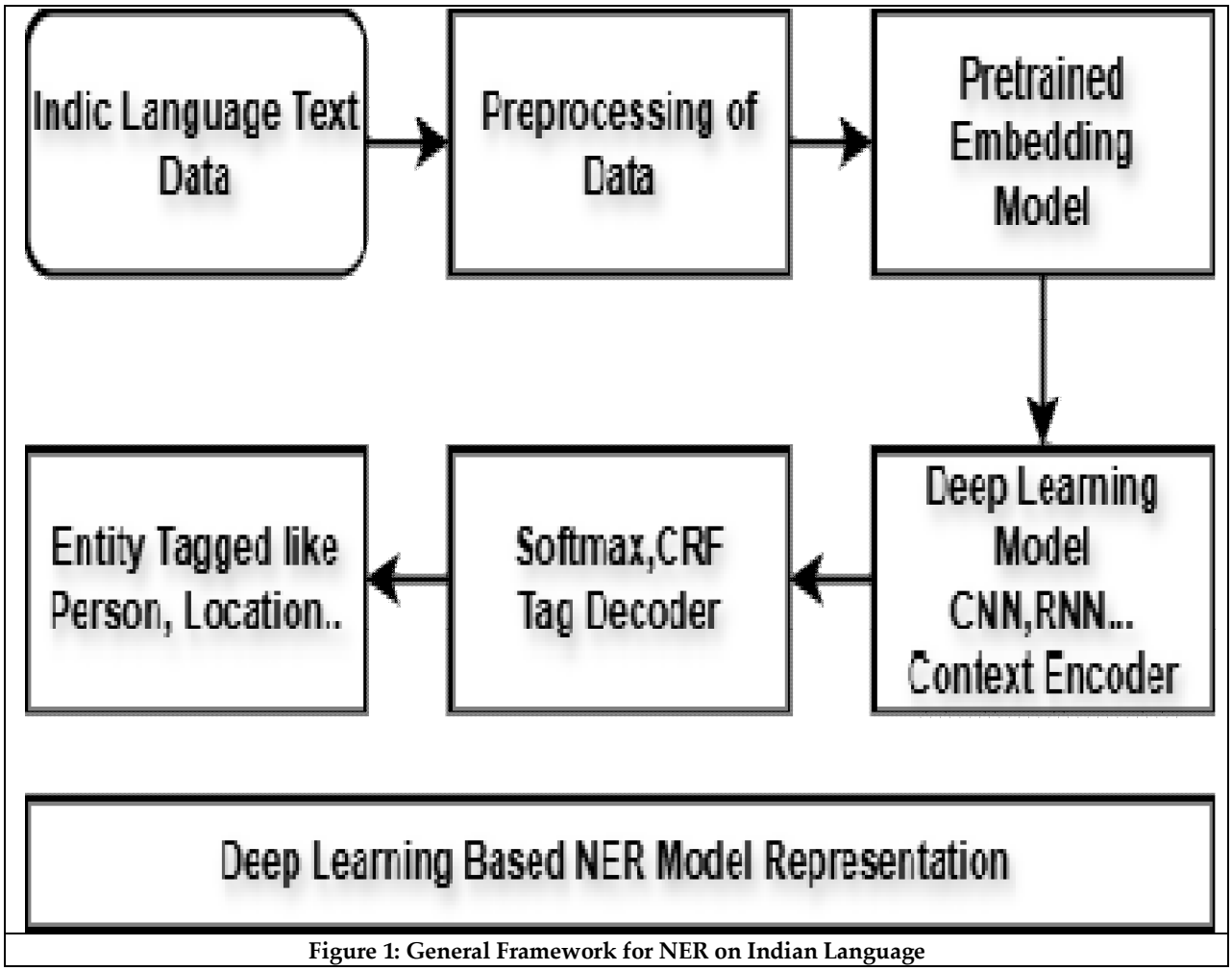


Figure 1: General Framework for NER on Indian Language

RESEARCH ARTICLE

A Study on Quality of Work Life among the Employees Working in Aavin

K Umar[1]*, and C.Sathish Kumar[2]

[1]Second Year MBA Student, School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.
[2]Assistant Professor (SG), School of Management, Hindustan Institute of Technology and Science (Deemed to be University), OMR Road, Padur, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
K Umar
Second Year MBA Student,
School of Management,
Hindustan Institute of Technology and Science (Deemed to be University),
OMR Road, Padur, Chennai, Tamil Nadu, India.

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ABSTRACT

Quality of work life refers to happiness or dissatisfaction with one's career. There is an attempt to examine the Quality of work-life among employees in Aavin. The research design chosen is descriptive in nature. The sample size taken to conduct the research is 100 employees. For this study, the sampling technique chosen is convenience sampling. A structured questionnaire was used for primary data collection. Secondary data was collected from earlier research work, various published journals, websites, and online articles. Simple Percentage is the tool used for data analysis. The investigation has pointed out that the Quality of Work Life factors include pay package, health and safety in the work environment, training and development, organizational environment, and stress involved in the work. This study also covers the employees' overall satisfaction in the organization.

Keywords: Employee Satisfaction, Organizational Environment, Quality of work life, Stress, Work Environment.

INTRODUCTION

Work is an integral part of our everyday life, as it is our life, career, or business. On average, we spend around twelve hours daily in the workplace, which is one-third of our entire lives; it influences the overall quality of our lives. It should yield job satisfaction, give peace of mind, a fulfillment of having done a task, as expected, without any flaw, and having spent the time fruitfully, constructively, and purposefully. Even if it is a small step towards our lifetime goal, it gives satisfaction and eagerness to look forward to the next day.

Problem of the Study

An organization's quality of work life is essential for its employees' smooth running and success. The work-life balance must be maintained effectively to ensure all employees run at their peak potential and are free from stress and strain. The quality of work life can affect such things as employees' timings, work output, available leaves, etc. Quality of work life helps employees feel secure and like they are being thought of and cared for by the organization where they work. An organization's HR department assumes responsibility for effectively running the Quality of Work Life for its employees. This is the real fact, and there was absenteeism and a lack of job satisfaction among the workers in the Co-operative Milk Society at Chennai. The current study aims to analyze the Quality of work-life among workers.

Need of the Study

Though monetary aspects play an essential role in motivating employees, organizations worldwide have come to understand that many other elements contribute to better employee performance. It is these aspects that form the basis for this study. In particular, this study aims to identify the various tangible and intangible. Aspects that contribute to the quality of the workplace. An organization needs to create a conducive working environment for employees. This study also points out the impact of stress on the quality of work life. This study must ensure that all employees are performing at their peak. Potential, being free from stress and strain, and ensuring all their needs are fully satisfied, leads to employee satisfaction. This study will be used as employee feedback to learn about their current workplace perspective and identify areas for improvement in the organization.

Objectives of the study

Primary objective

To find out the quality of working life of the employees in the Cooperative Milk Society.

Secondary Objectives

- To assess the Quality of Work Life among workers
- To comprehend the connection between the representatives and their workplace.
- To dissect working environment factors influencing the worker's execution.
- To recommend techniques for improving QWL by featuring workers' desires and required changes.

Scope of the Study

- The Scope of the study covers employees of all the departments to understand their point of view concerning the Quality of work-life.
- The scope of the study also includes the employees' response to the various measures taken by the management to make the employees comfortable at the workplace.
- The scope is also widened to determine the employees' expectations from management concerning the above concept.
- The scope also includes the importance of the grievance settlement procedure as a part of the quality of work life of the employees.

Limitation of the study

- Some of the employees are illiterate. So it wasn't easy to collect the exact data.
- There was a problem meeting the employees during the working hours of the factory.
- Some of the employees refused to answer the questions.
- The Employees' quality of work life cannot be analyzed with 100 samples.
- Data collection was challenging because there was no adequate leisure time to speak to the employees, and they hesitated to disclose the truth.

RESEARCH METHODOLOGY

The steps in which the study was carried out involved collecting both the primary and the secondary data. The secondary data was collected first. This data was collected by reading various materials such as books, journals, magazines, newspaper articles, etc., and looking for similar content online (i.e., on the Internet). The study was, therefore, carried out based on the data collected.

Research Design

The design of a research topic explains the type of research (experimental, survey research, correlational, semi-experimental, review) and its sub-type (experimental design, research problem, descriptive case study).
There are three main types of designs for research: Data collection, measurement, and analysis.

Descriptive Research

In a descriptive design, a researcher is solely interested in describing the situation or case under their research study. It is a theory-based design method created by gathering, analyzing, and presenting collected data. This allows a researcher to provide insights into the why and how of research. Descriptive design helps others better understand the need for the research. If the problem statement is not clear, you can conduct exploratory research.

Sampling Techniques

Sampling Methods are selecting a sufficient number of elements from the population. This is also understood as obtaining information about the entire population by examining only a part of it. So a sample can be defined as a subset of the population. The population (universe) of this study is 500. The sample size is 100(ie) of the population. A convenience sampling method was used to collect the data. Convenience sampling is a method researchers adoptto collect market research data from a conveniently available pool of respondents. It is the most commonly used sampling technique as it's incredibly prompt, uncomplicated, and economical. Members are often readily approachable to be a part of the sample.

Methods of Data Collection

In the questionnaire method, the respondents have to answer specific sets of questions. The questionnaire's effectiveness depends on the sincerity and dependability of the respondents. There are several ways in which the questionnaire can be presented, such as open-ended and closed-ended. A good questionnaire should have at least 12-15 questions, of which 8-10 should be closed-ended or multiple-choice questions and 2-3 should be open-ended questions. The questionnaire is mainly used for a sample population to get accurate, efficient, and effective results. The questionnaire is made according to a structured format. The response to the questionnaire can be collected in many ways, such asby mail and telephonic questionnaires.

Primary Data

The primary data are those collected afresh & for the first time, & thus happen to be original in character.

Secondary Data

The secondary data are those that have already been collected by someone else & which have already been passed through the statistical process. The secondary data is collected through the company records, books, journals & magazines.

Data Analysis and Interpretation

Correlation

Table 1: Analysis of Relationship Age of the Respondents and Iam Agree with the Content of the Cantenna Facilities Provided by Dairy Industries

Table 2: Correlations

Inference

There is a positive correlation between age, and I agree with the canteen facilities provided by dairy industries.

Chi-square test

Table 3: Representing the Relationship between the Gender of the Respondents and Whether You Feel Comfortable and agree with your job

H_0: There is no significant association between the two variables

H_1: There is a significant association between the two variables

Inference

The calculated value is more than the tabulated value, so we accept the null hypothesis. So, we infer that no significant relationship exists between gender and whether you feel comfortable and agree with your job.

ANOVA

Table 5: Representing The Differences Between The Qualifications Of The Respondents: Do You Need To Achieve Your Targets Within The Given Period

H_0: There is no significant association between the qualifications of the respondents and whether they need to achieve their targets within a given period

H_1: There is a significant association between the qualifications of the respondents and whether they need to achieve their targets within a given period

Inference:

The calculated value is more than the tabulated value, so we accept the hypothesis. So, we infer no significant variance between respondents' qualifications regarding whether you need to achieve your targets within a given period.

FINDINGS

- Majority 60% of the respondents are female
- 21% of respondents fall under the age group of above 29 years
- 31% of the respondents belong to UG
- 60% of the respondents are married
- 59% of respondents strongly agree that I have a healthy relationship with my superior
- 36% agree how often you will find your work stressful
- 50% are asked which factor motivates you the most
- 34% are how do you feel about the working hours of the organization
- 49% agree that I can maintain a good balance between work and family life
- 34% are you satisfied with how the dairy industry conducted the medical camp
- 40% of respondents are delighted with how confident you are with the machinery given in the working environment
- 32% agree to tell their opinion about the job security in the working organization
- 40% to tell their opinion about the training program conducted in the organization
- 30% agree that you feel comfortable and agree with your job
- 40% of respondents in general, how would you describe relations in your workplace between management and employees
- 34% are you satisfied with the opportunity for employees and career development prevailing in the organization
- 69% of respondents strongly agree that the organisation will provide the safety and health measures

- 60% of respondents say you need to achieve your targets within the given period
- 43% of respondents strongly agree that they agree with the canteen facilities provided by dairy industries

SUGGESTIONS

- Management could look into providing their employees with flexible work hours and stress breaks to reduce the stress experienced by the employees and improve the quality of work-life.
- The management can reduce the working days from 6 to 5, from Monday to Friday.
- The employee grievance handling procedure can be improved.
- The management can change the leave policy by providing adequate casual leave, or permission may be considered.
- The organization's infrastructure can be improved.
- There needs to be utmost care taken in choosing to adopt and implement the policies because they impact each worker and the organization.
- It will be the same. Job-life balance could also be an essential issue within the Human Resource Management field, significantly impacting the productivity and growth of every organization and worker.

CONCLUSION

After discussing with the employees of Aavin and analyzing the different factors contributing to their quality of work life, it became clear that the employees are satisfied but expect an improvement in their career,including an improvement in designation and a financial upgrade. A happy and healthy employee will give better turnover, make good decisions, and positively contribute to organizational goals. An assured good quality of work life will attract young and new talents and retain the existing experienced talents. Quality of work life can affect such things as employees' timing, their work output, their available leaves, etc. Work-life balance must be maintained effectively to ensure all employees run at their peak potential and are free from stress and strain. So, it is up to the organization to focus on its workers and improve their quality of work life so that attrition, absenteeism, and decline in workers' productivity can be checked.

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Table 1: Analysis of Relationship Age of the Respondents and I am Agree With the Content of the Cantenna Facilities Provided By Dairy Industries

Descriptive Statistics			
	Mean	Std. Deviation	N
Age	1.5091	.75109	100
I agree with the canteen facilities provided by dairy industries.	1.5818	.59635	100

Table 2: Correlations

		Age	I agree with the canteen facilities provided by dairy industries
Age	Pearson Correlation	1	-.012
	Sig. (2-tailed)		.902
	N	100	100
I agree with the canteen facilities provided by dairy industries.	Pearson Correlation	-.012	1
	Sig. (2-tailed)	.902	
	N	100	100

Table 3: Representing the Relationship between the Gender of the Respondents and Whether You Feel Comfortable and Agree With Your Job

	Gender * Do you feel comfortable and agree with your job					
		Do you feel comfortable and agree with your job?	Total			
		Strongly agree	Agree	Neutral	Disagree	
Gender	Less than 2yrs	32	49	6	1	88
	2-5 years	4	11	0	0	15
	5-10 yrs	4	3	0	0	7
Total		40	63	6	1	100

Table 4: Chi-Square Tests

	Value	df	Asymp. Sig. (2sided)
Pearson Chi-Square	3.937[a]	6	.685
Likelihood Ratio	5.196	6	.519
Linear-by-Linear Association	.962	1	.327
N of Valid Cases	100		
a. 8 cells (66.7%) have expected count less than 5. The minimum expected count is .06.			

Table 5. ANOVA

ANOVA						
		Sum of Squares	df	Mean Square	F	Sig.
need to achieve your targets within the given period	Between Groups	1.070	3	.357	.623	.601
	Within Groups	60.648	106	.572		
	Total	61.718	109			

RESEARCH ARTICLE

IoT Based Smart Building Management System for Optimized Energy Consumption and Remote Access

Karuna Nikum[1], Kavita Bani[1]* and Sunita Pachori[2]

[1]Associate Professor, Department of Engineering Sciences and Humanities, Thakur College of Engineering and Technology, Kandivali East, (Affiliated to University of Mumbai), Mumbai, Maharashtra, India.
[2]Associate Professor, Department of Engineering Sciences and Humanities, Thakur College of Engineering and Technology, Kandivali East,(Affiliated to University of Mumbai), Mumbai, Maharashtra, India.

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***Address for Correspondence**
Kavita Bani
Associate Professor,
Department of Engineering Sciences and Humanities,
Thakur College of Engineering and Technology, Kandivali East,
(Affiliated to University of Mumbai),
Mumbai, Maharashtra, India.
E.Mail: kavita.bani@tcetmumbai.in

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ABSTRACT

To implement a scalable, low-cost IoT-based Smart Building Management System to optimize energy consumption through automation of parking lights in commercial infrastructures. The study employed a distributed network of wireless motion sensors, Light Dependent Registers (LDR) controllers, and intelligent schedulers to automate parking light operation. The system was deployed in three towers at Magarpatta City, Pune. Energy data was monitored via a section controller hub, comparing pre- and post-automation usage. Offline operation support and switch operating rules were key enhancements. The system demonstrated significant energy savings: 84.5% in Tower 1, 67.6% in Tower 2, and 73.86% in Tower 3. These results were achieved using real-time motion detection, ambient light sensing, and automated scheduling, eliminating manual intervention. The use of offline-capable Integrated Command and Control Center (ICCC) and adaptive switch rules enhanced the system's robustness and deployment flexibility. The findings align with reported IoT benefits in energy systems, showcasing an effective, cost-efficient, and retrofittable solution for large infrastructures. Compared to traditional systems, this implementation reduces energy waste and improves accountability while offering modular scalability and centralized control. Offline-operable IoT system with dynamic scheduling and real-time sensing, applied at scale in an existing commercial infrastructure.

Keywords: Internet of Things, Smart Building, Energy Optimization, Remote Monitoring, Motion Sensors, Lighting Automation, Parking Management

INTRODUCTION

IoT is The Internet of Things (IoT) is an intelligent network that connects and communicates with physical devices through sensors, electronics, and software. It enables devices to be identified, located, tracked, monitored, managed, and securely interact with other systems and cloud-based platforms. As of 2025, IoT has become an integral part of modern life—transforming the way individuals live, work, and interact with their surroundings. The technology now supports a vast array of smart devices, routing and processing sensor data continuously, even in the absence of active internet connections. In industrial and commercial applications, IoT has revolutionized operations by enabling remote monitoring and control of appliances and machinery. Particularly in smart buildings and factories, automation facilitated by IoT has led to significant energy savings, cost reductions, and increased operational efficiency. These systems now empower users with real-time insights into device status and performance, allowing them to control single or three-phase AC appliances, manage electricity consumption, and ensure optimal functionality. With the advent of the Industrial Internet of Things (IIoT), the fourth generation of industrial machines now operate autonomously via cloud-connected frameworks. These machines are managed remotely, enhancing the productivity and quality standards of manufacturing processes. Manufacturers benefit from precise machine functioning, real-time tracking of inventory and devices, early fault detection, and uninterrupted monitoring—features that help prevent production downtimes and enable predictive maintenance. The global growth of IoT is evident. According to industry reports, IoT device adoption surged from 237 million in 2015 to over 923 million by the end of 2021. In 2025, this number continues to grow exponentially, with major industry players investing in smart device innovation and infrastructure expansion. IoT has proven to be a critical enabler of smart automation, scalable solutions, and cross-geographical control under unified platforms. Moreover, modern IoT solutions are designed to offer security, privacy, and flexible user-defined parameters—functioning even without internet or Wi-Fi routers in certain configurations. Ultimately, IoT has enhanced visibility, collaboration, and streamlined operations across sectors. Its role in driving digital transformation, especially in industrial automation and smart infrastructure, remains a cornerstone of future technological evolution. The following section provides a comparative analysis between the proposed IoT-based system and prior works in the field. Comparative Analysis of present work with earlier work Table 1.

Problem Statement

The biggest problem that not many people think of this as an opportunity to save electricity by Parking Lights automation (PLA). In reality, significant cost can be reduced if adopted the automation. In this work, there were multiple problems with the existing system listed as below

1. Almost all the time, all the lights were kept ON even when it was not required.
2. Due to huge parking area the operator fails to operate the switches which are required to be turned on at particular day/time.
3. No accountability was set upon the operators.
4. No information was available if the operators did not switch OFF the lights when needed.
5. It was unknown whether which section of lights was consuming how much electricity.

METHODOLOGY

IoT-Based Smart Building Management System (BMS) for Optimized Energy Consumption and Remote Access system model. Figure 1 shows IoT based smart building management system for optimized energy consumption through architecture diagram.

Remote sensor management

Following are possible Automation types based upon the requirement.

IoT based BMS: This is full-fledged Automation comes up with ICCC (Integrated Command and Control Center). More than one ICCC can also be setup based on the customer requirement

IoT based Chiller Plant System (CPS): Comprehensive solution for Chiller plant including AHU, FCU, VFD integration

IoT based HVAC Automation: Unique product having capabilities to automate all appliances in Heating, Ventilation and Air Conditioning

Wireless/Wired Motion Sensor with inbuilt switching module.

Each device will have an ON and OFF-time and the appliance is switched ON or OFF when the user time and real clock time is equal. By entering 2 different times, the kit will daily switch on the device at the ON-time and switch it OFF when the real time is the same as the off time of the device. This technology makes a system completely automatic and minimizes the need for any human interaction without having to manage any infrastructure physically. IoT based BMS is the most comprehensive product with complete focus on reducing electricity consumption. This BMS system is a wireless retrofit product capable of remotely operating all kinds of regular appliances like heavy HVAC systems (AHU, FCU Split AC), Lighting, Fan, heavy motors, pump etc. The ICCC to access all the appliances and brought together into the single umbrella operations. The complete setup of the ICCC will be wireless and therefore no digging on the walls or rewiring would be required. As per the customer needs multiple ICCC can also be setup based on the requirement. Remote Sensor Management Figure 2.

a. **Distributed Motion Sensor Installation:** Motion sensors controlled lights as per the traffic. It was always made sure that there is always luminescence on all the drive lanes. Now when the vehicle passes or any person walks by through a certain area, that area is luminated through the motion triggered by the wireless Motion Sensors. Also the way Distributed Motion Sensors were installed, the whole parking area was covered under a single umbrella that is unique and solves the real world problem without impacting the safety for the employees and other persons.
b. **LDR/Lux Controller Installation:** LDR Controllers senses the luminescence of the surrounding. As soon as the intensity reaches configured trigger value, lights are turned OFF and vice versa. This is extremely useful and saves a lot on cost when days are sunny but rather adjust the conditions immediately in cloudy conditions as well.
c. **Activating Schedulers:** Schedulers are like advanced timers which can be put to switch ON/OFF an appliance at a predefined time on a given set of day(s). Schedulers were put to switch OFF multiple lights that seemed not required to be ON during a given schedule (e.g. day time, weekends, etc). This resulted in saving electricity from Lights which were left ON unintentionally. These were created to switch ON or switch OFF the appliances at
d. A particular day and time. Also, some schedulers were created to switch ON the appliances at a particular time and then switch it OFF at another time. Through schedulers, we reduced the workload significantly in multiple ways, one of them is that on the exact timing the appliances are getting Switched OFF/ ON. This means that the appliances are now getting operated exactly on the time they were supposed to operate without a single minute delay.
e. This has reduced the labour effort required, prevented the time delay of operators in walking from the office to the LDBs, removed unknown delay in operating an appliance by electrician. In addition to these reasons, appliances are now getting centrally tracked, monitored as well as recorded for the reporting purposes as well.
f. **Activating Switch Operating Rules:** Switch Operating Rules are smart algorithms that can switch OFF any appliance after running for predefined time. At Magarpatta City, these helped switch OFF the lights which are left ON unintentionally. These rules can also be set based on multiple time slots and any particular days of week. After the automation at Tower 2 last year, this year we further optimized the automation at Tower 2 with changed installation approach and optimization in algorithms. Table 2 shows the number of available facilities with the present system and Table 3 shows the features of available facilities.

Implementation of Parking light

In this complete project for the parking light automation installed at Magarpatta City Hadapsar Pune, three Towers (Tower 1, Tower 2 and Tower 3) located at Pune. The Magarpatta City is a premier township with 27 commercial towers at the centre. Each commercial tower is equipped with a huge parking space and around 500+ lights in each. These lights were controlled by LDBs (Light Distribution Boards) through MCBs. Each MCB is operating a group of lights illuminating different sections of the parking area. In Magarpatta Parking, due to unnecessary usage of a large number of lights, receives high electrical bills. It broadly faces the issues with manual operations, lack of operational accountability for appliances, need of an automation system, and focus on electricity usage reduction. Since the solution was provided in phases, it details out the solution process in steps. As per the visit at the plant and observations, we provide different ways of implementing IoT based BMS in the facility. These types will vary in functionalities, features, Installation efforts and cost associated with it, Figure 3 is the representation of Integrated Command and Control Center (ICCC) for same case. Firstly, a survey was conducted, the layout of the area was made and all the parking lights were divided into various groups based on their position. After dividing the lights into various groups, each group was analysed individually based on certain parameters. Some of these parameters are current consumption, minimum illuminance, Motionsensor positioning, availability of sunlight etc. Once all these things were analysed, the motion sensors were ready for deployment. After deployment of motion sensors, all the motion sensors were configured based on the group they were deployed in. There was a deep thought given during the configuration of the sensors as motion sensors alone would result in 25 percent of the total saving. Once all the motion sensors throughout the area were configured, deployment of the LDR sensors (Light Dependant Resistor). The value of the LDR was used to monitor the minimum illumination criteria at all times. The system was then kept running and under observation for a week. Once the observation was complete further optimizations were performed for every group. Few motion sensor modules were added/removed based upon the observation. It was made sure that the whole area is completely covered and highly sensitive. Finally, after optimization, the positioning of all the motion sensors were added to the layout.

Installation and Optimization of Tower 1, Tower 2 and Tower 3

The PLA controls the parking lights of whole Tower 1, 2 and 3, parking space, which indeed has resulted in more savings to the client. The following steps are followed:

Step 1: As the installation of five electrical panels in series with the existing LDBs across Tower 1, 2 and 3. Also, for the reporting and monitoring purpose. section controller (The hub) was installed and given the internet through the Internet dongle. At this point, all the panels were installed and energy consumption is being monitored internally by hub (section controller). This time we have changed the approach and now we were allowed to cover the driveway paths as well. With significant technological advancement recently to save huge power consumption.

Step 2: In the next phase, the installation of motion sensors, LDR Controller, activated Section Controller (Hub), applied Schedulers and Switch Operating Rules and more importantly implemented the concept of Distributed Motion Sensor which is meant for large Parking areas.

At Tower 1 and 2 at Magarpatta City with two panels has been installed at A and B Wings whereas in Tower 3 three panels has been installed at A, B and C. After the installation of Panels and Wireless Motion Sensor, LDR and Routers a comparative study has been made and data was captured through a hub (Section Controller) with automation OFF and ON mode on to get a clear picture of effectiveness of the automation product. Figure 4 shows the actual image of tower installation. The percentage savings at tower 1, 2 and 3 has been shown in results section.

Results and Discussion of Parking Light Automation system

The data highlights the energy savings achieved by automating parking lights across Tower 1, Tower 2, and Tower 3. Each tower has multiple panels controlling the lighting systems. The key metric here is Units per Day (kWh) consumed before and after automation, and the resulting percentage of savings shown in Table 4.

Tower 1 achieved an 84.5% reduction in daily energy consumption — a substantial improvement, mostly driven by significant cuts in usage across both panels.

Tower 2 also saw major savings. While Panel 1 had better efficiency gains (75.8%), Panel 2's savings were more modest (53.1%), lowering the overall average to 67.6%.

Tower 3 had the highest total consumption before automation, but after automation, achieved a strong 73.86% savings, especially with Panel 3 showing exceptional efficiency at 86.6%.
Although the graph image Figure 5, it likely visualizes, Figure 6 shows the actual layout of PTA implementation.

- A bar chart comparing "Before" vs "After" energy consumption for each panel/tower.
- A line or secondary axis showing the percentage savings.

This type of visual would quickly convey

- How drastically consumption dropped.
- How consistent savings were across different panels.

To improve financial performance, reduce carbon emissions and energy costs, and reinvest savings into the guest experience. Light automation offers powerful and feature rich Light Automation Product which suits multiple industry needs. The product suits Street Light Automation, Parking Light Automation, Outdoor Luminaries Automation etc. By automating street lights, lighting and scheme for a diverse urban landscape whilst maintaining a responsible approach to minimizing manual intervention, providing centralized access, multiple channels to operate for functional purposes. The proposed system will be modular in nature, communication between various modules will be wireless and secured, along with low maintenance cost. This Automation Product is based upon the latest IoT(Internet of Things) Technology, based upon the wireless communication between various nodes/modules. Also, the product works flawlessly in the absence of the internet as well in Offline mode. The product - Light Automation simplify complexity with a single, integrated platform that connects all lights and distributed points for monitoring, analysis, and full remote control via rich user interface. This enables you to drive operational efficiency and surely lowering down the electricity bills. This system is designed with customizable GUI, inexpensive embedded system, and it is easy to install, control and monitor with an array of electronic devices widely used in everyday home chores. The microcontroller Raspberry Pi is also connected to the IOT module.

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Table 1: Comparative Analysis of present work with earlier work

Theme / Topic	Description	Supporting References
IoT in Smart Grids	IoT and cloud computing integration enhances demand-side energy management, improving monitoring and real-time control in smart grids.	[1]
Energy Metering and Power Quality	Development of compact, IoT-based energy meters enables better control of power quality and facilitates load optimization in	[2]

	commercial buildings.	
Transition to Smart Infrastructure	Migration from traditional to smart grids in developing nations is crucial for sustainable urban energy systems.	[3]
IoT for Smart Energy Control	Real-time energy monitoring and scheduling using IoT platforms can significantly reduce power wastage.	[4], [5]
Global IoT Market Trends	The IoT energy market is expected to grow at a CAGR of 11.8%, reaching USD 35.2 billion by 2025, indicating large-scale adoption.	[6]
Enterprise Deployment of IoT	A rising number of IoT projects across industries reflects maturity and scalability of solutions in commercial applications.	[7], [8]
Optical IoT Technologies	Advanced methods like free-space optical communication are being explored for robust energy grid integration.	[9]
Behaviour-Based Energy Analytics	Consumer electricity usage analysis enables demand forecasting and efficient energy distribution via IoT.	[10]
IoT Vision and Challenges	Early foundational work outlines the potential of IoT in industrial domains and flags key challenges like scalability, security, and latency.	[11], [12]
Green IoT and Sustainability	Energy-efficient IoT deployment is critical for sustainable development, especially in resource-intensive urban areas.	[13]
Social Integration of IoT	IoT systems increasingly connect with social systems for user-informed customization and feedback-based control.	[14]
IoT for Smart Cities	IoT deployment supports urban planning and infrastructure, contributing to the realization of fully smart cities.	[15]
IoT Deployment Issues	Barriers to IoT adoption include interoperability, standardization, and integration complexity in legacy systems.	[16]
China's IoT Perspective	Case studies from China reveal large-scale IoT application in energy efficiency and infrastructure modernization.	[17]
Intelligent Energy Gateways	Power supplies with embedded IoT capabilities enhance communication between devices and centralized control hubs.	[18]
IoT and 5G Synergy	Integration of IoT with 5G networks improves latency, throughput, and edge processing capabilities.	[19]
Edge Computing	Enables real-time local decision-making, reducing dependence on cloud and improving response time in energy systems.	[20]
Offline and Resilient Operation	Real-world implementations like the Magarpatta deployment emphasize offline operability and modular deployment.	Present study
Automation Results	Measured savings: Tower 1 (84.5%), Tower 2 (67.6%), Tower 3 (73.86%), validating performance of the IoT-based energy optimization system.	Present study
Novel System Contribution	This paper proposes a low-cost, scalable model integrating motion sensors, LDRs, and intelligent switching logic for smart energy management.	Present study
Support from Policy and Public Discourse	Public reports emphasize urgency in adopting tech-based solutions to systemic inefficiencies, aligning with the motivation of this work.	[21]

Table 2: Available Facilities

S.N	Key Product	Facilities
1	Wireless product line	Yes.
2	IoT Based Highly Scalable product	Yes
3	Advanced algorithm for most optimum utilization of	Yes

	resources (like Compressors)	
4	True Offline Product	Yes
5	Customization for any combination	Yes
6	Highest Number or Channels to operate	Yes(more than 14)
7	Access control mechanism + Device Binding	Yes
8	Real time energy monitoring + notifications	Yes
9	Failover mechanism	Yes
10	Real time theft notification (for electricity)	Yes
11	Advanced technologies like AI(Artificial Intelligence) + NLP (Natural Language Processing)	Yes. Used for issuing voice commands. Also for Real time monitoring.
12	Cost effective	Yes

Table. 3: FeaturesFeatures of Available facilities

1. Plug and Play Touch Screen

- Section Controller is a unique Plug - and - Play Touch Screen (Hub) without battery equipped (No other company is providing a true offline hub as a touch screen)
- Centralized status tracking with 12 active channels view.
- Supports True Offline operations, no need of internet or Router network.
- Also Supports Online operations.
- Offline Schedulers (Real Time Clock Support) and view all schedulers(both online/offline)

2. Offline Notifications

- Offline Notifications can be seen for schedulers, sensors, operating rules etc. When connected with internet, system synch up automatically to make the data available for other channels as well.
- Smart dashboard, self-changing slider to show Reports, Sensor readings, Active Operating Rules etc.
- Offline Notifications can be seen for schedulers, sensors, operating rules etc. When connected with internet system synch up automatically to make the data available for other channels as well.
- Smartdashboard, self-scrolling screen to show Reports, Sensor readings, active Operating Rules etc.

Mobile App can be operated in two modes, Online and Offline.

- Highest level of customization (Sensor/Bundle/IR/Voice /Operating Rules Configuration) (All Sensors, IR Remote, Voice Controller, Switches etc)
- Multiple Users can use the app and admin user can grant access on individual appliances to other users
- Multiple geographical locations can be controlled under single umbrella (single account)
- Customize Bundle (group of switches). And their flawless operations which includes scheduling them.
- Varieties of Usage Reports.
- Unique implementation of Sensor/Switch Operating Rules.
- Schedule appliances like hydraulic motor, water sprinklers etc.

3. Desktop Controller: Alexa Integration

- Perform operations via Voice in Hindi and English
- Getting analytical reports access over voice over interface.
- Customizable as per different business needs.

4. Alerts

WhatsApp Notifications, Calling Notifications, Email Notifications

5. 3 Face Motion Sensor Technology

The technology for 3 Face Motion Sensor **is** a key differentiator and provides huge benefits when it comes to an application requires person occupancy detection in premise. Following are the advantages of the patented technology for 3 face Motion Sensor.

- Covers 15 to 20-meter area in radius with 270-degree coverage
- This motion sensor module has 3 face with each face has motion sensor equipped on it and therefore covers huge area. This design eliminates the requirement of motion sensor modules which may require to achieve

the same results. (5 - 10 qtysof regular motion sensor module may require to achieve the same results)

- Wireless and Configurable Motion Sensor Module and reduce the cost of installation and wiring and become the clean installation even in existing buildings as well.
- The installation is retrofit and does not required change in the existing wiring connection nor required digging on the walls.
- There would be a separate retrofit installation of switching module which will be communicating wirelessly with the motion sensor module.

The configuration of motion sensor modules can be changed anytime wirelessly with custom module provided or by mobile Apps etc.

Table 4: Percentage of Savings before and after Parking Light Automation

Tower 1: Units/Day in kWH				Tower 2: Units/Day kWH			Tower 3: Units/Day kWH		
	Before	**After**	**Savings**	**Before**	**After**	**Savings**	**Before**	**After**	**Savings**
Panel 1	84.18	12.51	85.1	154.26	37.3	75.8	123.08	43.01	65.1
Panel 2	57.98	9.49	83.6	87.01	40.8	53.1	58.38	14.37	75.4
Panel 3	-	-	-	-	-	-	88.73	11.93	86.6
Total	142.17	22	84.5	241.27	78.1	67.6	265.19	69.31	73.86

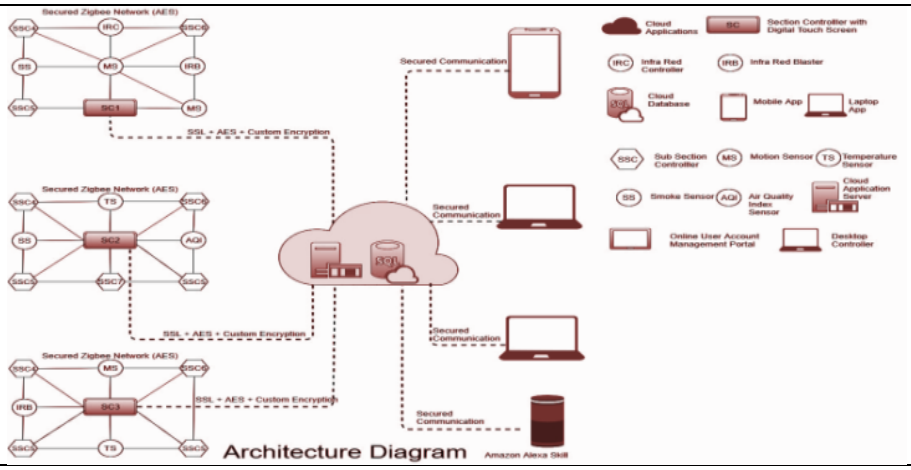


Figure 1. System Architecture

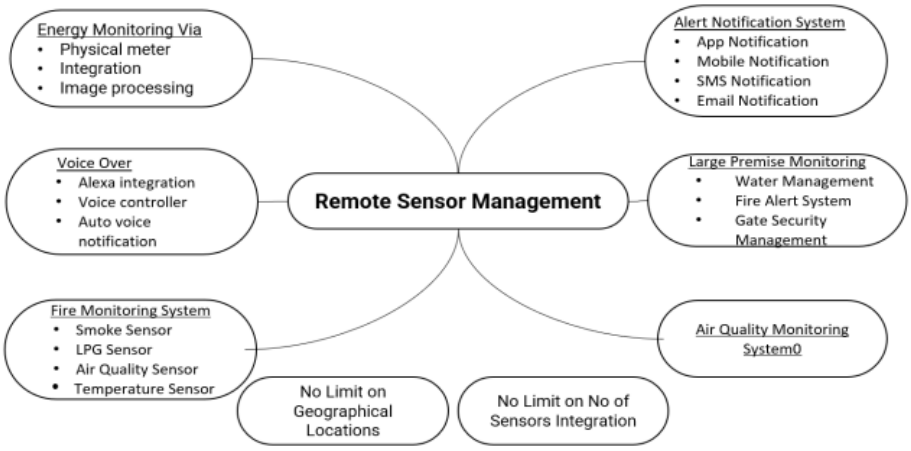


Figure 2. Remote Sensor Management

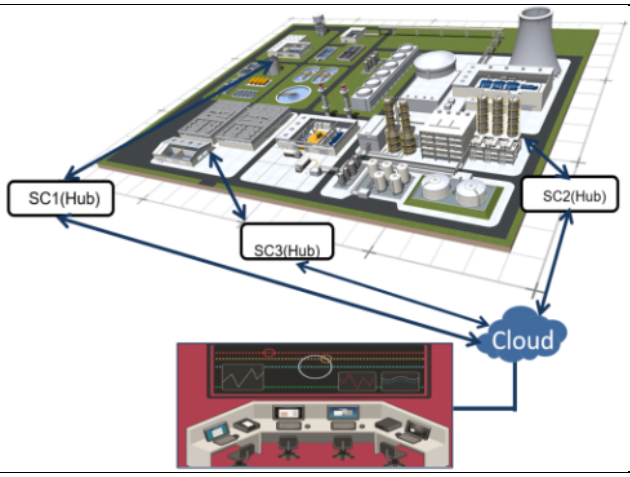


Figure 3. Integrated Command and Control Center (ICCC)



Figure 4. Tower Panel Installation

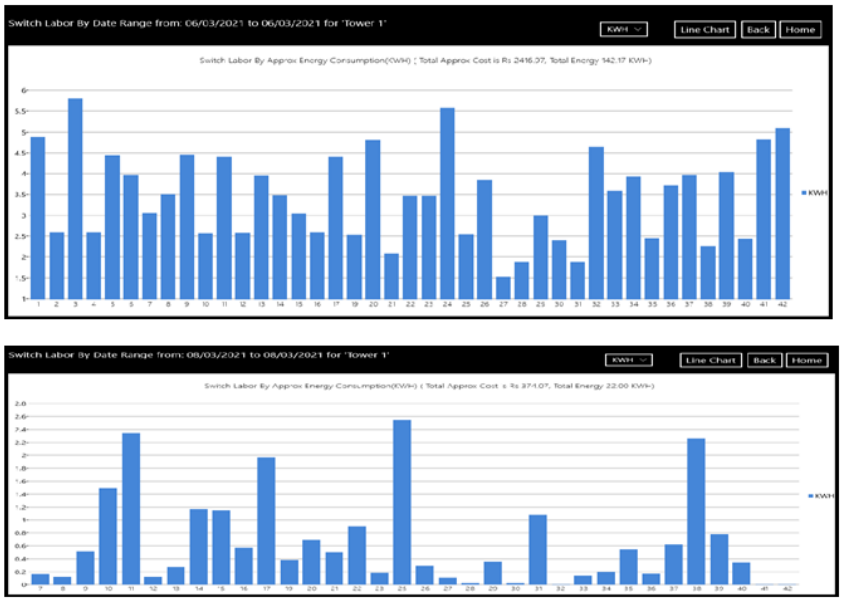


Figure 5. Before and after energy conservation

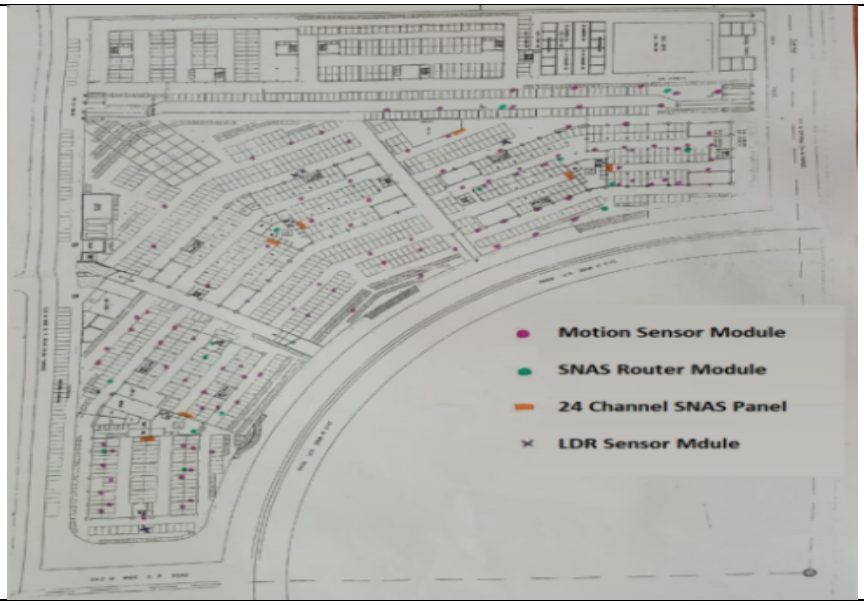


Figure 6. Actual layout of PTA.

RESEARCH ARTICLE

The Efficient Deep Learning Scheduling Algorithm for Internet of Things based E-Health Monitoring Approaches

S.Sindhu* and S.Suriya

Assistant Professor, Department of Computer Science and Applications, SRM Institute of Science and Technology (Deemed to be University), Ramapuram, Chennai, Tamil Nadu, India.

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***Address for Correspondence**
S.Sindhu
Assistant Professor,
Department of Computer Science and Applications,
SRM Institute of Science and Technology (Deemed to be University),
Ramapuram, Chennai, Tamil Nadu, India.
E.Mail: sindhuu89@gmail.com

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ABSTRACT

A Wireless Sensor Network (WSN) comprises a collection of sensors capable of sensing and transmitting real-time information to a central server. The sensor gateway scheduling persistent challenges are to optimize the necessary utilization of the channel within the sensor node. In a scheduling algorithm, the sensor gateway is predicted by the node's cycle time on duty for each idle period. The duty cycle scheduling impotently accepts the random-based data received from the sensor node using First In First Out (FIFO) queues, which are often utilized to arrange and manage the traffic loads of gateway findings. In this paper, we propose a customized schedule algorithm in duty cycle scheduling to tailor to diverse efficiency conditions and reduce the delay time and consumption time. Each sensor node adjustment is done dynamically at the time of data arrival rate from the preferred node and the specified gateway. To analyze the Network Simulator (NS-2) environment is to simulate and evaluate the performance of various scheduling algorithms, such as Sequential Model-based Algorithm Configuration(SMAC) and Tensor completion by parallel Matrix factorization (TMAC), providing further enhancements. This proposed methodology was implemented with the iSense simulator.

Keywords: Duty cycle scheduling algorithm, E-health monitoring system, Sleep time and wake-up sensor node, Network simulator, Scheduling algorithm.

INTRODUCTION

Human health monitoring is preferred for the purpose of timely management and provides better service to save their life. Electronic health(e-health) monitoring techniques are used to handle the data received from humans and

can be processed for the immediate decision-making system for solving each problem. Regular health monitoring helps detect diseases at an earlier stage, for prediction and health risk factors, embedded to provide early assistance and proactive actions. It improves the treatment outcome of each patient. The disease could be managed by ensuring that the health parameters are crucial for managing chronic conditions, such as hypertension, cardiovascular disease, and diabetes. To track the patient's health frequently to indicate the medication needed. It frequently monitors the blood pressure, heartbeat rate, cholesterol level, glucose level, and so on. Based on the disease, miniaturization could vary, to make decisions about human health and indicate changes in lifestyle and medication required to improve their health conditions. The indicators recognize the individual care and maintain the optimal health condition and well-being. To track their metrics, such as physical day-to-day routine activity, dietary habits, and weights. From the analyzed report, the patient should concentrate on which area requires the most attention to promote their health. These monitoring systems provide empowerment by individual self-care about self-health level information and tools to monitor their health frequently. The electronic health monitoring systems provide information and data to encompass the wide range of health care applications, which include electronic records(e-records) and mobile health devices, such as wearable and online portable patient individual portals. It commonly concentrates on improving human personal health by efficiently accessing the doctor by leveraging digital tools and providing a platform to monitor, store, and retrieve the necessary information about the patient securely, with enabled condition of care. It remotely accesses the patient's health record as an e-record for remote consultations to manage the chronic conditions efficiently. It increases the patient's health care and reduces costs by using digital technologies for health care and management. Wireless Sensor Networks (WSNs) find predominant usage in military operations and monitoring applications. These networks typically consist of numerous sensors distributed throughout the environment.
The e-health care can be classified into eight different categories as follows:

Telemedicine involves diagnosing and treating patients remotely through communication technologies like video conferencing, enabling consultations with healthcare providers from a distance.

Electronic Health Records (EHRs) are electronic formats of patients' medical histories, including diagnoses, medications, treatment plans, immunization records, allergies, radiology images, and laboratory test results.

Mobile Health (mHealth) refers to healthcare services accessible through mobile devices like smartphones and tablets. This includes health apps that monitor health metrics, offer medical information, track fitness activities, and send reminders for medication or appointments.

Wearable Health Technology comprises devices worn on the body, such as fitness trackers, smartwatches, and medical wear ables. These gadgets monitor various health metrics, such as heart rate, activity levels, sleep patterns, and other biometric data.

Health Information Exchange (HIE) systems enable the secure electronic sharing of healthcare data among different healthcare organizations. This allows for the seamless exchange of patient information across various providers and settings.

Remote Patient Monitoring (RPM) involves remotely monitoring patients' health using digital devices to collect data outside of traditional healthcare environments. For instance, patients with chronic conditions can have their vital signs monitored at home.

Health Information Portals are online platforms that grant patients secure access to their medical records, lab results, appointment scheduling, prescription refills, and communication with healthcare providers.

Digital Health Coaching entails virtual coaching and support programs delivered through online platforms or mobile apps. These programs assist individuals in achieving health-related objectives like weight management, smoking cessation, stress reduction, and managing chronic diseases. In Figure 1, the IoT devices are not always used

to monitor the data. It is activated by human intervention and the automatic scheduling algorithm from deep learning. This scheduling algorithm changes the mode of each device component from sleep to start mode for reading the data from the patient to care about their health, at a particular interval.

LITERATURE REVIEW

In the eHealth sector, IoT enables various stakeholders to access vital patient data and respond accordingly. Patients can receive notifications or updates on their smartphones from intelligent medical devices, reminding them to exercise[1], adhere to medication schedules, or schedule appointments with their doctors upon detecting signs of illness[2]. These IoT-enabled healthcare devices are made possible by advancements in healthcare development services, fostering a sense of empowerment among patients as they feel more in charge of their well-being[3]. The predominant IoT applications in healthcare often revolve around wearable devices such as smart bands, fitness trackers, and smart watches[4]. Despite their unassuming appearance, these devices signify wearable healthcare technology that holds significant utility for individuals[5]. Fitness trackers, for instance, can track users' physical activity levels, after which these intelligent medical devices offer health care recommendations to promote well-being[6].The SMAC protocol offers a predetermined active period interval[7], with the SYNC packet orchestrating this interval sharing. SMAC designates periodic, active, and sleep intervals. The SYNC packet within SMAC facilitates sleep-awake scheduling, enabling data transmission through the exchange of SYNC packets among sensor nodes. In case of RTS collision[8], sensor nodes enter a sleep phase until the next active period. Nonetheless, SMAC implementation leads to an increase in end-to-end delay[14]. In the existing TMAC algorithm, the transition from sleep state to awake state of sensors occurs if that sensor is not available or is not required for some time[9].TMAC mitigates energy consumption by facilitating sleep periods. During these sleep intervals, any data transmitted to the sensor node is buffered in the neighboring node's memory[10]. Upon the expiration of the sleep period, buffered data is transmitted from the neighboring node to the recipient node during the subsequent active phase. While TMAC reduces energy consumption, it introduces delays due to the earlier termination of the active period[11].DSMAC, on the other hand, dynamically adjusts its duty cycle by extending the active phase. This algorithm increases its active duration based on the application's requirements, consequently elevating energy consumption in tandem with the prolonged active phase duration[12].

Proposed Architecture

The duty-cycle-based scheduling algorithm is to enhance energy efficiency in e-health systems through intelligent management of IoT device duty cycles. The algorithm takes into account factors such as the wake-up probability of a sensor node, the maximum allowable traffic volume, and the expected data packet latency. The IoT device components continually adjust their state strategy by altering the mode from sleep to awake. The duty cycle time represents the propagated time for each node to compare its status as active or sleep, then the total cycle time is compared with the status of the sensor node, which comprises the time duration turnaround from the active and sleep operation of the sensors. The data is received from the sensor when it is in the active state. Enhances resource utilization and energy efficiency in IoT systems through dynamic adjustments of device operational duty cycles[10]. This kind of scheduling algorithm method is implemented within the duty cycle of individual sensors. The duty cycle represents the ratio of the active period to the cycle time, where the cycle time encompasses both the active duration and the sleep duration of sensor nodes. During active periods, each sensor node can transmit and receive data. During the sleep state of the sensor node, the ratio is calculated from the deactivated mode to the active mode. It avoids the data received from other nodes to create the inference from existing data. The sensor component battery consumption level, energy conservation is minimized. It extends the duration of the sensor in active mode to receive the proper data frequently to make a particular standard decision to monitor the patient in a good manner[11]. In the proposed algorithm, the time is calculated as the duty cycle time for each sensor node to find the gateway for receiving the sensed data in the queue. It is partitioned into three different segments, each of which should be governed by two predefined determinants with threshold values fixed according to requirements. The threshold values are dictated by the queue segment, and the arrival of data into the queue from the sensor is compared with the

threshold value for the final decision. In this study, the threshold value will be found at the earlier stage, denoted as Th1 in the initial stage, then the subsequent value as Th2, and so on. Every cycle, the threshold value could be stored in the cloud to make the final decision among the patient's health factors. The optimized data by specified requirements, considering the monitoring system for human health, Figure 2. Figure 2 shows the workflow of the proposed work. The sensor node depicts the flow of work from sleep to active state. To consider the rate of arrival data value for each node, it prolongs the sleep time and active awake time of a sensor node. The sensed data is classified into three different categories: high priority, low priority, and medium priority for the environment to monitor the temperature of a human. The duty of a sensor device's cycle time will be assigned based on the total number of times it can read the information per day and how many times it can read the information from the patients. The cycle time (Ct) is the ratio between the Active Time (A_t) and Total cycle time(T_t)-Equation 1.

$$C_t = \frac{A_t}{T_t}$$

Equation 1: Formula for calculating active time.

Equation 1 calculates the cycle time for the duty from the Active time and Total time. The Active time refers to the period when the device is actively engaged in transmitting or receiving data. The total time is the combined duration of both active time and sleep time, during which the device remains inactive. The mode is an existing mode(Figure 4). Based on the existing mode, the duty cycle time will be calculated, and information will be given to the gateway for activating or waking up the sensor node and keeping that node in sleep mode based on the priority of an individual sensor node. Every time all the sensors need not read the data from the patient, the required amount of time the device could read the data and keep it in sleep mode.

RESULTS AND DISCUSSION

The duty cycle will be calculated and implemented into an application to handle the IoT device components for their individual performance and management. establish these parameters for each IoT device in the e-health system, considering factors such as device capabilities, energy requirements, and communication patterns. The sensor that remains in sleep state (Figure 5) gets the highest priority to receive data from that sensor immediately. This sensor state immediately changed from sleep state to active state to adhere to the prescribed data receiving process. In this proposed methodology, to handle critical data received from the sensor node during the sleep and active period, the data is promptly converted and transmitted to the data gateway to minimize the risk factor for data loss. The sleep duration of the sensor node dynamically adjusts according to the detection of low and medium-priority data within the node.

When the threshold is reached, sleep time (St) is calculated for each low-priority data detection (Lt) using the following formula

$$S_t = 2\sqrt{L_t}$$

Equation 2: formula for sleep duration

The sleep duration is computed as (2√L) *1000, where L denotes the quantity of low-priority data instances identified by Equation 2. The count for low-priority data resets to zero if high-priority or medium-priority data is discovered. Upon detecting crucial (high-priority) data while in sleep mode, the node transitions to active mode and proceeds with regular operations (Figure 6).

CONCLUSION

The proposed methodology for the scheduling algorithm is to implement it with the medical record maintenance using a wireless sensor node, and it could operate with specified parameters from the sensor and the gateway for transmitting the data to a cloud for analyzing the received input data. The information received was kept in a queue

gateway with the priority information. This kind of approach improves the active time of a sensor and minimizes the gateway delay, and minimizes the usage of all sensors every time. To concentrate on the notification of a data loss from each node. Unlike SMAC, which employs fixed active and sleep durations, the proposed algorithm dynamically adjusts these intervals based on sensed data. At the time of sensor sleeping time, the sensor cannot transmit any required data to a gateway. To improve the sleep and active time duration, it dynamically varies from each requisition according to its usage. The specified critical data is received from the sensor means, this algorithm changes the time of sleep mode for an individual sensor to awake mode for higher priority of the data, to avoid the delay time.

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Figure 1. Workflow of the e-health data collection and preprocessing

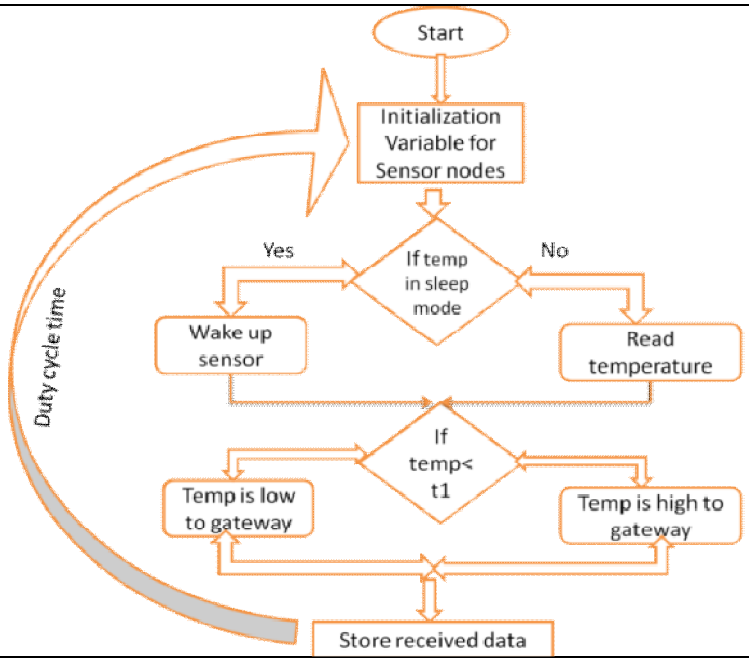


Figure 2. work flow of the proposed methodology

Component	Mode	Wakeup time	Sleep time
Temperature sensor(TMP 36)	Wakeup	1.02 ms	1.01 ms
Temperature sensor(TMP 36)	Sleep	1.602 ms	2.04 ms
photosynthesis	Wakeup	1.04 ms	1.89 ms
photosynthesis	Sleep	1.01 ms	2.87 ms
photoplethysmography	Wakeup	2.04 ms	1.02 ms
photoplethysmography	Sleep	1.89 ms	1.02 ms
piezoelectric	Wakeup	2.87 ms	1.602 ms
piezoelectric	Sleep	1.02 ms	1.04 ms

Figure 3. Temperature monitor IoT device component sleep time and wakeup time based on the mode.

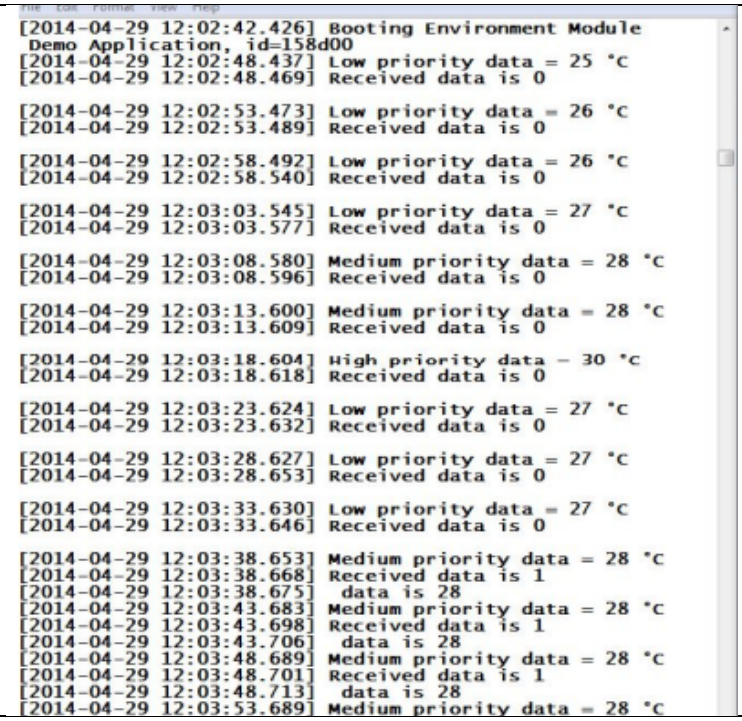


Figure 4. The outcome of the first threshold, reaching out from the cycle time

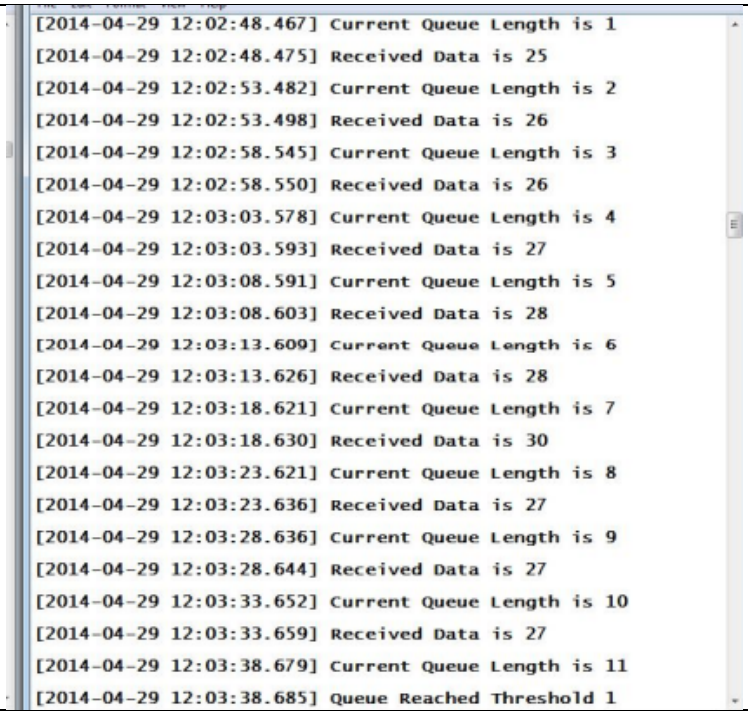


Figure 5. Sleep time threshold for a temperature sensor device component

RESEARCH ARTICLE

Comparative Analysis of Word Spotting Techniques for Indic Scripts with a Focus on Research Gaps in Gujarati Language Processing

Bijal N Dalwadi[1]* and Vatsal Shah[2]

[1]Ph.D Scholar, Department of Computer Engineering, Gujarat Technological University, Ahmedabad, Gujarat, India.
[2]Associate Professor, Department of Information Technology, BVM Engineering College, Anand, (Affiliated to Gujarat Technological University, Chandkheda, Ahmedabad), Gujarat, India.

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***Address for Correspondence**
Bijal N Dalwadi
Ph.D Scholar, Department of Computer Engineering,
Gujarat Technological University, Ahmedabad, Gujarat, India.
E.Mail: bijaldalwadi7710@gmail.com

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ABSTRACT

Word spotting has emerged as a critical technique in document image analysis, enabling efficient information retrieval from large-scale handwritten and printed text corpora. While substantial progress has been made in developing robust word spotting methods for widely used Indic scripts such as Devanagari, Bangla, and Tamil, the application of these techniques to underrepresented languages like Gujarati remains relatively unexplored. This paper presents a comprehensive comparative analysis of existing word spotting approaches—ranging from traditional handcrafted feature-based methods to modern deep learning architectures—across various Indic scripts. It explores the roles of convolutional neural networks (CNNs), recurrent neural networks (RNNs), and generative adversarial networks (GANs) in advancing recognition accuracy. Recent developments are examined within the context of multilingual and multiscript environments, and particular attention is given to underexplored scripts like Gujarati, which pose unique structural challenges. The integration of transfer learning into word spotting systems represents a significant step forward in enhancing document digitization and retrieval across diverse linguistic landscapes.

Keywords: Word Spotting, Indic Scripts, Gujarati Language Processing, Document Image Analysis, Script-Specific Challenges

INTRODUCTION

The rapid growth in digitization of historical manuscripts, administrative records, and cultural texts has created a vast archive of document images that require effective methods for text retrieval [1]. Traditional Optical Character

Recognition (OCR) systems, while widely used, face significant limitations in handling handwritten documents and diverse script styles, particularly in complex and degraded images [1]. As a result, OCR has proven less effective for languages with unique orthographic structures and complex fonts, such as Indic scripts, which often involve a variety of diacritics, ligatures, and character modifications [2, 16]. Convolutional Neural Networks (CNNs), Recurrent Neural Networks (RNNs), and Generative Adversarial Networks (GANs) have all been successfully applied in transfer learning contexts for word spotting [3, 4, 14]. CNN-based architectures such as TPP-PHOCNet, ResNet, and DenseNet have shown strong performance in capturing visual features across complex scripts, providing accuracy improvements over traditional feature extraction methods [3, 11, 12]. RNNs have also been effective, particularly for languages requiring sequential processing of character sequences, as they can retain context across varying word structures [4, 13, 20]. GANs have further contributed by generating synthetic datasets, which are valuable for expanding training sets in low-resource languages [6, 14, 18]. This is especially beneficial for developing accurate retrieval systems for underrepresented languages and scripts [6]. Despite these advancements, substantial research gaps remain, particularly in adapting these models for scripts like Gujarati, which exhibits unique structural complexities [5, 6]. The high variability in handwriting styles, use of conjuncts, and extensive modifiers require tailored approaches for effective retrieval [5]. Therefore, this review focuses on recent word spotting advancements using transfer learning, with a special emphasis on their application to Indic scripts and the need for further research to support underrepresented languages [2, 6, 15]. By highlighting both successes and research gaps in transfer learning for word spotting, this paper aims to pave the way for the development of robust document retrieval solutions that can handle the linguistic diversity and orthographic complexity inherent in multilingual and multiscript document collctions [2].

Literature Review of Word Spotting Techniques Using Transfer Learning

The development of word spotting techniques has been transformative in handling document images where traditional OCR falls short [1]. The introduction of transfer learning has further optimized these techniques, allowing pretrained models to be adapted for diverse languages and scripts [2]. This section provides an overview of key advances in transfer learning for word spotting, focusing on three main categories: Convolutional Neural Networks (CNNs), Recurrent Neural Networks (RNNs), and Generative Adversarial Networks (GANs) [3, 4, 14].

Convolutional Neural Networks (CNNs) and Transfer Learning

CNNs have become a cornerstone in word spotting due to their ability to capture spatial hierarchies in visual data, making them well-suited for recognizing intricate patterns in text images [3]. Early CNN architectures demonstrated moderate success in word spotting; however, recent adaptations leveraging transfer learning have achieved marked improvements [3]. Transfer learning enables CNNs to benefit from feature representations learned on extensive datasets, such as ImageNet, and fine-tune them for specific word spotting tasks on smaller, script-specific datasets [2]. This approach has proven highly effective in dealing with scripts that involve complex shapes, diacritics, and modifiers, as seen in Indic languages like Devanagari and Tamil [7, 16]. Popular CNN architectures in word spotting include TPP-PHOCNet, ResNet, and DenseNet [3, 11, 12]. Moreover, the Transformer-CNN hybrid models introduced by Kim *et al*. (2022) have shown promise in word spotting tasks by combining the attention mechanisms of Transformers with the spatial feature extraction of CNNs [9]. This hybrid approach significantly boosts retrieval accuracy for scripts with high modifier frequency, as seen in Japanese and Hindi datasets [9].

TPP-PHOCNet (Pyramidal Histogram of Characters): Building on established models like TPP-PHOCNet, recent studies have further optimized these networks with enhanced transfer learning techniques [3]. For instance, Chang *et al*. (2024) used multiscale CNNs fine-tuned with PHOC embeddings, specifically designed to process dense, modifier-rich scripts by emphasizing word shapes over individual characters [10]. This advancement achieved notable success on the challenging IAM and CVL datasets, which contain complex word shapes and varied script styles [3, 10, 21].

ResNet (Residual Networks): ResNet's architecture includes skip connections that mitigate the degradation problem commonly encountered in deep networks, allowing it to effectively handle complex image data [3, 12]. ResNet's



ability to maintain accuracy even at greater depths has proven advantageous in word spotting for challenging datasets like the IAM and George Washington collections [3, 12].

DenseNet: This architecture connects each layer to all subsequent layers, maximizing feature reuse and enhancing efficiency [3, 11]. DenseNet's compact structure has been particularly effective for handling high-density scripts and offers competitive results in word spotting for highly complex datasets, such as historical manuscript collections [3, 11].

Recurrent Neural Networks (RNNs) and Sequential Processing

RNNs, especially their variants like LSTMs and BiLSTMs, have shown potential in handling languages with high sequential dependency, making them useful for script-specific applications [4]. Since 2022, transfer learning in RNNs has expanded to support low-resource languages by fine-tuning models on synthetically generated data [4]. For example, Zhang and Huang (2023) implemented a BiLSTM-based transfer learning model adapted for Arabic and Tamil scripts, achieving high retrieval accuracy by leveraging pretrained language embeddings on large, generalized corpora [13]. The adaptation of BiLSTMs to fine-tuned language-specific embeddings enhances the model's ability to capture script-dependent patterns in sequences, enabling accurate retrieval of complex, context-dependent scripts [13]. A new Attention-LSTM model was introduced by Lee *et al*. (2023), focusing on Indic scripts with high diacritic and modifier usage, such as Bengali [13]. The model integrates attention layers that emphasize character contexts, making it effective for scripts with recurrent modifier shapes [13, 20]. This model has been successfully applied in identifying handwritten variations in low-quality historical texts, further validating the efficacy of RNN-based transfer learning in word spotting [13].

Generative Adversarial Networks (GANs) for Data Augmentation

Generative Adversarial Networks (GANs) have opened new avenues for enhancing word spotting through data augmentation [3, 6]. GANs generate synthetic training data, which is particularly useful for languages and scripts with limited available datasets [6]. By generating realistic handwritten or printed word images, GANs augment existing training data and improve the generalization capabilities of word spotting models [6, 14]. This technique is crucial for low-resource languages, such as Gujarati or other regional Indian scripts, where creating large, annotated datasets is a challenge [6, 18].

Conditional GANs (cGANs): Conditional GANs enable the generation of images conditioned on certain input parameters, such as text sequences or font styles [3, 14]. This is useful for producing variations in word images that can replicate real-world handwriting styles or printing variations, increasing model robustness [3]. Transfer learning allows cGANs to adapt from general image-generation tasks to script-specific applications, producing augmented data that is tailored for the unique challenges of specific scripts [3, 14].

CycleGANs: CycleGANs can convert images between two domains (e.g., printed to handwritten) without paired data [3, 18]. For instance, CycleGANs can transform synthetic printed word images into realistic handwritten forms, which expands the training set's diversity [3, 18]. This has proven useful in domains where handwritten data is scarce, allowing models to learn more effective features from larger datasets [3, 6].

Research Gap and Scope in Gujarati Word Spotting

Despite substantial progress in word spotting for widely studied languages, research focused on Gujarati—a regional script with unique orthographic characteristics—remains limited [5, 6, 15]. Gujarati, used by millions in India and parts of the diaspora, presents challenges in automatic retrieval due to its complex morphology, diacritics, and conjuncts [5]. This section explores the specific research gaps in Gujarati word spotting and proposes potential directions to address these challenges [6, 15].

Unique Characteristics of Gujarati Script

Gujarati script, derived from Devanagari, is structurally distinct in terms of its vowel modifiers and conjunct characters, which often appear as combined shapes rather than linear sequences [5]. Additionally, Gujarati uses a unique set of matras (modifiers) attached to base consonants, altering their visual form and complicating segmentation and recognition tasks [5].

Vowel Modifiers and Consonants: In Gujarati, vowels often modify consonants by attaching above, below, or beside them, changing the overall word shape [5].

Conjunct Characters: Some consonants form ligatures or conjuncts, which create completely new shapes that differ significantly from their isolated forms [5].

Challenges in Word Spotting for Gujarati

The characteristics mentioned above make word spotting in Gujarati particularly challenging [5]. Unlike English or other Latin scripts, Gujarati requires complex feature extraction techniques to distinguish between character shapes altered by matras, ligatures, and conjuncts [5]. Additional challenges include:

Shape Variability: Matras and conjuncts change the visual appearance of words drastically, even if the base consonants are similar [5].

Limited Dataset Availability: Unlike Hindi or Tamil, few large, annotated datasets exist for Gujarati [6]. The absence of extensive data limits model training and prevents robust evaluation [6, 15].

High Context Dependency: Gujarati words often depend on context to provide accurate retrieval, especially in the handwritten form, where variability is high [5].

Current Gaps in Gujarati Word Spotting Research

Several research gaps have been identified in recent literature that hinder the development of reliable word spotting for Gujarati [5, 6, 15]:

Insufficient Transfer Learning Applications: Despite the proven effectiveness of transfer learning in various computer vision tasks, its application in Gujarati word spotting remains underexplored [2, 6, 15, 22]. Most existing systems are trained from scratch, which demands large amounts of annotated data—a significant limitation given the scarcity of labeled Gujarati word image datasets, especially for handwritten forms [6]. Moreover, models trained on standard scripts like Latin or Devanagari are rarely adapted for Gujarati, missing opportunities to leverage cross-lingual feature representations [2, 15]. This lack of transfer learning not only hampers performance on limited-resource tasks but also slows down the development of generalized models capable of handling diverse styles and fonts across printed and handwritten domains [2, 6, 22].

Lack of CNN and GAN-Based Adaptations: Most models that work well on other scripts have not been adapted for Gujarati, especially in architectures like TPP-PHOCNet and DenseNet [3, 6, 11, 21]. Further adaptation is needed to account for Gujarati's unique morphological features [6].

Need for Synthetic Data Generation: The use of GANs for creating synthetic Gujarati datasets is highly underexplored [6, 14, 18]. GANs could generate realistic handwritten and printed Gujarati word images, providing the variability needed for robust training [6, 18].

Proposed Research Directions for Gujarati Word Spotting

To address these gaps, future research should prioritize the following approaches:

Developing Gujarati-Specific Datasets: Expanding existing datasets with more annotated Gujarati word images, especially for handwritten forms, will enable training and evaluation of more accurate models [6, 15].

Application of Transfer Learning with Devanagari Models(fig 1): Since Gujarati shares structural similarities with Devanagari, pretrained models on Devanagari datasets can be fine-tuned for Gujarati, reducing the need for large annotated data [2, 6, 15, 22].

Synthetic Data Augmentation Using GANs(fig 2): Applying GANs to generate realistic Gujarati word images in various handwritten styles could significantly improve model robustness [6, 14, 18]. This approach would address the scarcity of real-world Gujarati training data and aid in overcoming shape variability issues [6, 18].

Available Datasets for Word Spotting Research

The availability of high-quality, annotated datasets is crucial for the development and evaluation of word spotting techniques, particularly in low-resource languages like Gujarati [6]. While there has been significant progress in creating datasets for more widely spoken Indic languages such as Hindi, Tamil, and Bengali, the resources for Gujarati remain scarce [6]. This section reviews the key datasets available for word spotting research, with a focus on Indic scripts, and highlights the gaps that need to be addressed to improve word spotting for underrepresented languages like Gujarati [6, 15].

Datasets for Indic Scripts

Over the past decade, various datasets have been developed to support word spotting and optical character recognition (OCR) tasks for Indic scripts [2, 16]. These datasets include both handwritten and printed texts, covering multiple languages and scripts [2]. Many of the existing datasets focus on scripts like Devanagari, Tamil, and Bengali due to their widespread use and availability of digitized documents [2, 16]. However, even in these languages, the datasets are often limited in size, variety, and annotation quality, especially for handwritten forms [2].

Gujarati-Specific Datasets

Despite the significant progress made in creating datasets for Indic scripts, the availability of Gujarati-specific datasets remains limited [6]. The Gujarati Handwritten Corpus released in 2022 is one of the few datasets that focuses exclusively on Gujarati [6]. However, with only 10,000 annotated words, it is relatively small compared to the datasets available for other Indic scripts like Devanagari and Tamil [6]. This limited availability of Gujarati datasets is a major challenge for the development of robust word spotting techniques [6, 15].

Gaps in Existing Datasets for Gujarati

While there has been considerable progress in creating datasets for word spotting in Indic scripts, significant gaps remain in the availability of large, annotated datasets for Gujarati [6]. These gaps include:

Limited Handwritten Data: Existing datasets, such as the Gujarati Handwritten Corpus, are small and lack diversity in handwriting styles [6]. This is a major limitation for developing robust word spotting models, especially since handwriting variability is a key challenge in Gujarati word recognition [6, 15].

Lack of Conjunct and Modifier Variability: The complexity of Gujarati conjunct characters and modifiers is underrepresented in current datasets [5]. Many datasets do not capture the full range of possible conjuncts or the different ways modifiers (matras) can attach to base consonants [5].

Historical and Degraded Documents: There is a lack of datasets that focus on historical or degraded Gujarati documents, such as manuscripts or archival records [6]. These documents often suffer from ink fading, smudging, and other forms of degradation that make word spotting particularly challenging [1]. The inclusion of such documents in future datasets is crucial for improving model performance in real-world scenarios [6, 21].

Synthetic Data for Gujarati: While GANs (Generative Adversarial Networks) have been successfully used to generate synthetic data for low-resource languages like Tamil or Hindi, their application to Gujarati remains limited [6, 14, 18]. Synthetic data can help overcome the lack of real-world handwritten samples by generating diverse, realistic handwriting styles that can be used to augment existing datasets [6, 18].

In-Detail Reported Work for Indic and Non-Indic Languages, Comparison-Based Analysis of Different Word Spotting Techniques, and Their Results on Various Datasets

Word spotting techniques have advanced significantly over the past few years, particularly with the integration of deep learning models like CNNs, RNNs, and GANs [2, 3, 4]. These advancements have been applied across a wide variety of languages, both Indic and non-Indic, to address the challenges of document image retrieval [1].

Word Spotting Techniques for Non-Indic Languages

Word spotting in non-Indic languages, particularly European languages like English, French, and German, has been extensively studied [1]. These languages, which typically use relatively simple and uniform scripts (Latin alphabets), present fewer challenges compared to scripts like Devanagari, Tamil, or Gujarati [1]. Nonetheless, the techniques developed for non-Indic languages have laid the groundwork for more advanced approaches used in complex scripts [1].

DenseNet and PHOCNet: Models like DenseNet and PHOCNet (Pyramidal Histogram of Characters) were introduced to improve accuracy in word spotting tasks [3, 11, 21]. PHOCNet, in particular, became popular due to its ability to represent words as spatial histograms of characters, bypassing the need for explicit segmentation [3, 21]. These models achieved high accuracy on the IAM Handwriting Dataset (which includes handwritten English) and demonstrated their robustness on Latin-based scripts [3]. PHOCNet's hierarchical approach allowed it to recognize words holistically, making it suitable for scripts with variable character spacing [3, 21].

Performance on Non-Indic Datasets: On datasets like the IAM and Bentham handwritten datasets (both English-based), CNN-based models achieved accuracy rates upwards of 90% in word spotting tasks [3, 11]. These models handled variations in handwriting styles effectively and showed significant improvements over traditional segmentation-based methods [3].

Word Spotting Techniques for Indic Languages

Word spotting for Indic languages, such as Hindi, Tamil, and Bengali, presents significantly more challenges than for non-Indic languages due to the complexity of Indic scripts [2, 16]. These scripts often include multiple levels of diacritics (matras), conjuncts (joint characters), and modifiers, all of which complicate character segmentation and recognition [2].

PHOCNet for Indic Scripts: PHOCNet has been successfully adapted for Indic languages due to its ability to capture holistic word shapes [3, 21]. For instance, on the Indic Handwritten Dataset, PHOCNet achieved significantly higher accuracy than segmentation-based methods, particularly when applied to scripts like Tamil and Bengali, which feature frequent use of conjuncts [3, 16]. By representing word images as pyramidal histograms, PHOCNet could bypass the need for character segmentation, which is particularly prone to error in Indic scripts [3, 21].

DenseNet for Indic Scripts: DenseNet has also shown promise in word spotting for Indic scripts, especially in handling complex forms like Devanagari conjuncts and Tamil ligatures [3, 11]. In experiments conducted on the Devanagari Handwritten Dataset, DenseNet outperformed traditional models, achieving an accuracy of 87%, particularly for complex handwritten documents where modifiers and ligatures were common [3, 11].

GAN-Based Approaches for Low-Resource Scripts

For low-resource languages like Gujarati, where few large datasets exist, Generative Adversarial Networks (GANs) have been employed to generate synthetic training data [6, 14, 18]. By creating realistic samples of handwritten

Gujarati, GANs can augment the training dataset, enabling models like PHOCNet and DenseNet to be trained more effectively [6, 18].

CycleGAN for Synthetic Data Generation: A CycleGAN model trained on small amounts of real Gujarati handwriting has been used to generate synthetic handwriting samples [6, 18]. This technique helps overcome the lack of large annotated datasets and enables the creation of varied handwriting styles [6, 18]. When trained with synthetic data, models such as DenseNet showed improvements in accuracy, particularly in recognizing conjunct characters and complex matra placements [6, 11].

CONCLUSION

This review has explored the advancements and challenges in word spotting for the Gujarati script, identifying the unique complexities posed by its conjunct characters, modifiers, and extensive shape variability [5, 6]. Despite significant progress in deep learning, particularly through segmentation-free models and transfer learning techniques, current models often struggle to handle the nuances of Gujarati due to limited annotated datasets and high variability in character formation [6, 15].Models like PHOCNet and DenseNet have shown promise in recognizing entire word structures without relying on segmentation, making them well-suited for complex scripts like Gujarati [3, 11, 21]. Furthermore, generative methods, such as GAN-based synthetic data generation, provide a valuable resource to augment datasets, addressing the data scarcity that often impedes model development for low-resource languages [6, 14, 18].In conclusion, while challenges remain, the integration of advanced machine learning techniques and collaborative data collection initiatives can drive significant improvements in Gujarati word spotting [6, 15]. These efforts will contribute to the broader goal of digital preservation, enhancing accessibility to Gujarati texts and supporting the preservation of linguistic and cultural heritage [1, 6].

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Table 1: Datasets for Indic Scripts

Dataset Name	Language(s)	Type	Size	Features	Year
Indic Handwritten Dataset	Hindi, Telugu, Tamil	Handwritten	50,000+ words	Covers multiple scripts and includes challenging handwritten text	2020
IAM Handwriting Database	English, Hindi	Handwritten	1,000,000+ words	Large-scale dataset, mostly focused on English and Latin scripts	2017
MNIST-Indic Characters	Hindi, Tamil	Isolated characters	60,000+ characters	Isolated handwritten characters for deep learning tasks	2019
Bengali Character Dataset	Bengali	Handwritten, Printed	45,000+ words	Includes handwritten and printed forms, varying font styles	2021
CVL Handwriting Database	Multiple	Handwritten	300,000+ characters	Covers multiple languages, primarily used for handwriting research	2019
Devanagari Handwritten OCR Dataset	Hindi, Marathi	Handwritten	92,000+ characters	Focus on Devanagari, includes handwritten text samples	2019
Gujarati Handwritten Corpus	Gujarati	Handwritten	10,000+ words	Small dataset focused on handwritten Gujarati	2022

Custom Historical Documents Dataset	Multiple (incl. Hindi)	Printed	5,000+ pages	Focus on historical manuscripts, scanned from physical archives	2021

Table 2: Gujarati-Specific Datasets

Dataset Name	Type	Size	Features	Year
Gujarati Handwritten Corpus	Handwritten	10,000+ words	Limited handwritten samples with few variations in style	2022
Custom Historical Documents	Printed, Scanned	500+ pages	Focus on printed Gujarati documents, mainly from archived manuscripts	2021

Table 3: Performance on Non-Indic Datasets

Technique	Dataset	Language	Accuracy	Strengths	Limitations
Segmentation-Based (HMM)	George Washington Dataset	English	75%	Works well for isolated characters	Struggles with connected handwriting
PHOCNet (CNN)	IAM Handwriting Dataset	English	90%	Segmentation-free, handles word variability	High computational cost, requires large training data
DenseNet (CNN)	Bentham Handwriting Dataset	English	92%	Handles variable handwriting styles	Requires large-scale datasets for optimal performance

Table 4: CycleGAN for Synthetic Data Generation

Technique	Dataset	Language	Accuracy	Strengths	Limitations
Segmentation-Based (HMM)	Devanagari Handwritten Dataset	Hindi	65%	Useful for isolated characters	Poor performance on conjuncts and ligatures
PHOCNet (CNN)	Indic Handwritten Dataset	Hindi, Tamil	85%	Handles variable conjuncts and modifiers	Requires large datasets for effective training
DenseNet (CNN)	Devanagari Handwritten Dataset	Hindi, Marathi	87%	Effective for handwritten text with diacritics	Computationally expensive, requires GAN-based augmentation
CycleGAN (for Synthetic Data)	Gujarati Handwritten Dataset	Gujarati	80%	Generates diverse handwriting styles	Depends on quality of real training data

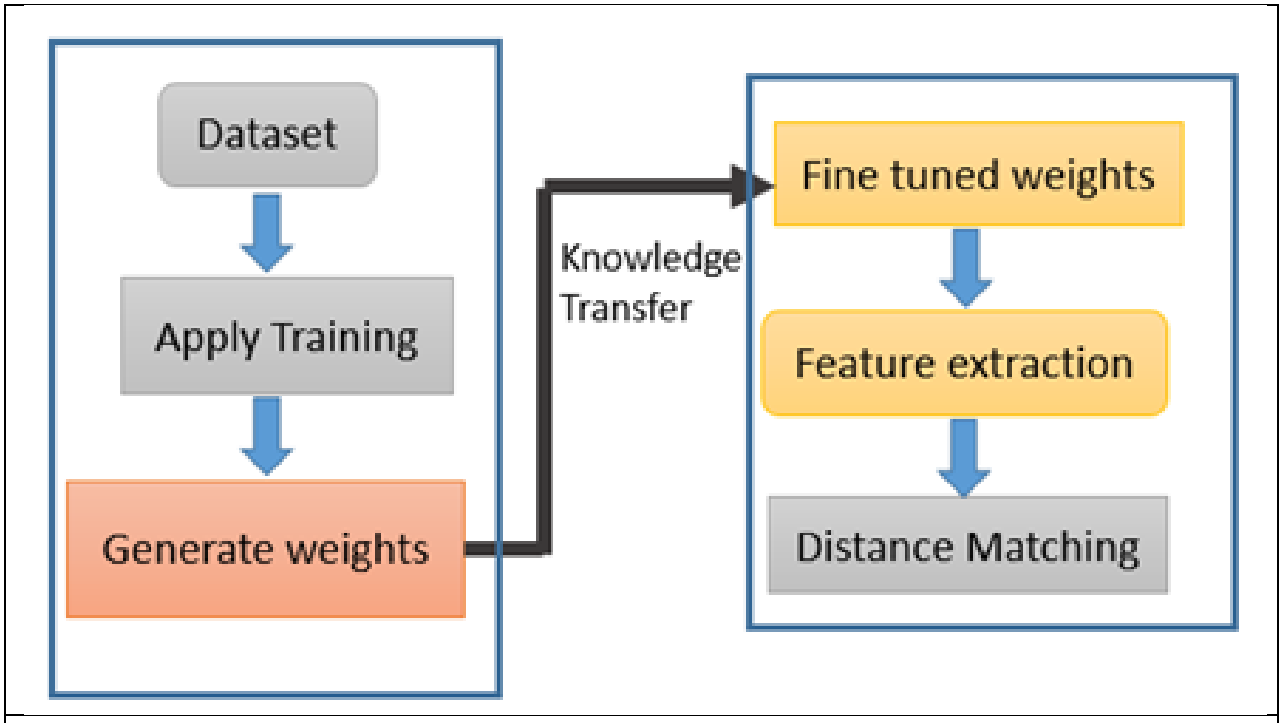


Figure 1: A transfer learning approach for word spotting

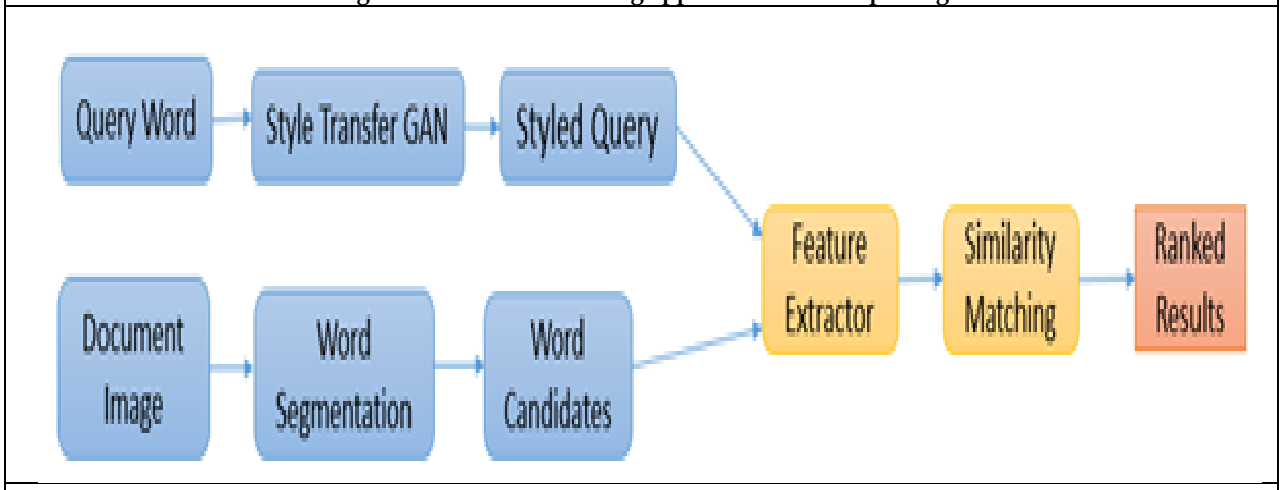


Figure 2: GAN model for word spotting

RESEARCH ARTICLE

Advancements in Nanotechnology for Cosmeceuticals

Ummae Kulsum[1], Sana Farheen[1], Simran[1] and Balusu Haarika[2]*

[1]M.Pharm Second Year Student, Department of Pharmaceutics, Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Tarnaka, (Affiliated to Osmania University), Hyderabad, Telangana, India.
[2]Vice Principal and Professor, Department of Pharmaceutics, Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Tarnaka, (Affiliated to Osmania University), Hyderabad, Telangana, India.

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***Address for Correspondence**
Balusu Haarika
Vice Principal and Professor,
Department of Pharmaceutics,
Sarojini Naidu Vanita Pharmacy Mahavidyalaya, Tarnaka,
(Affiliated to Osmania University), Hyderabad,
Telangana, India.
E.Mail: haarikabalusu09@gmail.com

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ABSTRACT

The cosmetic engineering is undertaking a substantial alteration motivated by advancements in nanotechnology, which manipulates materials at the nanoscale (1-100 nm). This field offers unique properties like increased surface-to-volume ratio, leading to enhanced skin penetration, long-lasting effects, and improved ingredient delivery in cosmetic formulations. Nanoparticles are categorized as organic, carbon-based, and inorganic, each offering distinct advantages. Key nanocarriers like liposomes, niosomes, solid lipid nanoparticles (SLNs), nanoemulsions, nanospheres, and dendrimers are crucial for delivering active cosmetic ingredients with precision and stability. Beyond formulations, bionanomaterials are revolutionizing tissue engineering, nanomedicine, and biosensing, fostering ethical product development. Advanced engineering methods, including 3D printing and microfluidics, permits the creation of personalized cosmetic products and highly uniform nanoparticles, respectively.

Keywords: Liposomes, Nano technology, Niosomes, Micro fluidization, 3d printing, Tissue engineering,

INTRODUCTION

The cosmetic industry is currently undergoing a transformative period, largely driven by the remarkable advancements in nanotechnology. In recent years, nanotechnology has captured global attention. This expansive field involves the strategy, development, and manipulation of constituents at the nanoscale (1–100 nm). At the nanoscale, materials exhibit altered characteristics, notably an increased surface-to-volume ratio, a higher quantity of elements per unit weightiness, and the quantum confinement effect. These unique assets make nanoparticles highly

suitable for diverse businesses, together with domestic utilizations, farming, impurity exclusion, food, announcement, medicine, transport, cosmetics, and cosmeceuticals. [GarimaNagpal *et al.*, 2023]. Nanomaterial-based cosmetics offer distinct rewards over their micro-scale matching part, primarily enabling continuing effects and improved stability. The cosmetic industry's key objectives in utilizing nanomaterials include achieving efficient skin penetration for improved ingredient delivery, developing novel color essentials (e.g., Lipsticks and nail polishes), ensuring clarity (e.g., Sunscreens), and providing more durable results [Georgia Fytianos *et al*., 2020] With the continued advancement of nano cosmetics through scientific research and technological development, we anticipate the introduction of increasingly sophisticated and personalized products, promising unprecedented efficacy, safety, and environmental accountability. [KetanSonawane *et al*., 2024] Nanoparticles are well-defined as tiny particles fluctuating from 1 to 100 nanometers, distinguished by their exclusive physical and chemical attributes, which stem from their elevated surface area and reactivity. Their classification is based on their sizes, classifying them as Zero-dimensional (0D), One-dimensional (1D), Two-dimensional (2D), or Three-dimensional (3D). Within cosmeceutical utilizations, nanoparticles are morecharacterized according to their morphology, size, and physicochemical features. [N. Ranjan *et al*., 2022]

Categories of Nanoparticles

Biological Nanoparticles: These nanoparticles are derivative from organic materials such as proteins, lipids, carbohydrates, or polymers. Distinguished examples include Dendrimers, Micelles, Liposomes (which may own a deep core), and protein network like ferritin. While recyclable and non-lethal, they displayvulnerability to current and electromagnetic radioactivity, as well as light and heat.

Carbon-Based Nanoparticles: This group is exclusively collected of carbon particles. Key instances encompass Carbon dark NPs, Fullerenes, and Quantum spots. Fullerenes are categorized by their equal, closed-cage carbon nanomaterial constructions, demonstrated by C60, C70, and C540. Carbon Black Nanomaterials are nebulous carbon totals that exhibit a grape-like morphology. Quantum Dots are quasi-spherical nanoparticles, characteristically sized amid 1 and 10 nm.

Inorganic Nanoparticles: Invented from inorganic materials, this grouping comprises metal-based, ceramic-based, and semiconductor-based nanoparticles.

Metal-Based Nanoparticles: These are entirely composed of metallic precursors, which can be mono, bi or polymetallic. Their diverse electrical and optical properties are influenced by phenomena such as surface plasmon resonance.

Ceramic Nanoparticles: These inorganic particles are formed from carbides, carbonates, oxides, and phosphates of metals or metalloids, such as calcium and titanium.

Semiconductor-Based Nanoparticles: Constructed from semiconductor materials, these nanoparticles possess properties of both metals and non-metals. They find utility in photocatalysis, electronic devices, and optical devices. [Joudeh, N *et al*., 2022]

NANOTECHNOLOGYINCOSMETICINDUSTRY/ THE EVOLUTIONAND IMPACTOFNANOCOSMETICS

The lasting human desire to preserve a decent presence is as old as humanity itself, serving as the primary driving force behind cosmetic use. The practice of cosmetics times back to earliest Egypt around 4000 BC, with civilizations like the Americans, Greeks, Chinese, Romans, and Japanese also adopting them. While household items served as early cosmetic solutions, the 20th century saw their widespread, unrestricted use. The 21st century marks a new era, characterized by the integration of advanced technology leading to inventive cosmetic formulations now in immense use. The minute these cosmetic products are encouraged with biologically dynamic therapeutic additives to improve appearance, they are called cosmeceuticals [9]. These products efficiently address various skin and hair worries,

including hyperpigmentation, wrinkles, irregular complexion, photoaging, skin aridness, dark spots, and hair injury. [Garima Nagpal *et al*., 2023]

Nanomaterials: A Game Changerin Cosmetics

Surveys reliably show that nanosized resources are now routinely working by all chief cosmetic producers across their product choices. The merging of chemical sciences with pharmaceuticals has led to a new nomenclature: Nano cosmetics. A diverse array of nanoparticles, counting metals (e.g., silver nanoparticles - AgNPs, gold nanoparticles - AuNPs), metal oxide nanoparticles (e.g., titanium dioxide nanoparticles - TiO2NPs, zinc oxide nanoparticles - ZnO NPs, iron (III) oxide nanoparticles - Fe2O3NPs), and carbon-based nanoparticles, are broadly used in cosmetic preparations. Chief cosmetic establishments such as Estee Lauder, L'Oréal, Nivea, Zelens, and Derma Swiss, among others, actively incorporate nanoparticles into their goods. The nanotechnology-based cosmetics manufacturing is a multi-million-dollar global marketplace. L'Oréal, the world's major cosmetics corporation, suggestively invests in nanoexclusive rights, having secured numerous exclusive rights for "nano some particles".

Nanocarriers used in Cosmetics

Carriers are used to distribute active substance for the delivery of nanocosmeceuticals. There are various types of carriers used they are:

Liposomes

Liposomes are most frequently used in cosmetic products. They are membrane-bound constructions with an aqueous central encased in anaquaphobic lipid bilayer. The possibility of negative effects is reduced since phospholipids, which comprise the widely held of the lipid bilayer in liposomes, are usually regarded as innocuous chemicals. To stop metabolic breakdown, liposomes encapsulate medications and deliver their active ingredients gradually [GarimaNagpal *et al*., 2023]. Liposomes are either unilamellar or multilamellar in structure, and the size is between 20 nanometers to 1 micrometer. Liposomes are used as potent carriers in cosmetic industry due to its characteristics like increased compounds solubility, accord with human skin, lessened harmful effects and enhanced drug buildup at the target spot[GarimaNagpal *et al*., 2023].

Niosomes

Niosomes are microscopic, self-assembling vesicles formed from the hydration of unionized surfactants, often united with cholesterol. They hold a bilayer construction, similar to liposomes, with a water-soluble head facing outwards and inwards, and a non-water-soluble tail forming the central of the bilayer. This exclusive amphiphilic nature permits them to capture both hydrophilic drugs in their aqueous central and lipid-soluble drugs within their double layer crust. Niosomes offer several compensations as drug and cosmetic delivery schemes. They are generally considered more stable than liposomes due to the biochemical nature of their non-ionic surface-active agent, making them less prone to oxidation and degradation. Their suitable for biological applications, decomposable, and low toxicity make them appropriate for several applications. Niosomes can improve the bioavailability of encapsulated substances, provide controlled and sustained release, and offer targeted delivery to specific sites in the body or skin. Their versatility in encapsulating a wide range of active compounds has led to their increasing use in pharmaceuticals, cosmetics, and even agriculture.

Solid Lipid Nanoparticles (SLNs)

Known for their biocompatibility and stability, these carriers offer a safe way to encapsulate diverse substances, including those that are water-loving, fat-loving, or have low solubility, thereby reducing potential toxicity. [KetanSonawane *et al*., 2024] They are collected of a solid matrix, which can be completed from several materials like polymers, lipids, metals, or inorganic compounds. These tiny carriers are expansively explored for their capability to encapsulate and transport a wide variety of active substances, including drugs, genes, and imaging agents.

Nano Emulsions

Nano emulsions are steady, finely dispersed mixtures of two non-miscible fluids, with droplet sizes usually between 20 and 200 nanometers. Surfactants stabilize them by lowering interfacial tension and stopping droplet coalescence. In contrast to microemulsions, they lack thermodynamic stability but show long-term kinetic stability because of their tiny droplet size. This compact size results in numerous benefits, such as superior optical clarity (often looking transparent), remarkable physical stability against creaming or sedimentation, and improved bioavailability of the encapsulated substances. They are commonly utilized in medication delivery to enhance the solubility and approval of drugs that are less soluble in water. In addition to pharmaceuticals, nano emulsions are utilized in cosmetics to improve skin absorption and feel, as well as in the food sector to encapsulate flavors and nutrients.

Nano Spheres

Nanospheres are spherical nanoparticles generally measuring between 10 and 200 nanometers in diameter. They are essentially solid matrix systems, indicating that the active substance is evenly distributed within or bonded to the polymeric or inorganic material that makes up the sphere. This design enables efficient encapsulation and safeguarding of medications or other therapeutic compounds. Their main benefits consist of outstanding stability, regulated release of the enclosed material, and the capability to expand the solubility and solubility of difficult-to-dissolve substances. Nanospheres are extensively researched in drug transport, especially for embattled treatment of particular cells or tissues, minimizing side effects. They are also utilized in diagnostics, imaging, and in areas such as catalysis and environmental clean-up owing to their large surface area and adjustable characteristics.

DENDRIMERS

They are used as a vehicle to enhance the absorption of vitamins into the profound layers of dermis. Dendrimers are intricately branched, tree-shaped macromolecules featuring an exact, symmetrical design radiating from a central core. They are developed in consecutive "generations," with each adding an additional layer of branching and enhancing the quantity of surface functional groups. This distinctive structure offers a high surface area relative to its volume and inner cavities, positioning them as outstanding options for encapsulating and delivering various molecules, such as drugs, genes, and imaging agents. Their specified dimensions, form, and many adjustable surface functionalities enable customized characteristics such as solubility, targeting, and controlled release, providing considerable benefits in nanomedicine and other areas

Bionanomaterials

Bionanomaterials are tiny, biologically-made materials. Their minuscule size gives them special properties—structural, chemical, physical, and more—that set them apart from larger versions of the same material. These unique traits allow them to be used in various biomedical applications, such as tissue engineering, drug delivery, cancer therapy, and treating neurological diseases and inflammation. They are made up of biological molecules like proteins, antibodies, enzymes, lipids, and nucleic acids.

Bionanomaterials: Diverse Applications

Bionanomaterials are vital in medicine, from building tissues and delivering drugs to nanomedicine and diagnosing diseases.

Nanomedicine

The landscape of medical practice is being reshaped by nanomedicine, allowing the satisfaction of healthcare needs that were once out of reach. It improves the body's ability to absorb, employ, and profit from even very harmful but otherwise beneficial treatments., such as Me pact, while introducing novel drugs for both diagnostics and therapeutics. This advancement is attributed to the unique properties of bionanomaterials, which enable crucial pharmaceutical and biomedical advantages. These include reduced toxicity and side effects through lower dosing, increased targeted drug effects, controlled site-specific drug release, and improved transport across biological barriers.

Bionanomaterials for Nanobiosensing

Combining biological components with transducers, a biosensor serves as an analytical device for detecting analytes or quantifying physiological signals by converting electrical, optical, and thermal inputs. Its core structure comprises a probe—responsible for detecting diverse biomolecules such as at their core, biosensors consist of a biorecognition element—drawing upon substances like DNA, RNA, antibodies, aptamers, enzymes, and proteins—and a transducer. This transducer is crucial for converting physiochemical information into quantifiable magnetic, electrical, optical, thermal, or electrochemical outputs.

Bio-Imaging

Molecular imaging, a crucial component of diagnostics, utilizes nuclear medicine and radiology to examine real-time molecular changes within a living organism during disease progression. It effectively observes the biochemical shifts that occur in the body's tissues or organs during illness. Significantly, bionanomaterials are currently a focus of intensive research to boost molecular imaging capabilities. Their advantageous properties, including a significant surface area for its given volume, excellent tissue compatibility, and careful affinity for biological molecule surface receptors, facilitate targeted attachment. This specific binding generates distinct signals, enabling the detection of minute molecular-level alterations. [Piyush Kumar Sadhu *et al*.,2023]

Tissue Engineering in Cosmetics

Tissue engineering in cosmetics represents a fascinating and rapidly evolving field, primarily focused on developing and utilizing laboratory-grown tissues to replace or reduce the need for animal testing and, more recently, to create advanced cosmetic ingredients and products. Moving beyond dependence on animal components or animal testing, cosmetic tissue engineering fabricates 3D skin models by culturing human cells, primarily fibroblasts or keratinocytes. These bioengineered constructs replicate human skin's natural structure and function, thereby enabling applications such as

Test product safety and efficacy: Companies can apply cosmetic formulations directly to these engineered skin models to assess irritation, sensitization, and penetration, providing a more human-relevant alternative to traditional animal tests.

Identify novel active ingredients: By observing how engineered tissues respond to various compounds, researchers can discover new molecules that promote collagen production, reduce inflammation, or offer antioxidant benefits.

Understand skin biology: Offering a precise experimental platform, these models enable comprehensive research into processes such as skin aging, variations in pigmentation, and a range of cutaneous disorders. Such studies yield enhanced insights into their mechanistic foundations, consequently aiding in the formulation of bespoke cosmetic treatments.

Develop "bio printed" cosmetics: While still largely experimental, the long-term vision includes bioprinting personalized skincare products or even "living" patches that can directly address specific skin concerns.

The core benefit of this approach is its ethical advantage, providing a cruelty-free method for product development. Furthermore, engineered human tissues often offer more accurate and predictive results for human application compared to animal models, leading to potentially safer and more effective cosmetic products. The field is continuously advancing, with efforts to create more complex tissue models that include multiple cell types, blood vessels, and even nerve endings, further enhancing their resemblance to in-vivo skin.

Applications of Nanomaterials in Tissue Engineering

Nanomaterials are revolutionizing tissue engineering by mimicking the natural extracellular matrix, crucial for cell behavior. Nanoparticles are instrumental in producing scaffolds that exhibit regulated porosity and specific mechanical characteristics, which in turn guides cellular development and differentiation. Their substantial surface area relative to volume promotes superior cell adhesion, proliferation, and signaling. Furthermore, these

nanoparticles offer the capability to encapsulate and precisely deliver growth factors, pharmaceuticals, or genes with fine-tuned spatial and temporal regulation. They also offer improved imaging capabilities for monitoring tissue regeneration in real-time. Specific nanomaterials like carbon nanotubes can provide electrical cues for nerve and cardiac tissue engineering. Metallic nanoparticles contribute antimicrobial properties, reducing infection risks in implants. The tunable surface chemistry of nanomaterials allows for biomolecule conjugation, enhancing specific cell interactions. They contribute to the assembly of elaborate 3D tissue models, playing a pivotal role in organ repair and transplantation. Taken together, nanomaterials empower the implementation of highly effective and focused methodologies for rejuvenating impaired tissues and organs.

Regarding scaffold fabrication: These agents facilitate the production of scaffolds designed to emulate the native extracellular matrix (ECM), ensuring comparable structure, porosity, and mechanical integrity. This biomimetic design is paramount for orchestrating cell adhesion, proliferation, and differentiation.

Enhanced Cell Interaction: Elevated surface area to volume proportion of nanomaterials provides abundant sites for cell attachment, promoting better cell growth, communication, and overall tissue development.

Controlled Delivery Systems: Nanoparticles act as efficient carriers for delivering growth factors, drugs, genes, and other bioactive molecules directly to the target site, allowing for localized and extended release, which is vital for tissue regeneration.

Improved Mechanical Properties: The inclusion of nanomaterials can markedly boost the mechanical properties and elasticity of tissue engineering scaffolds. As a result, these scaffolds become viable for use in load-bearing tissues, including bone and cartilage.

Electrical Cues: Due to their electrical conductivity, specific nanomaterials like carbon nanotubes are crucial for creating electrically functional tissues including nerve and cardiac tissue, given the supreme importance of electrical signaling in these contexts.

Antimicrobial Properties: Some metallic nanoparticles exhibit antimicrobial effects, which can be integrated into scaffolds to prevent infection, a common complication in tissue implants.

Bioimaging and Monitoring: Nanomaterials can be designed with imaging capabilities, allowing researchers to monitor cell behavior, scaffold degradation, and overall tissue regeneration in real-time within the body.

Polymeric Nanoparticles

Future scope of polymeric Nanoparticles in Nanotherapeutics

This field shows immense promise, with ongoing research and technological advancements continuously opening new possibilities and driving future growth.

Personalized medicine: An expanding field, centers on customizing treatments based on an individual's distinct genetic profile, disease features, and therapeutic responses. In this evolving domain, polymeric nanoparticles are gaining significant traction. They provide an accurate method for delivering bespoke combinations of drugs and gene therapies, thereby enabling highly targeted treatments**.**

Immunotherapy: These are a rapidly advancing area, revolutionizing cancer treatment and showing promise in other diseases too. A key development here is the ability to engineer polymeric nanoparticles. These nanoparticles can precisely deliver immunomodulatory agents directly to immune cells or to the tumor's immediate surroundings. This targeted delivery significantly boosts the body's natural defenses against diseases.

RNA Therapeutics: The rise of RNA-based therapies, such as mRNA vaccines and gene silencing treatments, has opened new frontiers in medicine. Polymeric nanoparticles are set to play a vital role here, acting as essential delivery vehicles for these delicate molecules. This capability allows for more efficient and targeted treatments across a range of conditions, from genetic disorders to viral infections.
[Piyush Kumar Sadhu *et al*.,2023]

3D Printing Technology in Nanoparticles

Through its method of layering materials based on digital blueprints, 3D printing (additive manufacturing) has utterly transformed how we approach design and manufacturing. This approach grants extensive design liberties, leads to decreased material consumption, and speeds up prototype development. As noted by Wohlers Associates, this technology is redefining industries where precision and tailored solutions are essential, notably healthcare, automotive, and aerospace.

3D Printing in Nanomaterials

- Defined by their fabrication at a scale of billionths of a meter, nanomaterials—a category including nanoparticles, nanotubes, and nanocomposites—possess unique attributes. These stem from their large surface area-to-volume ratio and pronounced quantum effects, leading to exceptional functionalities:
- **Mechanical strength**: Ideal for reinforcing 3D-printed structures
- **Electrical conductivity**: Useful in electronics and sensors
- **Thermal stability**: Crucial for aerospace and high-temperature applications

A review in the journal Nature Nanotechnology emphasizes that nanomaterials are "at the forefront of interdisciplinary research," with the potential to drive technological break throughs across sectors. [Piyush Kumar Sadhu *et al*.,2023]

Applications of 3D Printing Technology

By making it possible to create customized medicine formulations, on-demand manufacture, and intricate drug delivery systems, 3D printing, sometimes denoted to as preservative engineering, is totally changing the pharmaceutical sector. This technology allows for detailed control over dose, release profiles, and medicine combinations, which is especially valuable in personalized medicine.

Fused Deposition Modeling (FDM)

FDM is one of the utmost extensively used 3D printing practices in pharmaceutics due to its simplicity, cost-effectiveness, and versatility. The mechanism includes a thermoplastic filament, often loaded with an active pharmaceutical ingredient (API), is melted and extruded over a fiery outlet. Film by film deposition of this material will lead to formation of a 3D object—typically a tablet or implant.

Applications

Precision Dosing: FDM enables the fabrication of tablets with highly accurate drug dosages, tailored to individual patient needs.

Controlled Release: By modifying the internal geometry or polymer composition, FDM can produce tablets with immediate, sustained, or delayed release profiles.

Multi-Drug Integration: It allows for the integration of numerous drugs and excipients into one dosage form, reducing pill burden and improving patient compliance.
According to a Springer publication, FDM is particularly promising for oral therapies, transdermal patches, and implants, and is being actively explored for clinical and hospital-based drug manufacturing.

Bioprinting in Pharmaceuticals

Bioprinting is aprogressive form of 3D printing that uses bioinks, collected of living cells, biomaterials, and growth aspects to fabricate tissue-like structures.

Applications

- High-throughput drug screening using bio printed tissue models
- Personalized medicine through patient-specific tissue constructs
- Reduction in animal testing by providing physiologically relevant models [E-copy emerging trends of nanomaterials]

Microfluidics Technology

Microfluidics is a new and adaptable technology that is currently being applied in several fields. Microfluidic systems can manipulate fluid volumes as small as 10-9 to 10-18 L, with the help an integrated system of micrometer channels, microvalves, and micromixers. Microfluidics technology operates on the idea of controlled mixing, which employs the device microfluidizer. The device uses a positive displacement pump at high pressure which ranges from 500 to 20,000 psi. The product is forced through a special chamber that has tiny channels. As it goes through these micro-channels, it's then directed to an impact area. This impact creates extremely small, sub-micron particles.

Nano emulsions were manufactured using two methods

Single-channel and Dual-channel method

Single-channel method: This method is a two-step process. First, you mix the oil (lipid) and water (aqueous) parts with a high-speed blender at room temperature to make a rough, coarse emulsion. Then, you pump this coarse mixture through a special air-driven machine called a microfluidizer (with Y- and Z-type chambers) at different pressures for a single pass. This intense processing turns the coarse emulsion into a fine nanoemulsion.

Dual-channel method: This method is more direct. Instead of pre-mixing, you put the oil and water phases into separate containers. Then, you feed both phases directly into the same air-driven, high-pressure microfluidizer (also with Y- and Z-type chambers). As they pass through the machine together, they form fine emulsions (likely nanoemulsions) directly. [Piyush Kumar Sadhu *et al*.,2023]

Applications

Microfluidics Technology in Manufacturing Liposomes

The Microfluidic (MF) system is a unique approach that uses tiny channels, ranging from 5 to 500 micrometers, to process fluids. These narrow channels ensure a smooth, laminar flow, which significantly boosts how well microfluidic devices perform and mix substances. In the MF process, lipids are first dissolved in an organic solvent like ethanol or isopropanol. The amount of lipid compared to cholesterol is crucial, as it greatly affects the characteristics of the final product. The dissolved lipid phase is then directed into one chamber, while an aqueous (water-based) phase goes into another. The flow of both phases is precisely controlled by adjusting their flow rate ratio (FFR) and total flow ratio (TFR). Within this controlled environment, the organic and aqueous phases are continuously mixed. This mixing causes the phospholipids (a type of lipid) to spread into the aqueous phase, prompting the lipids to naturally arrange themselves into liposomes.

Microfuidics Technology in Manufacturing SLNs

Solid lipid nanoparticles are lipid nanocarriers designed to transport hydrophobic bioactive chemicals with increased loading capacity and stability. Solid lipid nanoparticles enhance the loading and bioavailability of several plant bioactive components. For its manufacture, the lipid and the drug are dissolved in an organic solvent. This mixture then goes into the microfluidic (MF) device as the inner liquid. At the same time, a separate water-based solution with a surfactant flows in as the outer liquid. Both these liquids are precisely pumped from individual syringes into

the MF device's channels and onto a chip. Once the SLNs form, the organic solvent is evaporated, and the particles are then chilled to 4°C to solidify them.

Microfuidics for Polymeric Nanoparticles

Polymeric nanoparticles, because of their ability to effectively encapsulate a variety of drugs and modify their release by modifying the use of the polymer characteristics, have caught a lot of attention in research fields. For instance, Polymeric nanoparticles due to its slow rate of degradation, has found its application in sustained or extended-release formulations. The conventional method of manufacture has a number of limitations; hence microfluidics technology emerged as a modern and advanced method for the manufacture of polymeric Nps. MHF method is employed. This microfluidic approach involves dissolving a suitable polymer in an organic solvent and then mixing it with an aqueous solution at specific flow rates (TFR and FFR). This method promotes the self-assembly of lipids, leading to the production of monodispersed nanoparticles without the need for solvent evaporation.

CONCLUSION

The cosmetics industry is currently experiencing a major transformation, largely due to the remarkable progress being made in the field of nanotechnology. This field, operating at the nanoscale, has unlocked new possibilities for cosmetic formulations, offering enhanced efficacy, stability, and personalized solutions. Nanoparticles and diverse nanocarriers are now widely used to deliver active ingredients more effectively, improving everything from skin penetration to product longevity. Beyond direct product applications, bionanomaterials are revolutionizing ethical ingredient sourcing and testing. Furthermore, 3D printing and microfluidics technology are enabling customized production and precise nanoparticle manufacturing. This ongoing innovation promises a future of increasingly sophisticated, safe, and environmentally responsible cosmetic products, continually enhancing well-being and appearance.

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Conflict of Interests

Declared none

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Table 1: Benefits and limitations of liposomes

BENEFITS OF LIPOSOMES	LIMITATIONS OF LIPOSOMES
Improved dermal layer stability and penetration to stop metabolic breakdown	Poor solubility and high production costs
Biocompatible and biodegradable	Drug leakage and insufficient stability
Enhanced effectiveness and lower toxicity	Sometimes oxidation and hydrolysis reaction and sensitive to osmosis

Table 2: Benefits and limitations of niosomes

BENEFITS OF NIOSOMES	LIMITATIONS OFNIOSOMES
High chemical and physical stability	Potential for aggregation/fusion
Improved skin penetration	Possible drug leakage
Biocompatible and biodegradable	Limited drug loading for some APIs
Encapsulates hydrophilic and lipophilic drugs	Physical instability (temperature and pH)
Enhanced bioavailability	Batch to batch variability
Controlled and sustained release	Hydrolysis of surfactants over time

Table 3: Benefits and limitations of SLNs

BENEFITS OF SLNs	LIMITATIONS OF SLNs
Increased bioavailability and controlled release	Drug filling capacity is low
Increased drug penetration	Drug filling magnitude is decreased due to partitioning effect
Increased stability	Gelatin liability is uncertain
Large scale extensibility is easy	Better release takes place

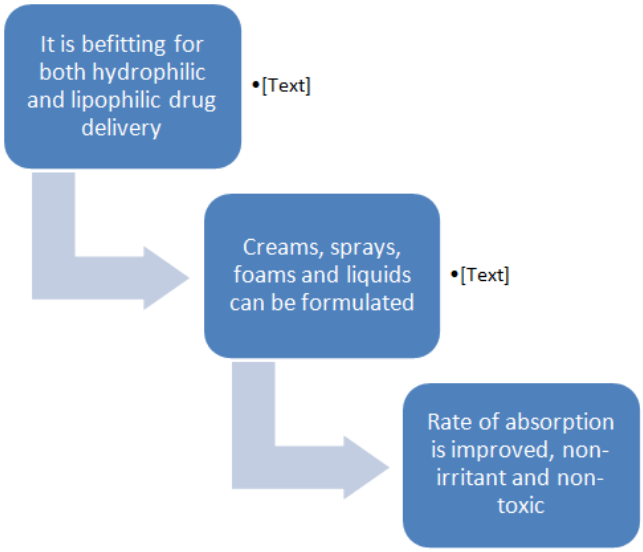


Fig1: Merits of Nano emulsions.

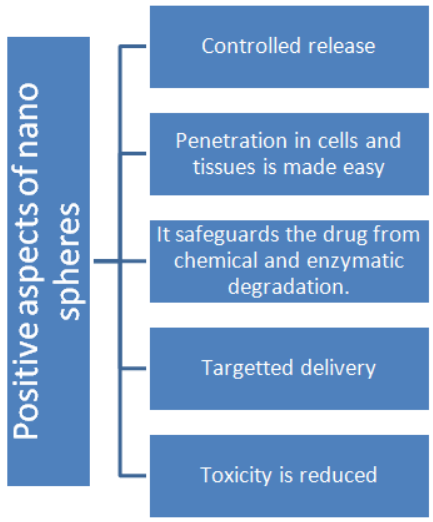


Fig 2: Positive aspects of Nano spheres

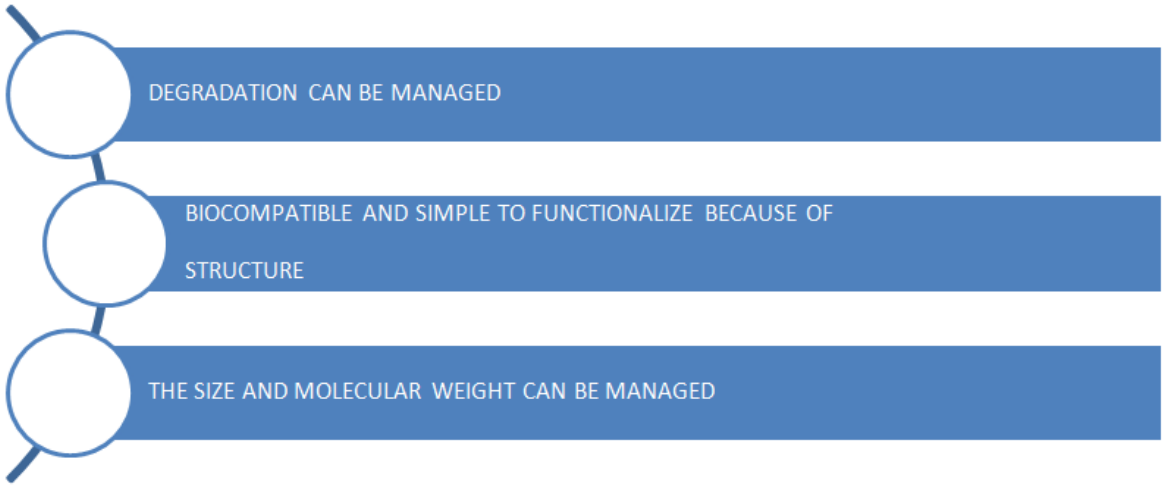


Fig 3: Merits of Dendrimers

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RESEARCH ARTICLE

Antibacterial Investigation of Urine Sample Collected from Domesticated Animals of Chalisgaon Region

Patil Mukesh Shantaram[1]*, Patil Saurabh Sunil[2] and Mahajan Snehal Dnyaneshwar[2]

[1]Assistant Professor, Department of Zoology, B.P.Arts, S.M.A. Science and K.K.C. Commerce College, Chalisgaon, (Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon), Maharashtra, India.
[2]Student, Department of Zoology, B.P.Arts, S.M.A. Science and K.K.C. Commerce College, Chalisgaon, (Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon), Maharashtra, India.

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***Address for Correspondence**
Patil Mukesh Shantaram
Assistant Professor,
Department of Zoology,
B.P.Arts, S.M.A. Science and K.K.C. Commerce College, Chalisgaon,
(Affiliated to Kavayitri Bahinabai Chaudhari North Maharashtra University, Jalgaon),
Maharashtra, India.
E.Mail: mukkeshpatil@gmail.com

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ABSTRACT

Urine samples of Cow, Buffalo, Goat and antibiotic cephataxim were collected from different localities of chalisgaon region. The study explores the antibacterial activity against *Salmonella abony*, *Escherichia coli, Pseudomonas aeruginosa, Bacillus cereus* and *Lactobacillus sporogenes*. Antibacterial activity was done by agar well diffusion method. The Statistical Analysis Performing one way ANOVA in conjunction with Tukey's Honestly Significant Difference (HSD).Typically, a significance level (α) of 0.05 is used. The Q statistic measures the difference between the means of the treatments compared. The one-way ANOVA test indicates that there are significant differences in the means of antibacterial activity across the bacterial species. The Tukey's HSD results of this analysis provide important insights into bioactive compound variability across species.

Keywords: Antibacterial activity, Tukey's HSD, Cephataxim, Agar well etc

INTRODUCTION

The antibacterial properties of animal urine, particularly that of domesticated animals like goats, cows, and buffaloes, have long been studied in traditional medicine and agriculture. However, recent research has started to provide

scientific evidence for these claims by identifying a number of antimicrobial compounds in these animals' urine that effectively combat bacterial infections. Studies have shown that phenols, one of the bioactive substances included in cow urine, have potent antibacterial properties against both Gram-positive and Gram-negative bacteria, such as *Escherichia coli* and *Staphylococcus aureus*[2]. Similarly, research on buffalo urine has shown that its peptide extracts are effective against bacteria, particularly Gram-negative ones like *E. coli* and *Streptococcus agalactiae*[4].These findings suggest that urine-derived compounds may have bacteriostatic or bactericidal effects, contingent on the bacterial strain and urine concentration. Recent studies have brought attention to a study gap in evaluating the relative antibacterial activity of urine from different domesticated species, such as dogs, cats, sheep, and horses. Although cow and buffalo urine has been examined more extensively, nothing is known about the antibacterial qualities of urine from other domestic animals. Although thorough comparative studies have not been conducted, some study suggests that the peptides and organic acids present in these animal urines may be essential in inhibiting the proliferation of germs[4]. Nevertheless, there aren't many systematic comparisons between different bacterial strains and animal species. This study aims to bridge that gap by examining the antibacterial activity of urine from a number of domesticated animals (goats, cows, and buffalo) against particular Gram-positive and Gram-negative bacteria. By comparing the results across species treated with antibiotics, this study will increase our understanding of the potential use of animal urine in agricultural applications and antibacterial therapies.

MATERIALS AND METHODS

Urine Sample Collection

Three domesticated animals, including cows, buffalo, and goats, had urine samples taken from them at different sites throughout the Chalisgaon district. To prevent contamination, the collection was carried out in sterile containers. Before undergoing additional analysis, the samples were kept at 4°c after being transferred to the lab in a refrigerated environment. The appropriate animal was labeled on each sample[8].

Bacterial Strains Used

The study will focus on Gram-positive p (*Bacillus cereus* and *Lactobacillus sporogenes*.) and Gram-negative (*Salmonella abony* (NCTC 6017), *Escherichia coli*(ATCC 8739), *Pseudomonas aeruginosa*(ATCC 9027)bacteria, which will be sourced from School of life sciences, K.B.C.North Maharashtra University, Jalgaon.

Agar Well Diffusion Method

The agar well diffusion method will be used to examine urine's antibacterial activity, bacterial strains will be added to nutrient agar plates, and a sterile cork borer will be used to create wells. Each well will get 100 µl of urine. Zones of inhibition will be evaluated when plates are incubated for 24 hr.At 37°C [3].

Control and Replication

For comparison, wells with sterile water and the common antibiotic cephataxim will serve as controls. Each strain's mean zone of inhibition will be determined by conducting the experiments in triplicate [3].

Statistical Analysis

The data will be statistically analyzed using one-way ANOVA to determine the significance of the antibacterial effects of the urine on each bacterial strain. Performing one way ANOVA[7]in conjunction with Tukey's Honestly Significant Difference (HSD) test[1]to identify which specific group means differ from each other. Typically, a significance level (a) of 0.05 is used. The Q statistic measures the difference between the means of the treatments compared

Formulate Hypotheses: p

Null Hypothesis: There is no significant différence in antibacterial activity among cow, buffalo, and goat urine for each bacterial species.

Alternative Hypothesis: There is a signifiant différence in antibacterial activity among cow, buffalo, and goat urine for at least one bacterial species.

Observation Table

Table 1: Shows the Antibacterialactivity of selected animal urine samples.
Table. 2: Anova: Single Factor
Table. 3: Scheffé multiple comparison
Table. 4: Tukey HSD results
Table. 5: Bonferroni and Holm multiple comparison

RESULTS AND DISCUSSION

We reject the null hypothesis, which indicates that the antibacterial activities of the goat, buffalo, cow, and antibiotic Cephataxim groups are not all the identical, because the one-way ANOVA's p-value (Table 2) of 0.031647 is less than 0.05. There is proof that the antibacterial activity of at least one of the groups differs significantly. The calculated F-value of 3.78444 is greater than the F critical value of 3.238872, confirming the significance of the group differences. We perform a post-hoc test to identify which specific urines are different from one another. The results of the Tukey HSD Test (Table 4) reveal that all comparisons have p-values greater than 0.05, indicating no significant difference in antibacterial activity between these pairs, with the exception of the comparison between buffalo urine and antibiotics, where the p-value is 0.0248179, which is less than 0.05, indicating a significant difference in antibacterial activity between the two. This suggests that buffalo urine and the antibiotic cephotaxim exhibit varying degrees of antibacterial activity against gram-positive and gram-negative bacteria. This study proves that animal urine works as an antibacterial agent by showing that it can fight against dangerous pathogens. According to the same research, camel and sheep urine have antibacterial qualities that help prevent common illnesses[6]. The antimicrobial properties of sheep and camel urine. A study on antimicrobial peptides in cattle urine by Zhi Li et al. in 2018 emphasises the importance of these substances. By serving as a protective mechanism against microbial diseases, these peptides may improve animals' overall health. Antibiotic residues in animal waste, including pee, may be the cause of the antibacterial activity in urine samples. It is necessary to look into the antioxidants found in the urine of various domesticated animals since they may also have antibacterial effects[5].

CONCLUSION

The results of the ANOVA and post-hoc tests can be used to form conclusions about the antibacterial qualities of the urine of cows, buffaloes, and goats as well as the antibiotic Cephataxim for the bacterial species under investigation against *P. auruginosa*. There was no discernible difference in the antibacterial activity of the urines of cows, buffaloes, goats, and the antibiotic cephataxim. The *P. auruginosa,E. coli, L. sporogens, B. cereus,* and *S. abony*. Post-hoc testing revealed that cephataxim had significantly greater antibacterial activity against *B. cereus* than buffalo urine (Tukey HSD, Scheffé, Bonferroni, and Holm) (Table 4 and Table 5)Cephataxim, an antibiotic, demonstrated consistent and effective antibacterial action against all of the tested bacterial species; nevertheless, it did not significantly outperform goat or cow urine in most situations.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this paper

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Table 1: Shows the Antibacterialactivity of selected animal urine samples.

SI. No.	Bacteria Strain	Average Zone of Inhibition in mm (Dilution factor 1:1)			
		cow urine	Buffalo urine	Goat urine	Cefotaxim (100 µg/mL)
1.	*Pseudomonas aeruginosa*	1.2	0	1.1	1.5
2.	*Escherichia coli*	1	0.9	1.9	1.8
3.	*Lactobacillus sporogenes*	0.8	0.9	0	1.5
4.	*Bacillus cereus*	1	0.8	0	1.5
5.	*Salmonella abony*	1.45	0	1	1.32

Table. 2: ANOVA: Single Factor

ANOVA						
Source of Variation	*SS*	*df*	*MS*	*F*	*p-value*	*F crit*
Between Groups	2.759935	3	0.919978	3.78444	0.031647	3.238872
Within Groups	3.88952	16	0.243095			
Total	6.649455	19				

Table. 3: Scheffé multiple comparison

Treatments pair	Scheffé -statistic	Scheffé p-value	Scheffé inferfence
Buffalo vs. Cephotaxim	3.2197	0.0415799	* p<0.05

Table. 4: Tukey HSD results

Treatments pair	Tukey HSD Q statistic	Tukey HSD p-value	Tukey HSD inferfence
Buffalo vs. Cephotaxim	4.5533	0.0248179	* p<0.05

Table. 5: Bonferroni and Holm multiple comparison

Treatments pair	Bonferroni and Holm T-statistic	Bonferroni p-value	Bonferroni inferfence	Holm p-value	Holm inferfence
Buffalo vs. Cephotaxim	3.2197	0.0321085	* p<0.05	0.0321085	*p<0.05

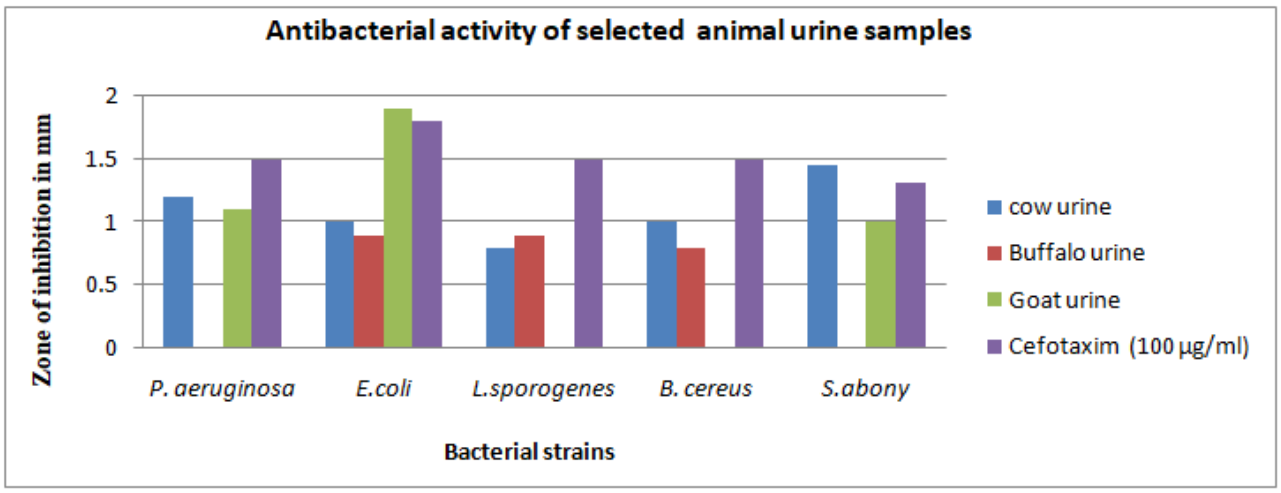


Figure 1: Shows the Antibacterial activity of selected animal urine samples.

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RESEARCH ARTICLE

Exploring Marine Algal Endophytes for Novel Antimicrobials Targeting β-Lactamase and Biofilm-Producing Bacterial Isolates

Thillainayagi .M[1]* and Bharathidasan .R[2]

[1]Research Scholar (Reg. No.: BDU2210282779810), PG and Research Department of Microbiology, Maruthupandiyar College, Thanjavur, (Affiliated to Bharathidasan University, Tiruchirapalli), Tamil Nadu, India.
[2]Assistant Professor, PG and Research Department of Microbiology, Maruthupandiyar College, Vallam, Thanjavur, (Affiliated to Bharathidasan University, Tiruchirapalli), Tamilnadu, India.

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***Address for Correspondence**
Thillainayagi .M
Research Scholar (Reg. No.: BDU2210282779810),
PG and Research Department of Microbiology,
Maruthupandiyar College, Thanjavur,
(Affiliated to Bharathidasan University, Tiruchirapalli),
Tamil Nadu, India.
E.Mail: thillainayagi.mb@gmail.com

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ABSTRACT

The emergence of multidrug-resistant (MDR) and biofilm-forming bacteria poses a significant challenge to current antimicrobial therapies. This study investigates the antibacterial potential of endophytic bacteria isolated from marine algae collected from Rameshwaram, Tamil Nadu. A total of 35 clinical isolates representing seven genera—including *Escherichia coli*, *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*—were screened for β-lactamase production and biofilm formation. Simultaneously, 28 endophytic bacterial isolates were obtained from six marine algal species and evaluated for antimicrobial activity using the agar well diffusion method. Isolates 12, 19, 22, and 23 exhibited broad-spectrum activity, particularly against *P. aeruginosa*, *E. faecalis*, and *A. baumannii*. MIC analysis revealed isolate 12 to be the most potent, with values as low as 0.3125 mg/mL. 16S rRNA gene sequencing identified isolate 12 as *Bacillus cereus*, and phylogenetic analysis confirmed its close evolutionary relationship with known *B. cereus* strains. These findings suggest that endophytic bacteria from marine algae, particularly *Bacillus cereus*, represent a promising source of novel bioactive compounds capable of inhibiting MDR and biofilm-forming pathogens. Their natural origin and broad-spectrum efficacy highlight their potential for development as alternative therapeutic agents in the fight against antibiotic resistance.

Keywords: Endophytic bacteria, Marine algae, Antibacterial activity, Biofilm formation, *Bacillus cereus*, ESBL.

INTRODUCTION

The alarming rise in antibiotic resistance is a critical global public health challenge, with experts cautioning that we may be approaching a return to the pre-antibiotic era. Community- and hospital-acquired infections caused by multidrug-resistant (MDR) organisms—such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant *Enterococci* (VRE), vancomycin-intermediate *S. aureus* (VISA), and extended-spectrum β-lactamase (ESBL)-producing Gram-negative bacteria like *Klebsiella pneumoniae*—are increasingly common [1]. The resistance mechanisms employed by ESKAPE pathogens include enzymatic inactivation, target site modification, reduced cell permeability, overexpression of efflux pumps, and protection through biofilm formation. These mechanisms, combined with the indiscriminate and prolonged use of antimicrobial agents, contribute to the widespread emergence of resistant pathogens [2]. Microbes develop resistance through various mechanisms, one of which involves producing extended-spectrum β-lactamases (ESBLs) that break down β-lactam antibiotics. Additionally, biofilms formed by these microbes pose a major global health challenge by promoting chronic infections and resisting antibiotics, host immune responses, and environmental stressors [3]. This matrix limits antibiotic penetration and creates microenvironments where cells either grow slowly or enter a dormant "persister" state. This further reduces the effectiveness of antibiotics. In fact, bacteria found in biofilms can exhibit up to 1,000 times greater antibiotic tolerance compared to their free-floating (planktonic) counterparts [4]. Compounding the issue are the adverse side effects of synthetic antibiotics and the emergence of infections previously considered rare. Consequently, there is growing interest in identifying new antimicrobial agents that are not structurally related to existing drugs. Natural compounds, particularly those produced by endophytic microorganisms, have shown promise as alternatives. Endophytes are microorganisms (bacteria or fungi) that colonize the internal tissues of plants without causing visible disease. They produce a wide array of secondary metabolites with antimicrobial, anticancer, and other therapeutic properties [5; 6] Marine and aquatic plants, including algae and seaweeds, are increasingly recognized as rich sources of these endophytes [7]. Marine ecosystems support a vast diversity of life, including complex microbial communities associated with macro algae. These include epiphytic and endophytic microbes that participate in nutrient cycling, biofilm formation, and symbiotic interactions [8, 9]. Many marine bacteria associated with seaweeds produce antimicrobial compounds that protect the host from pathogenic microbes [10]. For example, *Ulvaohnoi*-associated bacteria have demonstrated significant antimicrobial activity against a range of human pathogens, including *E. coli*, *Vibrio spp.*, *Salmonella*, and *S. aureus*, as well as the yeast *Candida albicans* [11]. In the current scenario where the effectiveness of existing antibiotics is declining, it has become essential to explore new biological resources, particularly endophytic microbes derived from marine environments. These organisms have the potential to produce novel bioactive compounds, making them promising candidates for the development of next-generation antimicrobial and anticancer agents [12; 13; 14; 15]. The objective of this study is to isolate such endophytes and evaluate their pharmaceutical applications, particularly their activity against biofilm formation and ESBL-producing bacterial strains.

MATERIALS AND METHODS

Collection of Clinical Isolates

To evaluate the antibacterial activity, seven bacterial isolates—*Staphylococcus aureus, Pseudomonas aeruginosa, Escherichia coli, Enterococcus faecalis, Salmonella sp*, *Acinetobacter baumannii* and *Klebsiella pneumoniae*—were collected from a clinical laboratory in Coimbatore. All isolates were confirmed using selective media and chromogenic media. At least more than one microorganism was isolated from each genus. The confirmed isolates were then used to assess the antibacterial activity of the endophytic bacterial isolates.

Assessment of Biofilm and ESBL Producing Bacterial Isolates

Biofilm formation was assessed using the Congo Red Agar method, [16] where BHIA with 5% sucrose and 0.08 g/L Congo red was prepared, plated, inoculated with bacterial isolates in duplicates/triplicates, and incubated at 37 °C for 24 h; black, dry, crystalline colonies indicated strong biofilm production. ESBL production was screened using the

phenotypic confirmatory test by placing amoxicillin (30 µg) and amoxicillin–clavulanic acid (30/10 µg) discs on Mueller-Hinton agar inoculated with test organisms, followed by incubation at 37 °C for 24 h; an increase of ≥5 mm in the inhibition zone for the combination disc confirmed ESBL production [17].

Collection of algal samples and Isolation of Endophytic Bacteria

Algal samples were collected from rocky coastal regions of Rameshwaram, Tamil Nadu, at an approximate depth of 50 m. The samples were aseptically transferred in sterilized plastic bags filled with seawater, rinsed with freshwater in the laboratory to remove salts and contaminants, and shade-dried for 48–72 h to preserve structural integrity [18]. Species identification was performed using taxonomic keys [19] and confirmed through detailed morphological analysis at the Chromopark Research Centre, Plant and Algal Section. For isolation of endophytic bacteria, surface-sterilized algal tissues were aseptically crushed in 5 mL sterile saline, and 100 µL of the extract was spread onto nutrient agar plates. Plates were incubated at 25 °C for 72 h, with daily observation for bacterial colonies, which were then sub cultured and preserved for further study.

Extraction of Bioactive Compounds from Endophytic Bacteria

The endophytic bacterial isolates were inoculated into tryptic soy broth (TSB; Merck) and incubated at 30 °C for 48 hours. After incubation, the culture supernatant was mixed with an equal volume of ethyl acetate in a separating funnel and shaken vigorously. The organic (ethyl acetate) layer was collected and allowed to evaporate under open air. The dried residue was then dissolved in DMSO and stored at 4 °C for further analysis [20].

Evaluation of Antimicrobial Activity of Bacterial Isolates

The antimicrobial potential of the bacterial isolates against clinical pathogens was assessed using the agar well diffusion method. Experiments were carried out on solid Mueller–Hinton agar (HI Media), which was sterilized, poured into sterile Petri plates, and allowed to solidify. Overnight cultures of the test pathogens were uniformly swabbed onto the agar surface. Sterile wells were then created using a borer, and 50 µL of secondary metabolite extracts from different bacterial isolates were carefully loaded into each well. The plates were incubated at 37 °C for 24 hours, and antimicrobial activity was evaluated by measuring the diameter of the clear zones of inhibition surrounding each well. Isolates exhibiting the largest inhibition zones against all tested pathogens were selected for further analysis [21].

Determination of minimum inhibitory concentration (MIC)

The minimum inhibitory concentration (MIC) of extracts from potential endophytic isolates was determined using the resazurin-based micro titer dilution method [22] Clinical bacterial isolates were cultured overnight in nutrient broth, adjusted to 0.5 McFarland (~1 × 10^6 CFU/mL), and tested in 96-well plates containing 100 µL nutrient broth, varying extract concentrations, 10 µL resazurin (0.01%), and 10 µL bacterial suspension (~5 × 10^6 CFU/mL). Positive controls lacked extracts, and negative controls lacked bacteria. Plates were incubated at 37 °C for 24 h and inspected for color change—purple to pink/colorless indicated growth, while unchanged purple indicated inhibition. The MIC was the lowest extract concentration preventing visible growth.

Identification of Potential Isolate

The potential isolate was identified by 16S rRNA gene sequencing. Genomic DNA was extracted, confirmed on a 1% agarose gel, and the 16S rRNA gene (~1500 bp) was amplified with universal primers. The purified PCR product was bidirectionally sequenced, and forward/reverse reads were assembled into a consensus sequence. BLAST analysis against the NCBI GenBank nr database identified closest matches, which were aligned with ClustalW. A phylogenetic tree and distance matrix were constructed using the Neighbor-Joining method in MEGA 11.

RESULT AND DISCUSSION

In the present study, 35 bacterial isolates representing seven different genera were obtained and identified using selective media and standard biochemical tests. The confirmed genera included *Escherichia coli, Enterococcus faecalis, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiellan pneumoniae, Salmonella spp*., and *Acinetobacter spp*. All isolates were confirmed with selective media and chromogenic media, according to the morphological characterization, isolates were confirmed. Both selective and chromogenic media; enabled clear, efficient isolation and presumptive identification of clinically relevant bacteria, while biochemical confirmation ensured reliability. This dual strategy optimized workflow and diagnostic precision. The β-lactamase production phenomenon plays a critical role in mediating resistance to β-lactam antibiotics, including penicillins and cephalosporins. These enzymes hydrolyze the β-lactam ring, rendering the antibiotic ineffective and compromising therapeutic outcomes. In the present study, all bacterial isolates were screened using a phenotypic method for β-lactamase activity, except *S.aureus*. Out of these, 9 isolates (30%) were identified as ESBL producers. Among the species tested, *Enterococcus faecalis* showed the highest prevalence, with 40% of isolates from this genus testing positive. This trend suggests that resistance mechanisms are becoming increasingly common not just among Gram-negative bacteria but also in clinically important Gram-positive pathogens. These findings align with previous reports in India, 92.2% ESBL positivity among urinary *E. coli* isolates in a tertiary care hospital in Vellore, much higher than the 20% observed in our study, likely due to differences in isolate origin, sample size, or detection methods [23]. A similar study from northeastern India highlighted widespread ESBL and carbapenemase-producing *Entero bacteriaceae* detected via phenotypic assays and mPCR. ESBL-producing *E. coli* and *K. pneumoniae*, major agents of CA-UTIs, are increasingly reported worldwide, often showing co-resistance to non-β-lactam antibiotics. Additionally, the recent rise in multidrug-resistant (MDR) uropathogenic *E. coli* (UPEC) with ESBL producing isolates in the community has created significant challenges for clinicians in selecting appropriate empirical therapy [24]. Among the various virulence factors, biofilm-forming infections are challenging to treat due to reduced drug penetration and increased resistance. *Staphylococcus aureus* and other pathogens are well-known biofilm producers, contributing significantly to treatment failure. According to the U.S. National Institutes of Health, over 60% of deaths from such infections are associated with drug resistance [25]. In the present study, 17 out of 35 isolates (48.5%) were identified as biofilm producers. *Enterococcus faecalis* showed the highest rate (80%), which is consistent with the findings of Upadhyaya *et al*. [26] and Nair *et al*. [27]. Another report has also documented biofilm production in 65% of *Salmonella* spp isolates [28, 29]. Other species such as *E. coli*, *P. aeruginosa*, *S. aureus*, and *K. pneumoniae* showed 40% biofilm positivity. Triveni *et al*. [30] reported 38.29% biofilm production in *S. aureus*, while Saxena *et al*. [31] observed 64.7% in *P. aeruginosa*. In the Western Ghats, *K. pneumoniae* exhibited 51% moderate and 27% strong biofilm production. Biofilm formation and β-lactamase production are key resistance mechanisms, enabling bacteria to evade antibiotics and immune responses while degrading β-lactam drugs [32]. This highlights the urgent need for new antibiotics targeting biofilm-forming, drug-resistant isolates. In this context, the present study explores the antibacterial potential of endophytic bacteria from algae. A total of six algal genera belonging to three families were collected: green algae (*Halimeda gracilipes* and *Chaetomorpha linum*), red algae (*Gracilaria edulis* and *Kappaphycus alvarezii*), and brown algae (*Padina pavonica* and *Sargassum polycystum*) (Figure.1). Identification of the collected algae was confirmed using the taxonomic keys described by Jha *et al* [19]. The following parameters were utilized for their characterization: thallus structure, color, branching pattern, holdfast type, habitat, and special features. From these six algal samples, a total of 28 bacterial isolates were obtained. All endophytic isolates were screened for antibacterial activity against ESBL-producing and biofilm-forming clinical isolates. The antimicrobial activity of 28 endophytic isolates, along with two controls (C1 and C2), was tested against seven clinically relevant pathogens using the agar well diffusion method. Among these pathogens, *P. aeruginosa*, *E. faecalis*, and *A. baumannii* were the most susceptible to the endophytic extracts. Notably, *P. aeruginosa* was inhibited by 24 of the 28 isolates, with inhibition zones ranging from 11 mm to 16 mm. *E. faecalis* was suppressed by 17 isolates, with isolate 27 showing the strongest activity (18 mm). Likewise, *A. baumannii* showed considerable sensitivity; especially Isolate 7 was the most effective against *A. baumannii*, with a 16 mm inhibition zone. These three pathogens were the most responsive, suggesting their higher vulnerability to bioactive metabolites from endophytes. *E. coli* was inhibited by 12 isolates, with zones ranging between 11 and 13 mm. Isolates of 16, 20, 23,

and 24 were showed moderate effectiveness. *K. pneumoniae* displayed minimal susceptibility, only isolates 19 and 20 showing inhibition (12–13 mm). *S. aureus* was more resistant, with inhibited only with 22 and 23, while controls (C2: 10 mm) showed minimal activity. Similarly, *Salmonella spp.* showed limited inhibition by isolates 6 and 12 (11 mm), and C2 (10 mm). Among all, isolates 12, 19, 22 and 23 stood out for their broad-spectrum activity, showing significant zones of inhibition against 5 pathogens (Table.1). These isolates may hold strong potential as sources of novel antimicrobial agents, especially against multidrug-resistant bacteria. These findings align well with published studies showing endophytic microbes isolated from marine algae or medicinal plants as prolific producers of secondary metabolites with broad-spectrum antibacterial activity. For example, isolate the *Acinetobacter indicus* from marine Sargassum sp and *Turbinaria ornate,* which showed strong active against to *S. epidermidis* and *S.aureus* [33]. Another study, actinomycetes from marine algal sources in India showed strong inhibition of MDR Gram-negative uropathogens, including *P. aeruginosa* and *E. coli* via agar well diffusion assays [34]. Similarly, a systematic screening of endophytic actinobacteria from medicinal plants revealed that approximately 30–40% of isolates exhibited bioactivity against pathogens like *E. faecalis*, *S. aureus*, and *P. aeruginosa* [35, 36]. The Minimum Inhibitory Concentration (MIC) results of the four endophytic bacterial isolates—Isolate 12, Isolate 19, Isolate 22, and Isolate 23—against seven pathogenic bacteria revealed varying degrees of antimicrobial efficacy (Table.2). Isolate 12 showed effective inhibitions against *E. coli*, *E. faecalis*, *P. aeruginosa*, *Salmonella sp.*, and *A. baumannii*, with MIC values ranging from 0.3125 to 1.25 mg/mL, but exhibited no activity against *K. pneumoniae* and *S. aureus*. Isolate 19 displayed the broadest spectrum of inhibition, including activity against *K. pneumoniae* and *A. baumannii*, although its MIC values were generally higher (1.25–2.5 mg/mL), indicating moderate potency. Isolate 22 was particularly effective against *E. faecalis*, *P. aeruginosa*, and *S. aureus* (MIC = 1.25 mg/mL or lower) but showed no inhibition against *K. pneumoniae* and *Salmonella sp*., and required higher concentrations (2.5 mg/mL) to inhibit *E. coli* and *A. baumannii*. In contrast, Isolate 23 demonstrated limited antimicrobial activity, with MIC values of 2.5 mg/mL against most susceptible pathogens and no activity against *K. pneumoniae* and *Salmonella sp.* Overall, Isolate 12 showed the highest potency (lowest MIC), while Isolate 19 had the broadest range of antibacterial action.

1-28: Extract of endophytic isolates (2.5mg), C1: DMSO control, C2: Ciprofloxcin -5mcg

Similarly, Makuwa *et al*. [37] and Maela *et al*. [38] determined the antimicrobial activity and MIC values of endophytic bacteria using a resazurin-based microdilution assay. This method has been extensively validated for assessing antimicrobial activity against both Gram-positive and Gram-negative bacteria [22]. The microtiter plate-based resazurin assay is simple, rapid, sensitive, and reproducible for evaluating the antimicrobial potential of secondary metabolites. Building on this, Elshikh *et al*. [39] optimized a 96-well microdilution protocol using resazurin and confirmed its reliability through consistent MIC values for standard antibiotics such as tetracycline and gentamicin. The potential isolate was identified by 16S rRNA sequencing, producing a consensus sequence with 99.93% identity to *Bacillus cereus* strain BCd16 (Accession No. KP717563.1). Other close matches showed ≥99.11% similarity. Phylogenetic analysis using the maximum likelihood method (Tamura-Nei model) confirmed its clustering within the *B. cereus* clade, with minimal evolutionary divergence (pairwise distance 0.000–0.002) (Figure 2). Thus, Isolate 12 was identified as *Bacillus cereus*, supporting its role in antibacterial and MIC studies. A similar finding was reported by previous study, they were demonstrated the antimicrobial activity of an endophytic *Bacillus cereus* isolate against *E.coli*, *P.aeruginosa*, *S.aureus*, *S.typhi*, and *K.pneumonia* [40]. In another study observed that endophytic isolates of *Bacillus* sp. also exhibited antifungal activity [41]. In the present study, no clear dominance was observed in the inhibition of either Gram-positive or Gram-negative bacteria. This result contrasts with the findings of previous study, which was reported that endophytic isolates exhibited stronger inhibitory activity against Gram-positive bacteria compared to Gram-negative bacteria [42]. The antibacterial activity exhibited by the crude extracts of endophytic bacterial isolates in this study highlights their potential to suppress pathogenic bacteria, supporting their possible application in the development of novel antimicrobial agents. In conclusion, this study highlights the significant antimicrobial potential of marine algal endophytic bacterial isolates, particularly *Bacillus cereus*. These isolates exhibited broad-spectrum activity against both Gram-positive and Gram-negative pathogens, including biofilm-forming and β-lactamase-producing strains, underscoring their promise as alternative therapeutic agents. The ability of these endophytes to inhibit biofilm formation and overcome β-lactamase resistance further supports their clinical relevance. Importantly, compounds derived from marine algal endophytes present a natural, safer, and

potentially less toxic alternative to conventional antibiotics. These findings emphasize the value of marine algal endophytes as sustainable and effective sources of novel antimicrobial agents in the ongoing fight against antibiotic-resistant infections.

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Table.1: Antibacterial activity of endophytic isolates against ESBL and Biofilm producing bacteria

Isolates name	Various endophytic isolates Zone of inhibition measured in mm																													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	C1	C2
E.coli	-	-	-	12	12	-	-	-	-	-	-	12	-	-	12	13	-	-	11	13	-	11	13	13	-	12	11	-	-	10
E.faecalis	-	-	-	11	-	-	14	13	13	12	12	12	12	-	-	-	-	-	11	12	-	14	16	16	13	15	18	14	-	10
K.pneumoniae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	12	13	-	-	-	-	-	-	-	-	-	11
P.aeruginosa	13	12	13	12	12	-	-	15	14	-	14	16	13	12	13	13	12	11	12	-	12	14	13	13	14	16	15	16	-	11
S.aureus	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	14	14	-	-	-	-	-	-	10
Salmonella sp	-	-	-	-	-	11	-	-	-	-	-	11	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	10
A.baumannii	12	12	11	-	12	-	16	11	-	-	11	12	-	-	12	12	11	12	14	11	15	13	11	11	13	-	-	13	-	10

Table.2: Determination of minimum inhibitory concentration of endophytic isolates

Bacterial pathogens	Endophytic bacterial isolates MIC in mg			
	Isolate 12	Isolate 19	Isolate 22	Isolate 23
E. coli	1.25	2.5	2.5	2.5
E. faecalis	1.25	2.5	0.625	0.625
K. pneumoniae	NA	1.25	NA	NA
P. aeruginosa	0.3125	1.25	1.25	2.5
S. aureus	NA	NA	1.25	1.25
Salmonella sp.	1.25	NA	NA	NA
A. baumannii	1.25	0.625	2.5	2.5



1. Halimeda gracilipes, 2.Gracilaria edulis, 3.Kappaphycus alvarezii, 4.Padina pavonica, 5.Sargassum polycystum, Chaetomorpha linum

Figure.1: Collection of algal samples

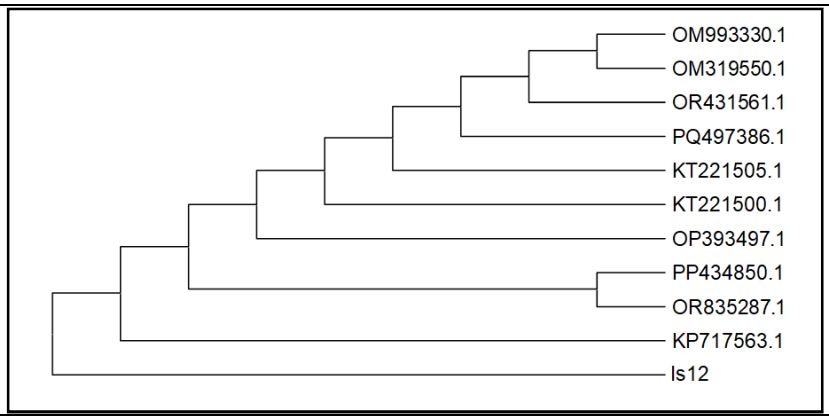


Figure.2: Phylogenetic tree analysis

RESEARCH ARTICLE

An Economic Analysis on Utilization of Milk Production and Milk Products in Tirupattur District of Tamil Nadu

R. Kurinji Malar*

Assistant Professor, Department of Economics, Sacred Heart College (Autonomous), Tirupattur, (Affiliated to Thiruvalluvar University, Serkkadu, Vellore), Tamil Nadu, India.

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***Address for Correspondence**
R. Kurinji Malar
Assistant Professor,
Department of Economics,
Sacred Heart College (Autonomous), Tirupattur,
(Affiliated to Thiruvalluvar University, Serkkadu, Vellore),
Tamil Nadu, India.
E.Mail: kurinjimalar@shctpt.edu

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ABSTRACT

An economic analysis of utilization of milk production and milk products in Tirupattur district of Tamil Nadu's was the main emphasis of this study. 300 respondents were surveyed utilizing an interview schedule in January 2023 in order to get the primary data. Of the sample as a whole, 60 per cent of respondents were male and 40 per cent of respondents were female. According to the study, younger members of the village are more involved in diary production and animal husbandry than older residents, and those with high and middle level education are more involved in these fields. In animal husbandry, one third of the respondents made more than Rs. 40,000 per month. The respondents did not experience any losses in their fair revenue from either milk production or animal husbandry. Approximately 96 per cent of those surveyed said they always use the money they make from milk production and animal husbandry to generate assets. A significant portion of the rural population relies on milk production as their primary source of income and subsistence. Everyone now has a bank account for their savings from milk production and animal husbandry thanks to the Jan Dhan Yojana.

Keywords: Economic analysis, milk production, milk products, animal husbandry, income, Tirupattur.

INTRODUCTION

In India there is a deep relationship between the sectors of agriculture and dairy industry. Dependence between both sectors is very high. Over 50 per cent of the Indian population is depending on the agricultural sector compared to dairy sector. In recent years, the dairy industry has taken over the agricultural sector and its contribution is more.

The dependency level on milk in India is very high when we compare to the other countries of the world. Indians consume milk in their day to day life in the form of pure milk or through the byproducts of milk since they consider milk as their traditional beverage from their ancient period. Many ancient literatures in India exhibit the uses of milk and the consumption level of milk during the ancient periods. The production of milk in the world has increased by 59 per cent from 530 million tonnes to 843 million tonnes in the year 2018. Approximately about 150 million people in the world are involved in the milk production. India is the world's largest milk producer which contributes about 22 per cent of the total milk production followed by United States, China, Pakistan and Brazil. Indian government is initiating many measures and schemes for the production of milk and animal husbandry since they are the main sources of income to rural people. Our Indian government has announced many schemes like Kamdhenu Yojana, Rashtriya Gokul Mission, Gokul Gram which help the farmers to get easy access into the dairy industry. The government of India provides loans for cattle through NABARD and subsidies through local banks. Globally export of diary production was about 54,800 million tonnes and 240 million USD by the financial year 2020-2021. In India, Rajasthan and Andhra Pradesh contributes more for milk production compared to other states. The cattle not only help in the dairy industry but also in the agricultural sectors for ploughing the agricultural fields. India is not only exporting milk, but also exports milk byproducts like ghee, butter, ice cream etc. In Tamil Nadu, cow and buffalo milk make up the majority of the milk produced. The size of the Tamil Nadu dairy market is expected to reach INR 1,058 billion in 2022, according to a recent estimate. The dairy market in Tamil Nadu would expand by 15.5% between 2023 and 2028 to reach INR 2,473 billion. So, considering the above points in view, this study focused on the economic analysis on utilization of milk production and animal husbandry in Tirupattur district of Tamil Nadu. This study was aimed with the following objectives. To probe into the socio-economic background of the population involved in milk production and milk products in Tirupattur district. To determine the distribution of animals across the sample households in the study area. To determine the utilization pattern of milk and milk products across various consumers in the study area. In view of this Kumar and Singh (2008) have stated in their study that animal husbandry and diary plays a vital role in the economic development of a country. India stands first in the milk production when compared to the other countries.

During the year 2007-08, the milk production of India is about 100.90 million tones. Many welfare schemes were implemented to initiate the production of milk throughout the country. Pramoud and Rajasekar (2011) has highlighted the various uncertainty and risks faced in the diary industry in the perspective of various stages of supply chains, the supply chain between the milk producer, the processor and the end-consumer are facing many difficulties in the supply chain. Deepthi Gour (2013) had made an attempt to analyze the development of dairy cooperative in India and how they help in the development of diary. The cooperatives play a commendable role in the upliftment of the rural people through the diary industry. The available opportunities for the dairy farming in the rural areas are plenty when compared to the urban areas. Muraliappa Reddy (2013) has founded that the development of dairy industry is in the three tire structure. The primary is at the milk producing society in the rural villages, the secondary level is at the cooperative milk collection sector from the rural areas and the tertiary level is at the processing of the milk and converting the milk into the finished products to reach the product to the people in the urban areas. However, the ability of such sectors can be influenced by the quality of the products. Doe *et al.* (2012) have concluded in their study that the cooperative society created market for the raw milk and also provided opportunities for the employment in rural areas which has increased the revenue level of the people from the village areas. Manvir Kaur (2011) has concluded that the milk cooperative unions at the district level maintained the distribution of milk within the district. The milk unions even process the milk or send the milk to other districts where the need for the milk is available through the variable milk processing milk plants. The unions are formed at the primary level at the village to collect the milk in an efficient manner. Meena and Jain (2012) have studied about the economics of milk production in Rajasthan and suggested that the adequate amount of schemes should be introduced for the cow upgradation programmes. They indicated a positive impact on the diary cooperative in the several parts of the rural area of production, consumption and market surplus of milk.

METHODOLOGY

Data Collection: The present study is based on primary data. The primary data collected from milk producers and agricultural labourers in Tamil Nadu. Partially the study was based on secondary data. The Tamil Nadu state has been separated in its seven agro-climatic zones. Tamil Nadu is a eleventh largest state in India by area. The Tirupattur district comes under North Eastern Districts of Tamil Nadu. It is a new born district from Vellore district. It consists of two revenue divisions, Tirupattur and Vaniyambadi, four taluks i.e., Tirupattur, Natrampalli, Vaniyambadi and Ambur is industrial denced area when compare to Tirupattur and Natrampalli. Multi-stage random sampling procedure was employed under district, taluks and villages. Five villages namely, Achamangalam, Karuppanur, Bommikuppam, and Annandapatti and Thiniyalam were selected in Tirupattur block randomly on the basis of agriculture and animal husbandry. To collect the primary data, the researcher selected Atchamangalam, Karuppanur, Bommikuppan, Annadapatti and Thiniyalam villages were selected in Tirupattur District of Tamil Nadu. These five villages come under the Gram Panchayats with the population of more than 4925 people consisting of 1,308 males and 3,617 females. These villages are surrounded by hills and hill base with green cultivation of millets and necessary agricultural cultivation surrounded by small temples on the base of the hills. A water falls also famous near to these villages named as Jalagamparai with full of herbal water and breezes in these hilly villages are the small identifications in these study areas. Due to its geographical location, these villages receive plenty of water during the rainy seasons and due to dam and lakes. Owing to the over availability of natural resources in these villages, animal husbandry and dairy farming are the major source of income to these village people. The researcher used the descriptive and analytical design. The primary data were collected from 300 respondents using interview schedule during month of January 2024. Research design employed here is descriptive analysis since this design describes the characteristics of individuals with behavior and their individual attitudes which would allow the researchers to complete the research without any errors. This study employed a multi-stage random sampling and 300 respondents were selected who are involved in the animal husbandry and diary production. To collect primary data a well-structured interview scheduled was framed to fulfill the objectives of the study. The secondary data were collected through newspapers, journals, books and web resources.

Analytical Framework: The production and utilization of milk and milk products were calculated using simple averages and percentage method for each category of every sampled unit. This study projected the utilization pattern of milk, milk products, animal husbandry and distribution of animals across the sample households.

Tabular Analysis: Tabular analysis was applied to the data in order to examine the production and utilisation patterns of the sample respondents. According to daily milk consumption, the amount of milk kept at home for liquid consumption or for processing into milk products was calculated. Milk and milk product consumption per capita was taken into account.

$$\text{Per capita per day milk consumption} = \frac{\text{Quantity of milk reserved at home}}{\text{Size of the family}}$$

Distribution and Utilization Pattern of Consumers: Using sample averages and percentages, the producers' overall milk production, distribution, and consumption patterns were calculated. Using sample averages and percentages, the producers' overall milk production, distribution, and consumption patterns were calculated.

RESULTS AND DISCUSSION

Production and Utilization Details of Milk and Milk Products among the Respondents Households

The socio-economic details of the sample respondents represented in Table 1. The primary data were collected from the 300 sample respondents from all the villages. Most of the respondents (60 per cent) comes under the age group of 30-40 especially in Karuppanur village followed by (55 per cent) under the age group of 40-50 in Achamangalam village. Regarding the gender details nearly 73.33 per cent of the respondents are males especially in Bommikuppam

village. Because the village is getting sufficient water resource from herbal water falls named as 'Ezhuaruvi.' This may be a reason for maximum involvement in animal husbandry and milk production. Regarding the community details of the respondents most of the respondents (75 per cent) comes under the category of Most Backward Class (MBC) in Achamangalam village. Basically the district is dominated by SC population. Regarding the educational qualification majority of the respondents were studied up to high level (66.67 per cent) in Karuppanur village. When compare the educational qualification the maximum of 66.67 per cent of the respondents were studied up to high level of education in Karuppanur. In Achamangalam, nearly 46.67 per cent of the respondents were studied up to middle level of education. Only 3 to 5 per cent of the respondents were post graduates in Karuppanur and Achamangalam. In Thiniyalam village nearly 53.34 per cent of the respondents were comes under the age group of 30-40. In the same village 73.34 per cent of the female respondents involved in animal husbandry based actives. When we look at the data people did not concentrate much on education. The data on milk production and profit levels represented in Table 2, Fig. 1 and 2. From the five villages, Aachamangalam, Karupanur, Bommikuppam, Aanandapatti, and Thiniyalam show a significant variation in output and earnings through animal husbandry. In terms of milk production, Karupanur leads with the highest daily production of 55 litres, followed closely by Aachamangalam and Bommikuppam with 50 litres each. Aanandapatti and Thiniyalam lag behind with 30 and 35 litres respectively. The average milk production across all villages stands at 19.4 litres per day, indicating a modest overall output. When considering monthly profits from animal husbandry, Aachamangalam shows the highest profitability, with farmers earning up to ₹40,000 per month, while Karupanur and Aanandapatti reach up to ₹20,000. The other villages, Bommikuppam and Thiniyalam, earn lower maximum profits, at ₹15,000 and ₹12,000 respectively. The average monthly profit across all villages is ₹3,420, showing that while some individuals achieve high incomes, the majority earn modest amounts. The lowest reported monthly profits range between ₹500 to ₹1,000, highlighting the economic vulnerability for many farmers depending solely on animal husbandry. Regarding milk usage for domestic purposes, about 52.33 per cent of households across the five villages use a portion of the milk they produce for their own consumption. Aachamangalam shows the highest domestic usage rate at 66.67 per cent, whereas in Karupanur and Aanandapatti, a majority of the milk is not used domestically but likely sold for income. The average domestic consumption of milk across villages varies widely; Thiniyalam stands out with the highest average consumption at 38 to 40 litres, while in other villages like Aachamangalam and Karupanur, average domestic use is significantly lower, around 3.5 to 5 litres. When it comes to the distribution of milk, local people are the major consumers, accounting for 48.67 per cent of total milk distribution.

Notably, Aanandapatti shows the highest share of local distribution at 66.67%, while Karupanur and Thiniyalam rely heavily on co-operative societies, each with 56.67 per cent of their milk distributed through this channel. Some villages like Bommikuppam also engage private milk collection centers and tea shops to a small extent, but overall, private centers and tea shops play a relatively minor role, with only 6.67 per cent and 3 per cent distribution respectively. While milk production and profit levels are encouraging in villages like Aachamangalam and Karupanur, there is considerable variability among the villages in terms of domestic milk consumption patterns and marketing strategies. The reliance on local consumers and co-operative societies remains crucial for the livelihoods of these farmers, with private collection centers and tea shops serving as supplementary outlets. The data regarding involvement in animal husbandry and awareness on government schemes represented in Table 3. Across the villages namely, Aachamangalam, Karupanur, Bommikuppam, Aanandapatti, and Thiniyalam highlights significant disparities. In terms of receiving animals from government schemes, Aachamangalam (38.33 per cent) and Karupanur (35 per cent) report the highest access, showing that more than one-third of the respondents in these villages benefited from government-provided livestock. Aanandapatti has a much lower level of support, with only 15 per cent of the farmers receiving animals. On the other hand, both Bommikuppam and Thiniyalam recorded no beneficiaries (0 per cent), indicating a complete lack of government assistance in livestock distribution in these villages. When it comes to awareness about government schemes, Aachamangalam once again leads, with 41.67 per cent of respondents aware of available programs, followed by Aanandapatti at 38.33 per cent. Awareness levels drop sharply in Karupanur (10 per cent) and Thiniyalam (1.67 per cent), while Bommikuppam shows no awareness at all (0 per cent). The high lack of awareness in Bommikuppam and Thiniyalam correlates with the fact that no respondents from these villages received any animals from the government. Overall, the data reveals that the success

of government schemes in promoting animal husbandry depends heavily on both availability and awareness. Villages like Aachamangalam and Aanandapatti have moderately benefited, while villages like Bommikuppam and Thiniyalam remain marginalized, highlighting a pressing need for better outreach, communication, and program implementation in these areas. The Table 4 presented the data on the improved standard of living through animal husbandry across five villages namely Aachamangalam, Karupanur, Bommikuppam, Aanantappatti and Thiniyalam. Among them, Bommikuppam shows the most significant impact in terms of high earnings, with 51.67 per cent of respondents reported income improvement due to animal husbandry. In contrast, villages like Karupanur and Thiniyalam reported very low earnings improvement, indicating that animal husbandry has not been a major income source there. In terms of housing development, Aachamangalam leads with 63.33 per cent of households constructing their own homes, suggesting that animal husbandry income has contributed to improved living conditions. Other villages like Karupanur and Thiniyalam also show some improvements, though at a much lower scale. When it comes to educational benefits for children, Thiniyalam stands out, with 28.33 per cent of respondents acknowledging improved access to education through income from animal husbandry. In contrast, Karupanur shows no impact in this area. The data on savings indicates that Thiniyalam again performs better than other villages, with 23.33 per cent of respondents managing to save, whereas Bommikuppam reported no savings at all. Reduction in borrowings is relatively low across all villages, but Karupanur and Aanantappatti have slightly better outcomes. In terms of loan repayment, Karupanur emerges as the most successful, with 61.67 per cent of respondents using their earnings to repay agricultural loans, followed by Aanantappatti with 33.33 per cent. Thiniyalam reported no loan repayment at all, possibly due to lack of loans or the inability to repay. Participation in awareness programs like meetings on animal husbandry also varies significantly. Thiniyalam has the highest attendance (53.33 per cent), which may explain its relatively better performance in areas like education and savings. Karupanur has a moderate level of participation (30 per cent), while Bommikuppam and Aanantappatti recorded no participation, which could be a factor behind their weaker performance in some key areas. Overall, while some villages like Thiniyalam and Karupanur show progress in particular areas, the benefits of animal husbandry as a tool for improving living standards are unevenly distributed across these five villages.

CONCLUSION

The majority of the people from the rural area are involved in animal husbandry and milk production which acts as the major source of income for their livelihood. After the pandemic period, more people are getting involved in animal husbandry and milk production because these sectors always have their peak level and demand even in the pandemic condition. Due to the reasonable price in the cooperative societies and local distribution, most of the respondents prefer cooperative societies and local distribution for their distribution of milk. Both animal husbandry and milk production always provide a reasonable income without making any loss for the respondents. Majority of the respondents received highest profit which they have earned from the animal husbandry and milk production. Milk production acts as the main source of income and also livelihood for many people from the rural areas. Due to the implementation of Jan Dhan Yojana, everyone has their own bank account for their savings earned by milk production and animal husbandry. Many new schemes should be introduced by the government for the proper collection of milk from the producers and make a proper way to reach the consumers. A common price should be fixed to the milk by the government since their prices are varying from one milk collection centre to other. Subsidies should be given to the entrepreneur for starting animal husbandry and milk production centres which would allow the government to generate more revenue. A concentrated effort is being made to promote dairying in high-potential locations in order to enhance milk output. Unfortunately, post-production handling of excess milk is not being addressed in parallel. The amount of milk consumed has decreased recently. The most significant contributing elements appear to be rising costs and a declining level of living. We must deal with this issue as soon as feasible. Strategies for milk processing and marketing must be created, and small-scale farmers must be encouraged to establish producer-processor cooperatives at the local level. Milk must be collected and processed in a strategic location due to its weight and high perishability. Cost-effective strategies must be used to maintain dairy product costs at levels that the majority of consumers can afford. Promotion and advertising will play a crucial role.

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Table 1: Socio-Economic Details of the Respondents.

Social Details	Achamangalam	Karupanur	Bommikuppam	Aanantappatti	Thiniyalam
Age Details					
20-30	3 (5.00)	9 (14.51)	8 (13.33)	1 (1.67)	6 (10.00)
30-40	7 (11.67)	36 (60.00)	14 (23.33)	14 (23.33)	32 (53.34)
40-50	33 (55)	6 (10.00)	17 (28.34)	17 (28.34)	12 (20.00)
50-60	15 (25.00)	5 (8.33)	20 (33.33)	15 (25.00)	8 (13.33)
60-70	1 (1.67)	1 (1.66)	1 (1.67)	3 (5.00)	1 (1.66)
Above 70	1 (1.67)	3 (5.00)	0 (0)	3 (5.00)	1 (1.66)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Gender Details					
Male	31 (51.67)	27 (45.00)	44 (73.33)	42 (70.00)	16 (26.66)
Female	29 (48.33)	33 (55.00)	16 (26.67)	18 (30.00)	44 (73.34)
Total	60	60	60	60	60

	(100)	(100)	(100)	(100)	(100)
Community Details					
MBC	45 (75)	35 (58.33)	28 (46.67)	26 (43.33)	53 (88.34)
BC	10 (16.67)	21 (35.00)	27 (45)	23 (38.33)	0 (0)
SC	3 (5)	3 (5.00)	5 (8.33)	7 (11.67)	7 (11.66)
ST	2 (3.33)	1 (1.67)	0 (0)	4 (6.67)	0 (0)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Educational Qualification					
Primary	5 (8.33)	0 (0)	8 (13.33)	4 (6.66)	9 (15.00)
Middle	28 (46.67)	16 (26.67)	13 (21.67)	10 (16.67)	22 (36.67)
High	16 (26.67)	40 (66.67)	24 (40.00)	22 (36.66)	16 (26.66)
Higher SEC.	6 (10)	2 (3.33)	14 (23.33)	19 (31.67)	8 (13.33)
Under Grad.	2 (3.33)	0 (0)	1 (1.67)	5 (8.34)	4 (6.66)
Post Grad.	3 (5)	2 (3.33)	0 (0)	0 (0)	1 (1.66)
Others	0 (0)	0 (0)	0 (0)	0 (0)	0
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)

Table 2: Productions of Milk, Milk Products and Profits Details of the Respondents.

Total Litres of Milk Per Day (in Litres)							
Levels	**Achamangalam**	**Karupanur**	**Bommikuppam**	**Anandapatti**	**Thiniyalam**	**Total**	**Average**
Highest	50	55	50	30	35	220	44
Average	20	20	24	18	15	97	19.4
Low	6	3	10	10	4	33	6.6
Average Profit (Per Month in Rs.)							
Animal Husbandry							
Highest	40,000	20,000	15,000	20,000	12,000	1,07,000	21,400
Average	5,000	2,500	2,000	2,600	5,000	17,100	3,420
Low	500	600	1,000	1,000	800	3,900	780
Milk Used for Domestic Purpose							
Yes	40 (66.67)	25 (41.67)	32 (53.33)	28 (46.67)	32 (53.33)	157 (52.33)	-
No	20 (33.33)	35 (58.33)	28 (46.67)	32 (53.33)	28 (46.67)	143 (47.67)	-
Total	60	60	60	60	60	300	-

	(100)	(100)	(100)	(100)	(100)	(100)	
Average Consumption of Milk for Domestic Purpose (in Litres)							
Highest	20	5	7	4	40	76	15.2
Average	3.5	2	3	2	38	48.5	9.7
Low	1	1	2	1	5	10	2
Distribution of Milk							
Local People	30 (50.00)	23 (38.33)	30 (50.00)	40 (66.67)	23 (38.33)	146 (48.67)	-
Co-operative Society	27 (45)	34 (56.67)	20 (33.33)	10 (16.67)	34 (56.67)	125 (41.67)	-
Private Milk Collection Centers	3 (5)	0 (0)	8 (13.33)	9 (15)	0 (0)	20 (6.67)	-
Tea Shops	0 (0)	3 (5.00)	2 (3.33)	1 (1.66)	3 (5.00)	9 (3.00)	-
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)	300 (100)	-

Table 3: Involvement in Animal Husbandry.

Animals Received from the Government					
Details	**Achamangalam**	**Karupanur**	**Bommikuppam**	**Anandapatti**	**Thiniyalam**
Yes	23 (38.33)	21 (35.00)	0 (0)	9 (15.00)	0 (0)
No	37 (61.67)	39 (65.00)	60 (100)	51 (85.00)	60 (100)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Awareness about Government Schemes					
Yes	25 (41.67)	6 (10.00)	0 (0)	23 (38.33)	1 (1.67)
No	35 (58.33)	54 (90.00)	60 (100)	37 (61.67)	59 (98.33)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)

Table 4: Animal Husbandry and Standard of Living.

Improved Standard of Living through Animal Husbandry					
High Earnings	3 (5)	3 (5.00)	31 (51.67)	13 (21.67)	19 (31.67)
Own House Constructed	38 (63.33)	14 (23.34)	7 (11.66)	12 (20.00)	9 (15.00)
Education for Children	6 (10)	0 (0)	2 (3.33)	17 (28.33)	6 (10.00)
Savings	2 (3.33)	1 (1.66)	0 (0)	14 (23.33)	1 (1.67)
Reduction of Borrowings	1 (1.67)	5 (8.33)	4 (6.67)	4 (6.67)	5 (8.33)

Repayment of agricultural Loan	10 (16.67)	37 (61.67)	16 (26.67)	0 (0)	20 (33.33)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)
Attended Meeting Regarding Animal Husbandry					
Yes	8 (13.33)	18 (30.00)	0 (0)	32 (53.33)	0 (0)
No	52 (86.67)	42 (70.00)	60 (100)	28 (46.67)	60 (100)
Total	60 (100)	60 (100)	60 (100)	60 (100)	60 (100)

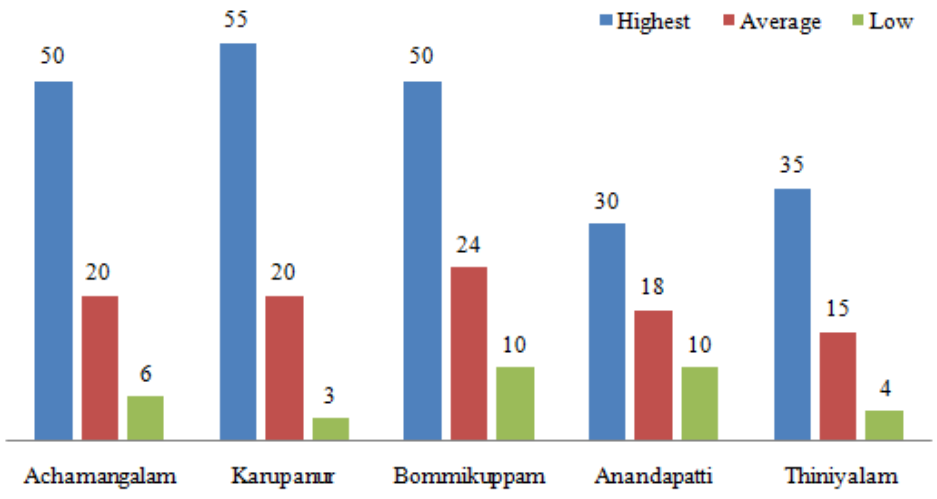


Fig. 1: Total Litres of Milk Production /day (L)

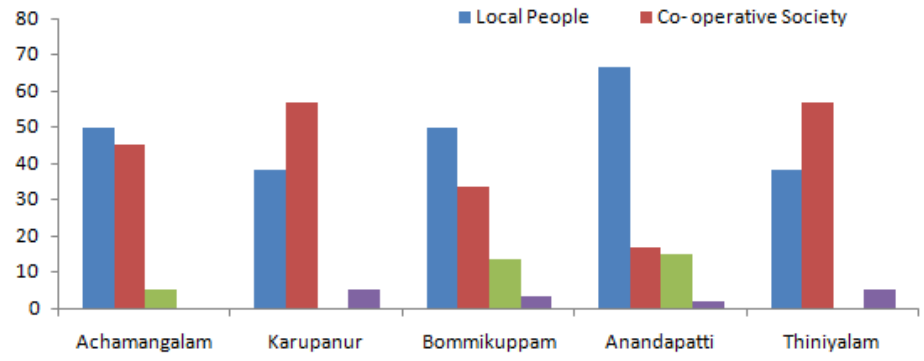


Fig.2: Distribution of Milk

RESEARCH ARTICLE

Experimental Determination of Depth Scaling Factor for PMMA Solid Phantom using High Energy X-Ray Beam of Linear Accelerator

Rahul Rai[1]*, Krishna Kumar Singh[2] and Lalit Mohan Singh[3]

[1]Ph.D. Research Scholar (Reg No: 24156100003), Department of Physics, FS University, Shikohabad, Uttar Pradesh, India.
[2]Assistant Professor, Department of Physics, FS University, Shikohabad, Uttar Pradesh, India.
[3]Medical Physicist and RSO, Department of Radiotherapy, Kailash Deepak Hospital, Karkardooma, Delhi, India.

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***Address for Correspondence**
Rahul Rai
Ph.D. Research Scholar (Reg No: 24156100003),
Department of Physics, FS University, Shikohabad,
Uttar Pradesh, India.
E.Mail: happyrahulrai@gmail.com

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ABSTRACT

Present study is done to determine the depth scaling factor using slab phantom made up of PMMA. Our goal is to determine the conversion factor which is to be used for measuring absolute dose in water. The use of scaling factor helps in reducing uncertainty of absorbed done in water for high energy photon beams. So, it's become highly imperative and necessary to determine depth of scaling factor before starting your absolute dose measurements. We have used 0.6cc PTW Farmer type ion chamber with PTW electrometer to measure the charge collected in nano Coulomb. Linear Accelerator or simply Linac producing high energy FFF beams mainly interacts with a medium by the Compton Scattering. That is why the scaling factor becomes most important parameter to be determined otherwise, it may cause uncertainty in dose measurement. As we know that every material has its own attenuation capabilities based on its density thickness (g/cm^2). The Compton mass attenuation coefficient is independent of Atomic Number *(Z)* and depends only on the number of electrons per gram. Although the number of electrons per gram of elements decreases slowly but systemically with atomic number, most materials except hydrogen can be considered as having approximately the same number of electrons per gram which is around $3x10^{23}$.For determining this scaling factor, we first measured the absorbed dose (cGy) in water phantom at the depth of 10cm with SSD of 100cm for 10x10 cm field size. Then the same measurement was done using slab phantom keeping the same depth, SSD and field size. From the ratios of charge collected by ionization chamber reading in water phantom to solid phantom the scaling factor were determined. Using this factor now we can measure absolute dose in water using a solid phantom with more accurately.

Keywords: Scaling Factor, Slab Phantom, Water Phantom, Linear Accelerator, Absorbed Dose

INTRODUCTION

Radiotherapy means use of radiation mainly X-rays of a very short wavelength and high energy. These high energy X-rays interacts with water present in our human body and damages the cell's DNA through physical, chemical and biological processes [1], [2], [3]. Radiation kills both cancerous and healthy cells, therefore precise dose control during radiation therapy is necessary to eliminate as many cancer cells while preserving as many healthy cells as possible. Radiotherapy's high energy photons, in particular, create a build-up region where the dose is minimal at the surface and rapidly grows from there to the maximal depth[4]. Additionally, even with slight variations in depth, the distribution of the absorbed dosage in the photon beam's build-up area exhibits highly rapid changes that are dependent on the energy and degree of irradiation. As a result, it is quite challenging to assess the absorbed dose distribution precisely. Precisely measuring the dosage of the treatment source of origin and evaluating the error are critical for the management of high energy radiation. The most human-like benchmark phantom currently in use for quality control of the absorbed dose management of the X-rays in the linear accelerator is the water phantom [5], [6]. However, because of the intricate implementation process, the test with the water phantom takes a long time to complete. This results in errors in the measurement depth because waves are formed from the water phantom's surface as a result of the ionization chamber's measurement position moving and the stability of water temperature. In the international code of practice published by International Atomic Energy Agency (IAEA) report TRS 398, water is the reference material for dosimetry of photon beams. As we know that many radiotherapy centers across the world using various types of phantoms of different materials to do their relative dosimetry measurements. Some of these materials which are most commonly used are like polystyrene, PMMA, Solid water, Virtual water, Plastic water phantom etc. Ideal condition all the phantom material should be water equivalence[7].

Various studies had been conducted to measure the water equivalency using scattered and absorbed properties as water for selected range of photon energy. For the treatment of cancer patient's high energyX-ray beams are used so as to give required dose to the target volume for this purpose mostly high energy X-ray beams are used. These high energy X-rays are produced by Linear Accelerator or simply called as Linacs. A Linac can produce X-rays beams of variable energies such as 4MV,6MV,10MV etc. depending upon the user's requirement or model provided by your manufacturer. For X-ray beams in the megavoltage range, where Compton effect is a predominant mode of interaction, the attenuation of the beam in any medium is governed by electron density (number of electrons per cm^3). Compton interaction involves essentially free electrons in the absorbing material, it is independent of atomic number Z. It follows that the Compton mass attenuation coefficient (σ_c/r) is independent of Z and depends only on the number of electrons per gram. Although the number of electrons per gram of elements decreases slowly but systemically with atomic number, most materials except hydrogen can be considered as having approximately the same number of electrons per gram as shown in the Table 1. Thus, σ_c/r is nearly the same for all materials[8][9]. The depth scaling factor is also known as fluence scaling factor in the IAEA TRS-398.This factor can be determined experimentally as a ratio of ionization chamber reading in water at a reference depth to reading in a solid phantom at an equivalent depth. The scaling theorem was used to determine the equivalent depth[10].In their report, depth can be scaled using a constant ratio of electron densities of two media. The scaling theorem assumes that photons interact with a medium only by the Compton scatter even if the pair-production accounts for 20% of total interaction for 10 MV photon in water. Therefore, all phenomena of interactions with a medium should be considered for the depth scaling for megavoltage photon beams[11], [12]. The study's goal was to compare the radiological doses of the solid and water phantoms based on depth and compute the depth scaling factor in order to evaluate the solid phantom's clinical use, which might offset the drawbacks of the water phantom.

MATERIALS AND METHODS

In the present study, 6 MV Flattening Filter Free (FFF) Halcyon linear accelerator manufactured by Varian Medical Systems is used for photon beam delivery. Firstly, the absolute dose in centi-Gray (cGy) measurement was carried out using 30x30x30 cm water phantom at the depth of 10cm with Source to Surface Distance (SSD) of 100cm for 10x10

cm field size as shown in Figure1(a) and its pictorial representation is shown in Figure2. A total volume of 0.6cc ion chamber calibrated with electrometer (PTW, Freiburg, Germany) were used to measure the charge collection in nano-Coulomb (nC). Measurement was done in the water medium as human body contain 80% of water and it is a standard protocol worldwide. Automatic-device or beam scanning system exists for rapid measurement of the beam profiler. Electrometers used with a water scanning system have degree of fidelity with a device dynamic range. They can measure charges in the range 10^{9}-10^{14}C.Electrometer should be rest to null or zero before scanning. Data acquisition process of sampling signals used during measurement of dosimetry. Water has an electron density 3.34 x 10^{23} per gram. After measuring the absolute dose, the same measurement was done using slab phantom keeping the same depth, SSD and field size. From the ratios of ionization chamber reading in water phantom to solid phantom the scaling factor were determined. Secondly, a solid phantom made of Polymethyl Methacrylate (PMMA) has been used as water equivalence, which has electron density3.248×10^{23}per gram. Different slabs of various thicknesses 1cm, 0.5cm, 0.3cm and 0.2 cm and area of 30x30 cm were used for dose output measurement. Same ion chamber and electrometer were used were for as shown in Figure1 (b), also the thickness of 10cm slab phantom was maintained at the bottom to reduce the effect of the backward scattering. Before ion charge collection in the process 10 minutes of warm up has been give up to reduce the uncertainty in background charge collection. Furthermore, the solid phantom was kept in the treatment room for almost two weeks in order to reduce the effect of temperature fluctuations on the results and minimize temperature variations during the measurement. To keep the water and treatment room thermally balanced when employing the water phantom, the water temperature was maintained within 0.1 degrees Celsius of the treatment room's temperature. In each instance, the depth was determined by measuring the distance between the ionization chamber's center and the ray source in each phantom. The experiment was conducted with an irradiation dosage of 200 MU and a dose rate of 800 Monitoring Units (MUs)/min. Various types of material have different values of mass densities, atomic number (Z) and electron density as shown in Table 1.A comparison of solid phantom with physical densities (ρ), electron densities (ρe), relative electron densities $(\rho e)_{pl,w}$ and effective atomic number (*Z*eff) have been shown in Table 2. For Intensity Modulated Radio-therapy (IMRT) beams, photon energy spectra are varied by transmitting through a multi-leaf collimator (MLC) and scattered photons in a medium. But, these effects on the depth scaling and the fluence scaling due to different density phantom have spectral variation on the depth scaling.

RESULT AND DISCUSSION

The depth scaling factor was determined from photon energy spectra for 10 × 10 cm open field. From the result for field size 10×10 cm and 100cm SSD at 10cm depth, ionizing charge at 10cm and Absorbed Dose rate at depth of dose maximum (D_{max}) for water phantom and slab phantoms were found to be 22.27nC, 21.99nC and 100.53cGy/100MU 98.86cGy/100MU respectively as shown in Figure 3. Ratio of ionization charge or scaling factor observed value was 1.013. Therefore, the percentage error of absorbed dose between water phantom and solid slab phantoms at 10cm depth was found to be around 1.63% as shown in Figure 5. This was a statistically significant difference, since electron density variation is more than unity in PMMA than water, so as the density increases, effect of attenuation increases, which reduces ion charge collection by ion chamber. By applying scaling factor correction, it was obtained that the ionization produced by 10 cm of water will be equivalent to that produced by 9.80 cm of PMMA solid phantom. Thus, by doing measurement for ionizing charge at 9.80 cm and Absorbed Dose rate at D_{max} for slab phantoms were found to be 22.42nC and 100.15cGy/100MU respectively as shown in Figure 4. As a result, the percentage error of absorbed dose between water phantom and solid slab phantoms was found to be around 0.34% as shown in Figure 5, which was not a statistically significant difference and clinically acceptable.

CONCLUSION

Our study showed that it is very necessary for users to first calculate the depth scaling factor accurately before using solid phantom for output measurement of Linac to avoid uncertainty in absolute dosimetry. Moreover, Solid

phantom dosimetry reduces the setup time compared to water phantom as well as convenient for user. In the future this study will be helpful for finding out the different material depth scaling factor for water equivalency.

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Table 1: No. of Electrons per Gram of Various Materials

Material	Density (g/cc)	Atomic Number	Number of Electron per Gram
Hydrogen	0.0000899	1	6.00×10^{23}
Carbon	2.25	6	3.01×10^{23}
Oxygen	0.001429	8	3.01×10^{23}
Aluminum	2.7	13	2.90×10^{23}

Copper	8.9	29	2.75×10^{23}
Lead	11.3	82	2.38×10^{23}
		Effective Atomic Number	
Fat	0.916	6.46	3.34×10^{23}
Muscle	1.04	7.64	3.31×10^{23}
Water	1	7.51	3.34×10^{23}
Air	0.001293	7.78	3.01×10^{23}
Bone	1.65	12.31	3.19×10^{23}

Table 2: Physical densities, electron densities and relative electron densities for solid phantoms

Elements	Water	WT1	457-CTG	RW3	MixDP	WE211	WE211R	Plastic Water	Plastic Water DT	Virtual Water	Polystyrene	PMMA	ABS
ρ[g cm^3]	0.998	1.020	1.043	1.045	1.000	1.018	1.018	1.030	1.039	1.030	1.060	1.190	1.050
ρe [$\times 10^{23}$ g^{-1}]	3.343	3.249	3.249	3.231	3.382	3.252	3.257	3.238	3.218	3.237	3.238	3.248	3.29
$(\rho e)_{pl,w}$		0.972	0.972	0.966	1.012	0.973	0.974	0.969	0.963	0.968	0.969	0.972	0.972
Zeff	7.22	6.35	6.35	5.83	6.38	6.34	7.07	6.83	6.83	6.35	5.61	6.24	5.67

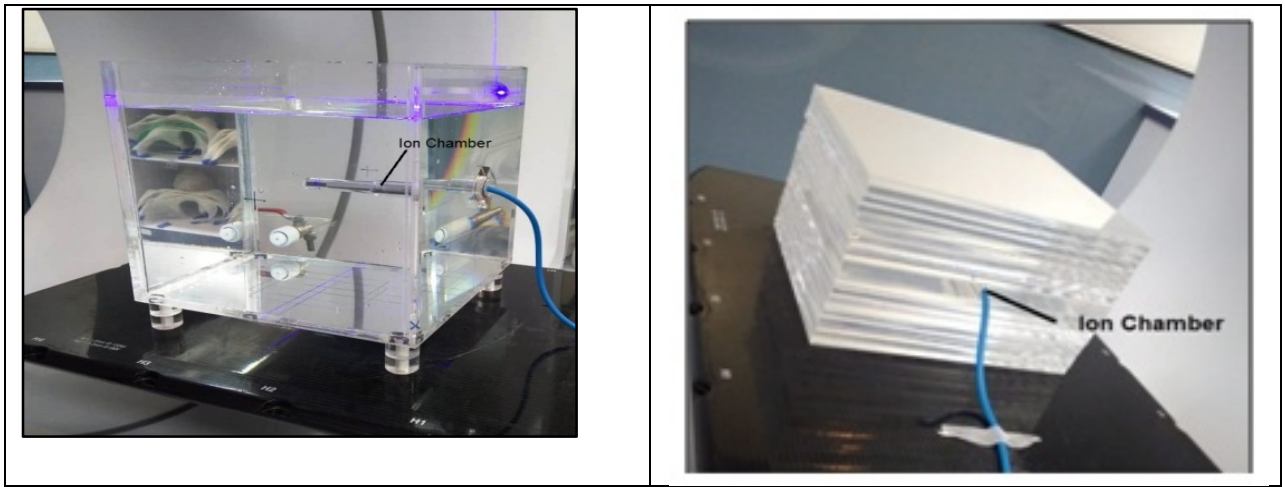


Figure1: (a) Setup of water Phantom with 0.6cc Ion Chamber at 10cm depth, (b) Setup of Solid Phantom (PMMA) with 0.6cc Ion chamber at 10cm depth.

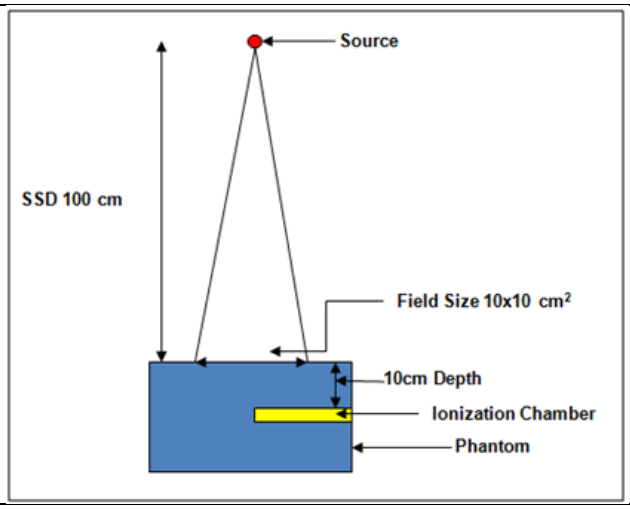


Figure 2: Showing pictorial representation of experimental setup for water and solid phantom.

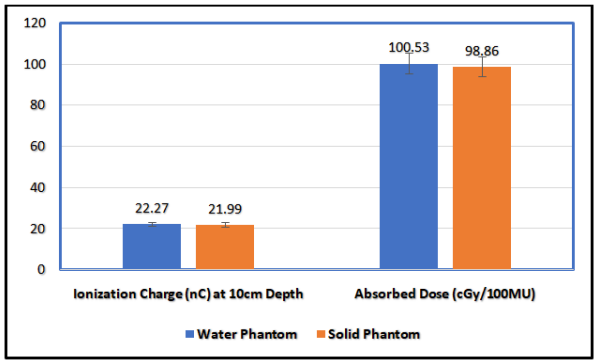


Figure 3: Showing comparison of Ionization Charge and Absorbed Dose Water Phantom and Solid Phantom at 10cm depth.

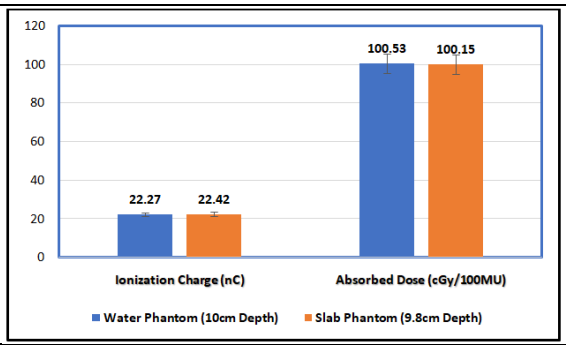


Figure 4: Showing comparison of Ionization Charge and Absorbed Dose for Water Phantom and Solid Phantom at 10cm and 9.8cm depth respectively.

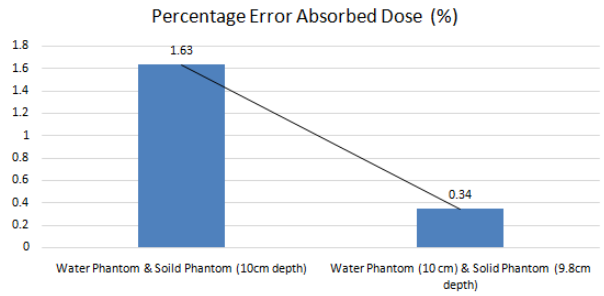


Figure 5: Showing comparison of Percentage Error Absorbed Dose for Water Phantom and Solid Phantom.

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REVIEW ARTICLE

Pyrazoline Derivatives as Privileged Scaffolds in Medicinal Chemistry: A Comprehensive Review of their Anticancer Potentials

Lekhashree Kirubanandan, Senthil Kumar Raju, Priyadharshini Selvaraj and Praveen Sekar*

Department of Pharmaceutical Chemistry, Swamy Vivekanandha College of Pharmacy, Tiruchengode, Namakkal, (Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.

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***Address for Correspondence**
Praveen Sekar
Department of Pharmaceutical Chemistry,
Swamy Vivekanandha College of Pharmacy,
Tiruchengode, Namakkal,
(Affiliated to The Tamilnadu Dr. M.G.R. Medical University, Chennai),
Tamil Nadu, India.
E.Mail: mrpraveensekar@gmail.com

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ABSTRACT

Despite significant advances in diagnostic technologies and targeted therapies, cancer remains a formidable global health challenge due to its complex biology and adaptive resistance mechanisms. Heterocyclic complexes are contemporary in almost 90% of novel medications, and they cross the chemistry-biology boundary where a prodigious deal of innovative systematic understanding, research, and application is occurring. One of the significant regularities in the medicinal chemistry is fused heterocyclic complexes, due to their broad-spectrum therapeutic functions. Amongst these, nitrogens-containing derivatives, like pyrazoline, isoxazoline, quinazoline and pyrimidine derivative are essential due to their multiple therapeutic applications including anticancer, anti-microbial, anti-alzheimier, anti-viral, anti-inflammatory, antimalarial and antitubercular properties. Additionally, to being effective against a wide range of newly emerging infectious diseases, heterocyclic compounds with pyrazoline moiety may also result in the development of potent derivatives, which are necessary for cancer treatment. 2-Pyrazolines are commonly investigated and more stable than 1- or 3-isomers of pyrazoline analogues. These derivatives can be synthesized via both conventional and greener techniques, including refluxing, microwave assisted synthesis, ultrasonic sonication mediated and oil bath mediated synthesis. This review focusses on the biological significance and anticancer applications of the pyrazoline derivatives. As a result, this review will be helpful in the future when designing new medications to treat cancer and its biological effects.

Keywords: Pyrazoline, 2-Pyrazoline, Heterocyclic chemistry, Anticancer, Breast cancer, Biological applications

INTRODUCTION

Cancer is the one amongst main diseases that cause mortality and morbidity all over, which poses a severe danger to people's ability to live healthily. Every year, almost 10 million people are diagnosed with various forms of cancer. They have a high level of molecular heterogeneity, which results in medication resistance to make the treatment challenging still [1]. Clinical markers can used to determine the developing tumors, based on initial diagnoses, track the tumor progression and assess the needed therapies to minimize the death rates due to the cancer [2]. Chemotherapy has been widely used for effective cancer treatments compared to targeted therapy, radiotherapy and surgery [3]. The World Health Organization reports that among emerging nations, cancer positions as the second principal cause of death. Researchers are working hard to create a new anti-neoplastic drug that is efficient and causes fewer toxic effects in the human body [4]. Heterocyclic compounds play a vital role in a diverse range of physiological functions linked to this class of molecules which makes it significant. The study of chemistry and biology in heterocyclic compounds is a fascinating field for future purposes [5]. The biological reactions frequently exhibit preference specificities due to the existence of heteroatoms or groups. The development of anticancer drugs becoming more interesting in naturally occurring heterocyclic molecules. The purpose of these novel strategies of heterocyclic compounds with numerous promising anticancer properties includes the development of activities by focusing on the tumor and other cancers such as lung, breast, colon and so on [6]. The heterocyclic compounds of pyrazoline and isoxazoline derivatives which belong to the azole family constitute extensive potential biological properties such as antioxidant, COX-2 inhibitor,antimicrobial and anticancer activities [7]. Pyrazoline is a five-membered heterocyclic molecule with nitrogen which offers a new source for synthesizing different derivatives with potent biological activities. Various types of heterocycles made up of pyrazoline derivatives are not only used in medications but also act as fungicides, insecticides, dyes and rubber chemicals [8]. Pyrazoline is a heterocyclic compound having two adjacent nitrogens at 1-2 positions with one endocyclic double bond with a wide spectrum of activity [9]. Its structure consists of a pyrazole ring with a saturated bond between those two positions.

The broad range of research on the biological activity of pyrazolines relies on cell-based assays and lacks molecular target identification. Pyrazoline has numerous pharmacological characteristics including anticancer, anti-inflammatory, analgesic, anti-depressant and anti-microbial properties [10]. Focusing towards anticancer activities, pyrazoline and their derivatives exhibited activities against breast cancer [11], cervical cancer [12], liver cancer [13], lung cancer [14], prostate cancer [15], colon cancer [13] and ovarian cancer [16]. Pyrazolines are closely tied with pyrazoles which have one fewer binary bond in their structure. Three isomers of pyrazoline exist, 1-pyrazoline, 2-pyrazoline, and 3-pyrazoline, distinguished by the presence or absence of a double bond. The most prevalent one was 2-pyrazoline. Derivatives of pyrazoline have a single endocyclic bond [9]. Pyrazolines are basic substances that can react with acids to produce salts. They have a potent fluorescence in solution, which makes them effective fluorescent brighteners. Due to their biological activity, pyrazolines and substituted pyrazolines have received a lot of importance among researchers [17]. The formation of pyrazolines can be accomplished through various methods, including the reaction of α,β-unsaturated ketones or aldehydes with hydrazines and 1,3-dipolar cycloaddition of nitrile Schiff bases (imines) to alkenes. Many compounds like ibipinabant, ramiphenazone, oxyphenbutazone, phenylbutazone and antipyrine contain the 2-pyrazoline scaffold [18]. The biologically important FDA-approved anticancer drugs bearing pyrazoline moiety along with the mechanism are given in figure 1. In this review, the literature survey was steered from PubMed, ScienceDirect, Embase and Google Scholar databases during the period of 2011-2025. As a search strategy, the keywords include pyrazoline, 2-pyrazoline, pyrazoline derivatives, anticancer activity, anti-breast cancer activity, cervical, lung, liver, prostate, ovarian and colon cancer, medicinal properties of pyrazolines and heterocyclic compounds. The keywords were utilized individually and in combination for collecting the literature. Following that, the titles and abstracts of each article were reviewed to remove any irrelevant ones. So, in order to help with cancer care, this review is going to concentrate on pyrazoline and its derivatives' structures, mechanisms, and features.

Pyrazoline Derivatives Against Cervical Cancer

Alam *et al*., 2017(12) tested a sequence of 1-(5-(3-(aryl)-1-phenyl-1H-pyrazol-4-yl)-3-(pyridin-3-yl)-4,5-dihydro-pyrazol-1-yl) ethanone derivatives (1-3) for their cytotoxicity via the 3-(4,5-dimethyl-thiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay against HeLa (cervical cancer) cell line. These compounds possessed potent anti-cervical cancer activity. Also, they exhibited superior IC_{50} values against the tested cancer cell line [12]. A series of hetero-aromatic scaffolds on curcumin containing pyrazoline substituted analogs were evaluated to determine the anti-proliferative activity against the HeLa cell line using MTT assay. (5-(5-Methoxy-1-methyl-1H-indol-3-yl)-3-(3-methoxyphenyl)-4,5-dihydro-1H pyrazol-1-yl)(6-methylpyridin-3-yl)methanone (4) has exhibited potent activity with better interactions and three-H-bond interactions higher than the parent molecule, determined by the molecular docking studies [19]. The pyrazoline derivatives associated N-methyl indole moiety were tested to assess the antineoplastic action towards the HeLa cancerous cell line by employing the MTT and colchicine assay. (5-(5-Methoxy-1-methyl-1H-indol-3-yl)-3-(3-methoxy phenyl)-4,5-dihydro-1H pyrazol-1-yl) (6-methyl pyridin-3-yl) methanone (5) showed better anti-cancer activity, lacking noxiousness to non-cancerous cells. The colchicine competition experiment proved that they have a potential tumor therapy by binding to tubulin's colchicine and blocking tubulin assembly. Analysis of the compound's molecular docking and interaction patterns revealed that it inhibits tubulin polymerization. The tested compound has also arrested the G2/M phase and induced cell apoptosis subsequently [20]. 1-Isobutyl-N-(4-(5-(4-methoxy phenyl)-4,5-dihydro-1H-pyrazol-3-yl) phenyl)-1H-imidazo [4,5-c] quinoline-4-amine (6) was investigated for its potential against cervical cancer. It was assessed using the Trypan blue exclusion assay and found to be possessing good anti-tumor activity against the HeLa cancer cell line [21]. The acetylated pyrazoline was synthesized by cyclization reaction and subjected to antiproliferative screening. Among the tested compounds, 1-acetyl-5-(2,4,5-trimethoxyphenyl)-3-(2-naphthyl)-4,5-dihydro-1H-pyrazole (7) has potent anti-tumor activity against the HeLa cancer cell line with a better IC_{50} value, assessed by the MTT assay [22]. 1,1′-(5,5′-(1,4-phenylene) bis (3- (naphthalen-1-yl) - 4,5-dihydro-1H-pyrazole-5,1-diyl)) diethanone (8) was produced and was tested against the HeLa cancer cell line. The synthesized derivatives were evaluated by Enzyme-linked Immunosorbent Assay (ELISA). Among the series of derivatives, it has a promising anti-cancer activity [23]. A novel series of D-condensed 2-pyrazoline was synthesized and evaluated by the ELISA.(3S,16R,17S)-3′-Methyl-1′-methyl-2′-pyrazolino[4′,5′:17,16]androst-5-en-3-ol (9) has a promising cell cycle disturbance and was examined by *in vitro* studies against the Human cervical cancer cell line HeLa, which has shown potent anti-neoplastic activity [24].

Patel *et al*., 2021[25] prepared a series of pyrazoline scaffolds by the process of cyclization that were screened using the MTT assay against a HeLa cancer cell line. The tested compounds exhibited better cytotoxicity and were found to have higher efficacy with better binding potential to the CDK2 protein, determined according to molecular docking studies. Among the tested compounds, 5-(9-Ethyl-9H-carbazol-3-yl)-3-(2-oxo-2H-chromen-3 yl)-4,5-dihydro-1H-pyrazole-1-carboxamide (10) and 3-(1-(3-Chlorophenyl)-5-(9-ethyl-9H-carbazol-3-yl) 4,5-dihydro-1H-pyrazol-3-yl)-8-methoxy-2H-chromen-2-one (11) were found to be most active and exhibits a potential to arrest cell cycle progression and to induce apoptosis [25]. A novel 2′-(2 ′′- 4 ′′- dinitrophenyl)-5α-cholestano [5,7-cd] pyrazoline (12) was investigated to govern the anti-tumor action in contrast to the HeLa cell line. This compound was screened by using the MTT cell viability assay and has a potent promising activity in the prevention of Human cervical cancer [26]. Using the MTT assay, the anti-cervical cancer potency of the steroidal pyrazoline cholest-5-en-7-one (13) was assessed against the HeLa cancer cell line using 5-FU and Doxorubicin as standard and 8-chloro-3-(1*H*-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydro[1]benzothiopyrano[4,3-*c*]pyrazole was found to be a potent anti-neoplastic compound with significant IC_{50} value [27]. A series of 3-(1H-indol-3-yl)-2,3,3a,4-tetrahydrothiochromeno[4,3-c]pyrazole derivatives were synthesized by Song *et al*., 2020[28] and tested their efficacy as topoisomerase II selective inhibitors. These substances have a higher IC_{50} value and demonstrate strong action against the cervical cancer cell line HeLa, according to the studies. Further, 6,8-dichloro-3-(1*H*-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydro[1]benzothiopyrano[4,3-*c*]pyrazole (14) and 6,8-dichloro-3-(1*H*-indol-3-yl)-2-phenyl-2,3,3a,4-tetrahydro[1]benzothiopyrano[4,3-*c*]pyrazole (15) induced G2/M phase cell cycle arresting and promising anti-cervical cancer activity via promoting apoptosis. These compounds also showed specific inhibitory effects towards topoisomerase II. DNA unwinding and cleavage reaction assays demonstrated that these compounds were a non-interactive topo II catalytic inhibitor, which agreed with the docking findings [28]. Yu *et al*., 2014 [29] tested a series of 4-(5-(Benzo[d][1,3]dioxol-5-yl)-3-(trifluoromethyl)-4,5-

dihydro-1H-pyrazol-1-yl)benzenesulfonamide (16) for cytotoxicity using the ELISA and Inhibitory assays. The chemical exhibited strong action towards the HeLa cells, according to the findings. This compound showed better inhibitory potential, QSAR also performed and found to be the inhibitor ofthe COX-2/B-Raf pathway [29]. The structures of compounds (1–15), which were previously described, are shown in figure 2, while compound (16) is shown in figure 3.

Pyrazoline Derivatives against Breast Cancer

The series of novel pyrazoline-containing compounds was designed and cytotoxicity against breast cancer was assessed using molecular docking studies with tamoxifen and erlotinib as standards. The study revealed that 5-(4-chlorophenyl)-1,3-diphenyl-4,5-dihydropyrazole (17) was established as most dynamic against P450, 4-(2-chlorophenyl)-2-(4-chlorophenyl)-1-phenyl-2,3-dihydropyrazole (18) was active against EGFR and 2,4-bis(2-chlorophenyl)-1-phenyl-2,3-dihydropyrazole (19) had exhibited higher potential against VEGFR1 and VEGFR2. The given compounds were also found to be exhibiting good pharmacokinetic properties and were predicted to be non-toxic [30]. 8-[1-(4-Chloro phenyl sulfonyl)-5-phenyl-4,5-dihydro-1H-pyrazol-3-yl]-7-methoxy-2H-chromen-2-one (20) was created and assessed for its capability to prevent the development of breast cancer cells (MCF-7) using the SRB assay; the results showed that it was an effective candidate. Also, it was proved that it can efficiently inhibit the PI3K Isoforms [11]. Lv *et al*., 2011[31] designed thiazolyl-pyrazoline derivatives and evaluated its anti-proliferative activity against the MCF-7 cancer cell line. Among different derivatives, 4-(4-chlorophenyl)-2-(3-(3,4-dimethylphenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H-pyrazol-1-yl)thiazole (21) has potent anti-breast cancer activity. According to the molecular docking studies, it was found to be having high potency as EGFR Kinase inhibitor and will exerts the potential towards the ATP binding site [31]. (5-(benzo(d)[1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)(3,4,5-tri methoxyphenylmethanone (22) was investigated for its potent anti-proliferative activity against the MCF-7 breast cancer cell line using MTT assay. Also, this compound induces the accumulation of cells in the G2/M phase of the cell cycle and promotes apoptosis [32]. Anticancer prospective of Bis-pyrazoline (23) was investigated against the MCF-7 cells using the MTT technique, compared with the standard 5-Fluorouracil. This compound was found to be potent against breast cancer and also found to be inhibiting the VEGFR2 Tyrosine Kinase receptor [33]. Rabou *et al*., 2018 [33] tested a series of bis-N-thioamide pyrazolines for the investigation of cytotoxicity using the MTT assay. They have possessed promising efficacy towards MCF-7 cells. Among the established derivatives, 1,4-bis (5-methyl-4-(5-phenyl-4,5-dihydro-1H-pyrazol-3-yl)-1H-1,2,3-triazol-1-yl)benzene (24) has exhibited significant activity. The molecular docking studies were also carried out and found to have significant inhibitory potential against VEGFR Tyrosine Kinase enzyme [33]. 1-[3-(5-bromo thiophen-2-yl)-5-(4-methoxy phenyl)-4,5-dihydro-1H-pyrazol-1-yl] ethanone (25) was synthesized by the process of condensation. Anti-cancer investigations on breast cancer cell line MCF-7 using Doxorubicin as the standard drug was evaluated by SRB assay and found to be more active [34].

The antiproliferative potential of the series of novel synthetic pyrazolines were assessed by using SRB assay. Among the tested compounds,1-(5-(4-Chlorophenyl)-3-(4-ethoxyphenyl)-4,5- dihydropyrazol-1-yl)ethanone (26) showed better activitywith significant IC_{50} value and provided the relationship between structure and activity for the design of anti proliferative agents [35]. Using the MTT assay towards MCF-7 cancer cells, investigators looked into the antiproliferative potential of a number of 5-substituted aryl-2-pyrazoline derivatives. Among them, 1-(2, 4-di-nitro phenyl)-5-(4-chloro phenyl)-3-(4- methoxy phenyl)-2-pyrazoline (27) and 1-(2,4-di-nitro phenyl)-5-(4-N,N-di-methyl amino phenyl)-3-(4-methoxy phenyl)-pyrazoline (28) remained as the effective compounds with better IC_{50} values. To identify the most effective binding mode and supportive mechanism, these compounds were docked with the Epidermal growth factor receptor (EGFR) and found to be potent EGFR inhibitors with better binding affinities [36]. The anticancer potential of 3-(4-methoxyphenyl)-5-substituted phenyl-2-pyrazoline-1-carbothioamide derivatives was screened by using SRB assay. 3-(4-Methoxyphenyl) -5-phenyl-2-pyrazoline-1-carbothioamide (29), 5-(3-Methoxyphenyl)-3-(4-methoxyphenyl)-2-pyrazoline-1-carbothioamide(30),5-(4-Chlorophenyl)-3(4methoxyphenyl)-2-pyrazoline-1-carbothioamide(31) and 3-(4-Methoxyphenyl) -5-(p-tolyl) -2-pyrazoline1-carbothioamide (32) have significant anti-cancer activity against the MCF-7 breast cancer cell line and observed an EGFR inhibitory activity [37]. The structures of compounds (17–30), which were previously described, are shown in figure 3, while

compounds (31) and (32) are shown in figure 3. To determine the efficacy of an array of new pyrazoline derivatives bearing naphthalin through inhibiting cell proliferation in the MCF-7 cells, researchers used the MTT assay in conjunction with inhibitory assays such as the EGFR/HER-2, HER-3, VEGFR-2, FAK, and BCATM protein assays. Among them, 3-(3,4-Dichlorophenyl)-5-(naphthalen-2-yl)-4,5- dihydro-1H-pyrazole-1-carbothioamide (33) has a better IC_{50} value and is devoted to have EGFR/HER-2 inhibitory activity [38]. Employing the MTT colorimetric technique, a battery of new pyrazolines was tested towards the anti-cancer potential against MCF-7 cells. Among the tested derivatives, 1-(2,4-dinitrophenyl)-3-((7-propylnaphthalen-1-yl)methoxy)-4,5-dihydro-1H-pyrazole-5-carboxylic acid (34) has high IC_{50} value. According to the molecular docking studies, this compound was found to act upon the ER-α receptor [39]. (E)-5-(4-Methoxyphenyl)-3-(4-methoxystyryl)-4,5-dihydro-1H-pyrazole-1-carboxamide (35), (E)-3-Phenyl-5-styryl-4,5-dihydro-1H-pyrazole-1-carbothioamide (36), (E)-3-(4-Chlorophenyl)-1-phenyl-5-styryl-4,5-dihydro-1H-pyrazole (37) and E)-1-(4-Methoxyphenyl)-3-phenyl-5-styryl-4,5-dihydro-1H-pyrazole (38) were prepared and found to be having promising cytotoxicity against the breast cancer cell line MCF-7, determined by the MTT assay with a standard reference staurosporine. According to the molecular docking investigations, these compounds were devoted as effective EGFR inhibitors [40]. 2-[3,5-bis(4-chlorophenyl0-4,5-dihydro-1H-pyrazol-1-yl]-5-(2-methoxybenzylidene)-1,3-thiazol-4(5H)-one (39) was prepared by Khalil *et al*., 2012 [41]. This compound was found to be a potent anti-cancer compound against the MCF-7 cancer cell line, compared with the standard Doxorubicin, determined using SRB assay [41]. The antiproliferative efficacy of the thiazolyl-pyrazolines was evaluated via MTT assay against the MCF-7 cell line (breast cancer). Based on the molecular docking and *in vitro* investigations, 1-(4-(4-Cyanophenyl)thiazol-2-yl)-3-(4- morpholinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (40) and 1-(4-(4-Cyanophenyl)thiazol-2-yl)-3-(4- piperidinophenyl)-5-(4-chlorophenyl)-2-pyrazoline (41) was identified as the potent molecules, which has significant inhibitory potential, along with high affinity towards the ATP binding sites of EGFR and HER-2 [42].

2-(5-(Benzo[d][1,3]dioxol-5-yl)-3-(4-bromophenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4-bromophenyl)thiazole (42) was found to have potent anti-proliferative activity against the MCF-7 cancer cell line with better IC_{50} value. This compound was also found to be inhibiting the HER2 receptor, determined by the molecular docking studies [43]. Jasril *et al*., 2020 tested a pyrazoline analog against an MCF-7 cell line, evaluated using the MTT assay technique. 5-(4-fluorophenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1*H*-pyrazole (43) was estimated as highly effective and it was evidenced that the compound has hydrogen bonding interactions with Arg 791 and also a hydrophobic interaction with His 790, estimated via molecular docking studies [44]. A novel pyrazoline derivative, 5-(4-fluorophenyl)-3-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazole (44) was tested for its cytotoxic potential towards the MDA-MB-231 cell line and has found to possessing promising anti-cancer efficacy, which was evaluated by Scratch-wound and Transwell assays. It induces apoptosis via arresting the G0/G1 phase cell cycle [45]. 1-(2-93,5-diphenyl-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl) imidazolidine-2,4-dione (45) has a promising anti-cancer potential against the MCF-7 cells, assessed by the MTT cell viability technique. This compound has a potent possession in breast cancer activity. Studies conducted using *in vitro* methods revealed increased DNA damage, Caspase activation, Cell cycle arrest in the G0/G1 phase and a decrease in the expression of the anti-apoptotic protein BCL-2 [46]. The structures of compounds (33–45), which were previously described, are shown in figure 4. A novel steroidal glycoside pyrazoline functionalhybrid,4-((17-(5-(2-chloro-4-methylphenyl)-4,5-dihydro-1H-pyrazol-3-yl)-8,14-dihydroxy-3((2R,4S,5R,6R)5-(((1S,3S)-4-hydroxy-3-methylcyclohexyl)oxy)-4methoxy6methyltetrahydro2Hpyran2yl)10,13dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-12-yl)oxy)benzoyl chloride (46) was prepared and evaluated for cytotoxicity against the MCF-7 cell line and the compound exhibited promising potential with potent IC_{50} value [47]. 4-(5-(4-chlorophenyl)-3-(pyridine-2-yl)-4,5-dihydro-1H-pyrazol-1-yl) benzene sulfonamide (47) acts as an effective agent towards the MCF-7 breast and MDA-MB-468 triple negative breast cancer cell lines. According to the molecular docking studies, this compound has a high docking score against the PARP1 and acts as potent PARP1 antagonist [48]. A series of new pyrazoline hybrids were efficiently synthesized by Kumari *et al*., 2020 [7]. (2R,3S,4R,5R,6S)-2-(hydroxymethyl)-6-((5-(5-nitro-1H-indol-3-yl)isoxazol-3-yl)methyl)tetrahydro-2H-pyran-3,4,5-triol (48) was found to be potent promising anti-cancer compound against the MCF-7, MDA-MB-453 and MDA-MB-231 breast cancer cell lines, assessed by the MTT assay. The molecular docking findings also agreed with the results of the MTT assay [7]. The structures of compounds (46–48), which were previously described, are shown in figure 5.

Pyrazoline Derivatives Against Colorectal Cancer

3-Anthracen-9-yl-5-phenyl-4,5-dihydro-1H-pyrazole (49), 3-Anthracen-9-yl-5-(4-chloro-phenyl)-4,5-dihydro-1H pyrazole (50), 3-Anthracen-9-yl-5-p-tolyl-4,5-dihydro-1H-pyrazole (51) and 3-Anthracen-9-yl-5-(3-chloro-phenyl)-4,5-dihydro-1H pyrazole (52) were evaluated and identified as promising anti-cancer compounds against the HCT-116 cell line, escorted by better IC_{50} values. These compounds were screened by using an MTT assay using curcumin as a standard [13]. 3-(5′-chloro-2′-hydroxy-4′,6′-dimethylphenyl)-5-(4-(N,N-dimethylamino)phenyl)-1-(p-sulfamylphenyl) —pyrazoline (53) was evaluated for anti-cancer activity against COLO 205 cell line and found to be a potent against the treatment of colorectal cancer [49]. 1,3-Diphenyl-4-(4-fluoro phenyl)-5-(4-fluoro benzoyl) pyrazoline (54) and 4-(4-Fluoro phenyl)-5-(4-methoxy benzoyl)-1-phenyl-3-(styryl) pyrazoline (55) were prepared and their potency against the HT29 cell line was assessed using the SRB assay. The produced compound was found to be a promising anti-colon cancer compound with better IC_{50} value [50]. 1,3,5-triaryl-2-pyrazoline (56) was prepared and gauged for its colon cancer efficacy against the HCT116 cells using the SRB assay. 1-(4-bromophenyl)-4-(4-fluorophenyl)-3-phenylimidazolidine was found to be more potent, yielding a more favorable IC_{50}value. It showed a better structural and molecular features, related to its biological activity [51]. The HCT 15 cell line was used to assess the cytotoxic potential of many new pyrazoline derivatives through the use of the ATPase test and the DNA-Topo II cleavable complex assay. Among the tested derivatives, Benzo [d] thiazol-2-yl-(3-(4-chloro phenyl)-5-(4-methoxy phenyl)-4,5-dihydro-1H-pyrazol-1-yl) methanone (57) showed a better IC_{50} value. The endogenous topo mediated DNA relaxation studies were also performed, it indicated that this compound can effectively inhibited endogenous topo-mediated pBR322 plasmid relaxation than the standard etoposide [52]. 2-[5-(4-Fluorophenyl)-3-(4-tolyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6- dihydropyrimidine-5-carbonitrile (58) and 2-[3-(4-Chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (59) was appraised using the HT 29 colon cancer cell line by Cell Titer Glo technique. From this assay, these compounds were found to possess potent anti-proliferative activity [53]. The structures of compounds (49-58), which were previously described, are shown in figure 5, while the compound (59) is shown in figure 6.

Using the Sulforhodamine B assay, novel 17 -pyrazolinyl derivatives of pregnenolone were explored to appraise their cytotoxic action towards HT-29 and HCT-15 cell lines. 1-(5-(3-Fluorophenyl)-4,5-dihydro-3-((10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren 17-yl)pyrazol-1-yl)ethenone(60),1-(5-(4-Fluorophenyl)-4,5-dihydro-3- ((10R,13S)2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17- yl) pyrazol-1-yl) ethenone (61), 1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3-hydroxy-10,13dimethylcyclopenta [a] phenanthren -17- yl) -5-o-tolylpyrazol-1-yl) ethenone (62), 1- (4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9, 10,11,12, 13,14,15,16, 17-tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-m-tolylpyrazol-1-yl) ethenone (63), 1-(4,5-Dihydro-3-((10R,13S)-2,3,4,7,8,9,10,11,12,13,14,15,16,17- tetradecahydro-3-hydroxy-10,13-dimethyl-1H-cyclopenta[a]phenanthren-17-yl)-5-(4-methoxyphenyl) pyrazol-1-yl)ethenone (64) and 1-(5-(2-Chlorophenyl)-4,5-dihydro-3-((10R,13S)- 2,3,4,7,8,9,10,11,12,13,14,15,16,17-tetradecahydro-3- hydroxy-10,13-dimethyl-1H-cyclopenta[a] phenanthren-17-yl) pyrazol-1-yl)ethenone (65) presented substantial anti-cancer action towards the tested colon cancer cells [54]. 2-(5-(Benzo [d][1,3]dioxol -5-yl)-3-(naphthalen-1-yl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(4substitued phenyl)thiazole (66) was produced by Mansour *et al.*,2019 [55] and tested their promising anti-proliferative activity against HCT -116 cancer cell line. This potent compound was screened by *in vitro* methods with a better IC_{50} value [55]. An innovative thiazolone-containing compound encompassing a pyrazoline moiety was screened by using a cytotoxicity assay. 5-(4-Hydroxy-3,5-dimethoxybenzylidene)-2-[5-(2-hydroxyphenyl)-3-phenyl-4,5-dihydro-1H-pyrazol-1-yl]-1,3-thiazol-4(5H)-one (67) has significant anti-cancer activity against selective influence on HT 29 (colon cancer cell line) [56]. The anti-proliferative activity of N-acetyl and N-formyl pyrazoline derivatives was evaluated using the SRB assay against the HCT-15 cell line.5-(4-Chlorophenyl)-3-[4-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde(68), 5-(4-Chlorophenyl)-3-[3-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde (69), 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-phenyl-4,5- dihydro-1H-pyrazole-1-carbaldehyde (70), 3-[3-(7-Chloroquinolin-4-ylamino) phenyl]-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (71) and 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-(3,4,5-trimetho-xyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (72) were found to possess potent anti-proliferative compounds for the colon

cancer therapy [57]. The structures of compounds (60-69), which were previously described, are shown in figure 6, whereas the compounds (70-72) in figure 7.

The potency of a variety of 1-acyl-2-pyrazoline derivatives towards the HCT-1 cell line was studied. The repressive impact of the derivatives that were synthesized was appraised via the MTT assay. Among them, (2-(2,3-Dimethyl phenyl amino)phenyl) (4,5-dihydro-5- (4-methoxyphenyl)-3-phenylpyrazo-1-yl) methanone (73), 1-(4,5-Dihydro-3,5-diphenylpyrazol-1-yl)-2-(4-isobutyl phenyl) propan-1-one (74) and 1-(4,5-Dihydro-5-(4-methoxyphenyl)-3-phenylpyrazol-1-yl) 2-(4-isobutyl phenyl)propan-1-one (75) was found to be possessing potent and promising activity [58]. A series of N-acyl derivatives of ferrocenyl pyrazoline was tested via MTT assay and the compound (76) was a potent anti-cancer agent against HCT-116 human colorectal cancer cells [59]. A novel pyrazoline derivatives was efficiently synthesized and gauged for their antiproliferative action towards the HCT-116 colorectal cancer cell line using Sulforhodamine B assay. Among them, 1-(3-(4-(benzyloxy)phenyl)-5-(4-chlorophenyl)-4,5-dihydro-1H-pyrazol-1-yl) ethenone (77) and 1-(3-(4-(benzyloxy)phenyl)-5-(4-fluorophenyl)-4,5-dihydro-1H- pyrazol-1-yl) ethenone (78) was identified to be the potent candidates. The binding interaction of compounds with proteins (RCSB PDB ID: 1M17 and 5ZTO) has been studied. According to the molecular docking studies, it showed that the compounds can efficiently interacts with target proteins and also exhibited better binding affinities [60]. Using the MTT assay Tilekar *et al*., 2020 [61] assessed the anticancer potential of 1-(2-(3-9-fluorophenyl)-5-(p-tolyl)-4,5-dihydro-1H-pyrazol-1-yl)-2-oxoethyl)pyrrolidine-2,5-dione (79) against the HT 29 colon cancer cell line. It was found to possessing potent activity and discovered to interfere with the growth phases in cell cycle analysis, causing the cell population to increase in the G_1/G_0 Phase and decrease in the G_2/M phase [61]. The structures of compounds (73-79), which were previously described, are shown in figure 7.

Pyrazoline Derivatives against Prostate Cancer

A variety of pyrazoline scaffolds were explored by to appraise their cytotoxicity against the PC-3 Prostate cancer cell line. 1-[5-(4-chlorophenyl)-3-(4-[(4-fluorophenyl)methoxy]phenyl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (80), 5-(4-fluorophenyl)-3-(4-[(4-fluorophenyl)methoxy]phenyl)-4,5-dihydro-1*H*-pyrazole-1-carbothioamide (81), 3-(4-[(4-fluorophenyl)methoxy]phenyl)-5-phenyl-4, 5-dihydro-1*H*-pyrazole (82) and 5-(4-chlorophenyl) -3- (4- [(4-fluoro phenyl)methoxy]phenyl)-4,5-dihydro-1*H*-pyrazole (83) have the promising anti-cancer activity and exhibits better IC_{50} value. Furthermore, the molecular docking analyses of these compounds with the EGFR and VEGFR ATP binding sites were performed and found to be potent EGFR inhibitor [15]. A series of pyrazoline derivatives were synthesized and evaluated their anti-proliferative activity against the PC-3 cancer cell line by using an MTT assay. (3-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-5-(4-dimethylamino-naphthalen-1-yl)-4,5 dihydro-pyrazole-1-carboxylic acid (4-chloro-phenyl)-amide (84), 3-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-5-(4-dimethylamino-naphthalen-1-yl)-4,5 dihydro-pyrazole-1-carbothioic acid amide (85), 1-(2,3-Dihydro-benzo[1,4]dioxin-6-yl)-3-4-dimethylamino-naphthalen-1-yl)-propenone (86),3-Benzofuran-2-yl-5-(4-dimethylamino-naphthalen-1-yl)-4,5-dihydro-pyrazole-1 carboxylic acid (4-chloro-phenyl)-amide (87) and 3-Benzofuran-2-yl-5-(4-dimethylamino-naphthalen-1-yl)-4,5-dihydro-pyrazole-1 carbothioic acid amide (88) were found be the significant anti-cancer compounds. According to molecular docking studies, these compounds can efficiently inhibit tubulin polymerization [62]. 3,5-diaryl N-acetyl pyrazolines (89) were synthesized and assessedtheir cytotoxicity against the PC-3 Prostate cancer cell lines using SRB assay and the tested compound exhibits the promising anti-neoplastic activity with better IC_{50} value [63]. The structures of compounds (80-89), which were previously described, are shown in figure 8. Using the Sulforhodamine B assay, we tested an assortment of pyrazoline derivatives for their cytotoxic effects on the DU145 prostate cancer cell line. (E)-3-(3-((1-(4-fluorobenzyl)-1H-1,2,3-triazol-4-yl)methoxyphenyl) -1- (3,4,5-trimethoxyphenyl) prop-2-en-1-one (90) was identified as an effective anti-neoplastic agent with a significant GI_{50} value. This compound was responsible for tubulin polymerization inhibition and cell accumulation in the G_2/M Phase [64]. A novel pyrazolines were prepared and evaluated their antiproliferative potential towards PC-3 cells using *in-vitro* SRB assay. Among those derivatives, 9-[(1,3-Diphenyl-4,5-dihydro-1H-pyrazol-5-yl)methyl]-6-piperidinyl-9H-purine (91) exhibits the compelling antiproliferative action and used in the prostate cancer therapy. This compound also revealed that it has high lipoxygenase inhibitory activity in combination with significant anti-thrombin activity, suggesting that this compound can effectively inhibits tumor growth and development [65]. Using the SRB assay method, 5-

(benzo[d][1,3]dioxol-5-yl)-3-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (92) was investigated for its promising anti-tumor activity against the DU-145 prostate cancer cell line. Also, it was found to exhibit a superior GI_{50} value against the tested cancer cell line [16]. A furan-based N-acetyl pyrazoline derivative was produced and evaluated for potent proliferative efficacy on PC-3 cells using DNA intercalation assay. 1-[3-(2,5-dimethylthiophen-3-yl)-5-(2-nitro phenyl)-4,5-dihydropyrazol-1H-1-yl]ethenone (93), 1-[3-(2,5-dimethylthiophen-3-yl)-5-(4-nitro phenyl)-4,5-dihydropyrazol-1-1H-yl] ethenone (94) and 1-[3-(2,5-dimethylfuran-3-yl)-5-(5-hydroxy-2-nitrophenyl)-4,5-dihydro-pyrazol-1H-1-yl]ethenone (95) were found to be significant anti-cancer compounds. The apoptotic effect using Topo II decatenation assay was determined and found to arrest the cell cycle at the G_1 phase [66]. The structures of compounds (90-95), which were previously described, are shown in figure 9.

Pyrazoline Derivatives against Lung Cancer

The MTT assay was used to evaluate the anti-cancer activity of pyrazoline-linked carboxamide derivatives that were developed and tested against the A549 human non-small-cell lung tumor cell line. Among them,3′-[4-(Benzyloxy)phenyl]-1′-phenyl-5-(pyridin-3-yl)-3,4-dihydro 1′H,2H-[3,4′-bipyrazole]-2-carboxamide (96) and 3′-[4-(Benzyloxy)phenyl]-1′-phenyl-5-(pyridin-4-yl)-3,4-dihydro1′H,2H-[3,4′-bipyrazole]-2-carboxamide (97) exhibits the promising anti-neoplastic activity. These compounds efficiently binds to the ATP binding site of EGFR Kinase like the erlotinib standard drug, determined according to the molecular docking studies [14]. The anti-lung cancer activity of a variety of pyrazolines was tested using the Cell titer Glo assay method on the A549 cancer cell line. These pyrazolines were made via a cyclocondensation technique. Among them, 2-[5-(4-Methoxyphenyl)-3-phenyl-4,5-dihydropyrazol-1-yl]-1-methyl-6-oxo-4-phenyl-1,6-dihydropyrimidine-5-carbonitrile (98) was found to be a potent anti-cancer compound with better IC_{50} value [53]. N-acetyl-5-ferrocenyl-3-(2-thienyl)-2-pyrazoline (99) was synthesized and evaluated by the methyl thiazol tetrazolium assay. This compound has been confirmed as a potent anti-cancer agent to prevent lung cancer by acting against the A549 cancer cell line [67]. A novel thienopyrimidine containing pyrazoline unit was prepared and appraised its potency towards the A549 cells using the MTT assay. N1-(2-(5-(4-Methoxyphenyl) -3-phenyl-4, 5-dihydro-1H-pyrazol-1-yl) thieno [2,3-d]pyrimidin-4-yl) -N1,N2,N2 trimethylethane-1,2 diamine (100) was identified as an effective anti-proliferative candidate. Furthermore, it was found to have better inhibitory potential against the P13K α\mTOR protein kinase. Annexin V-FITC/PI staining also revealed that this compound can induces apoptosis [68]. The structures of compounds (96-100), which were previously described, are shown in figure 9.

Using the MTT assay, we evaluated the potential anti-cancer activity of N-formyl derivatives that were developed and synthesized by the cyclization procedure against the A549 cells. 3-(4-chlorophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H- pyrazole-1-carbaldehyde (101) and 3-(4-bromophenyl)-5-(4-(dimethylamino)phenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (102) were found to be the Topo-isomerase II inhibitor. Also, the molecular dynamics simulation studies revealed the stability of the complexes [69]. A series of pyrazoline analogues were prepared and assessed for *in vitro* anti-cancer activity towards A549 small cell lung cancer cell line. Among them, 5-(4-Fluorophenyl)-N-(naphthalen-1-yl)-3-(3,4,5 trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (103), 5-(4-(Methylthio)phenyl)-N-(naphthalen-1-yl)-3-(3,4,5 trimethoxyphenyl) -4,5- dihydro-1H-pyrazole-1-carbothioamide (104) and 5-(4-Methoxyphenyl)-N-(naphthalen-1-yl)-3-(3,4,5trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbothioamide (105) have revealed a promising anti-cancer activity with better GI_{50} values [70]. A novel thiazole containing pyrazoline compounds was designed and screened for cytotoxicity towards the A549 cell line (NSCLC) by using both lactate dehydrogenase assay as well as SRB assay method. 2-(3-(4-chlorophenyl)-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazol-1-yl)-4-(pyridin-4-yl)thiazole (106) was found to be the potent anti-neoplastic agent and also inhibits the activity of mTOR via FKBP12, which can be reversed by 3BDO, an mTOR activator and autophagy inhibitor [71]. 1-acetyl-3-(2-thienyl)-5-aryl-2-pyrazoline derivatives were tested and assessed for their anti-cancer action towards the A549 cells consuming MTT assay. Among them, 1-[5-(4-methylphenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (107), 1-[5-(4-bromophenyl)-3-(thiophen-2-yl)-4,5-dihydro-1*H*-pyrazol-1-yl]ethan-1-one (108) and1-(5-(benzo[d][1,3]dioxol-5-yl)-3-(thiophen-2-yl)-4,5-dihydro-1H-pyrazol-1-yl)ethanone (109) were found to be promising anti-cancer agents. It was also revealed that these

compounds can indices apoptosis via caspase-3 activation [72]. The structures of compounds (101-107), which were previously described, are shown in figure 10, whereas the compounds (108 and 109) in figure 11.

Pyrazoline Derivatives against Liver Cancer

The MTT assay was used to explore a range of anthracenyl pyrazoline derivatives. 3-Anthracen-9-yl-5-(3-nitro-phenyl)-4,5-dihydro-1H-pyrazole (110), 3-Anthracen-9-yl-5-furan-2-yl-4,5-dihydro-1H-pyrazole (111), 3-Anthracen-9-yl-5-(3,4,5-trimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (112) and 3-Anthracen-9-yl-5-93,4-dimethoxy-phenyl)-4,5-dihydro-1H-pyrazole (113) revealed that they have a significant cytotoxic activity in HepG2 cells with better IC_{50} values [13]. A novel pyrazoline coumarins were produced and investigated for anti-proliferative action towards the HepG2 hepatocellular cancer cell line by using MTT assay. 7-methoxy-8-(5-[4-(methylthio)phenyl]-4,5-dihydro-1H-pyrazol-3-yl)-2H-chromen-2-one (114) showed better anti-cancer activity. The apoptotic potential was determined by Annexin V FITC/PI staining and it shows that this compound can effectively induce apoptosis [73]. A series of isolongifolanone pyrazoline derivatives were prepared and assessed their anti-proliferative potential against the HepG2 cells. The compounds were also screened via both apoptosis assay as well as MTT assay using Etoposide, 5-Fluorouracil as a controlled drug. The MTT assay showed that 3-(4-methoxyphenyl)-N,5,5,9,9-pentamethyl-4,5,7,8,9,9a-hexahydro-5a,8-methanobenzo[g]indazole-2(6H)-carbothioamide (115) was identified to be a potent candidate against liver cancer. Also, this compound potently induces apoptosis in HepG2 cells by enhancing intracellular reactive oxygen species [74]. In order to determine whether or not these pyrazolines had any anti-cancer effects, we used the MTT assay on the HepG2 cells. 3-(3-[4-Chlorophenyl]-5-[4-(trifluoromethyl)phenyl]-4,5-dihydropyrazol-1-yl)-6-phenylpyridazine (116) was found to be promising anti-cancer compound and exhibits equal apoptotic and necrotic properties [75]. Benzo[b]thiophen-2-yl-[5-(4-hydroxy-3,5-dimethoxy-phenyl)-3-(2-hydroxy-phenyl)-4,5-dihydro-pyrazol-1-yl]-methanone (117) was established as a promising agent against the HepG2 cells with significantly better IC_{50} value and compared using cisplatin as a controlled drug. It significantly arrested the cell cycle in the G2\M phase and induced apoptosis, as determined via the cell cycle analysis and apoptosis/necrosis evaluation [76]. The structures of compounds (110-117), which were previously described, are shown in figure 11.

Pyrazoline Derivatives against Ovarian Cancer

Utilizing the sulforhodamine B assay, an array of N-acetyl and N-formyl-pyrazoline derivatives were produced through the cyclocondensation technique and tested on the OVCAR-3 and ADR-RES cell lines, which comprise an ovarian cancer panel. Among the tested compounds, 5-(4-Chlorophenyl)-3-[4-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde (118), 5-(4-Chlorophenyl)-3-[3-(7-chloroquinolin-4-ylamino) phenyl]-4,5-dihydro-1H-pyrazole-1-carbaldehyde (119), 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-phenyl-4,5 dihydro-1H-pyrazole-1-carbaldehyde (120), 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-(4-methoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (121) and 3-[3-(7-Chloroquinolin-4-ylamino)phenyl]-5-(3,4,5-trimethoxyphenyl)-4,5-dihydro-1H-pyrazole-1-carbaldehyde (122) revealed the promising anti-tumor activity against the tested cell lines with significant IC_{50} values [57]. A novel steroidal glycoside pyrazoline functional hybrid, 17-(5-(2-chlorophenyl)-4,5-dihydro-1H-pyrazol-3-yl) -8,14-dihydroxy-3- ((4-((5-hydroxy-4-methoxy-6-methyltetrahydro-2H-pyran-2-yl)oxy)-5 methoxy-3-methyl tetrahydro-2H-pyran-2-yl)oxy)-10,13-dimethylhexadecahydro-1H-cyclopenta[a]phenanthren-12-yl benzoate (123) was synthesized and appraised its antiproliferative action towards the OVCAR-3 cells by using cell viability assay and Lactate dehydrogenase assay. The tested compound was found to be potent anti-cancer agent and possess higher growth inhibitory potential than the cisplatin standard drug [47]. The structures of compounds (118 and 119), which were previously described, are shown in figure 11, whereas the compounds (120-123) are shown in figure 12.

CONCLUSION

A five-membered heterocyclic structure containing nitrogen, pyrazoline is well-known in medicinal chemistry. This adaptable structure, which is semi-saturated, offers a distinct spatial configuration that enables different patterns of substitution to have significant pharmacological effects. These effects can include anticancer, antitrypanosomal,

antimicrobial, anti-inflammatory, analgesic, antidiabetic, antiviral, antiepileptic, etc., as well a selective activity, such as MAO inhibitory, cannabinoid CB1 receptor antagonist and NOS inhibitory activity. The chemical reactivity of pyrazolines has led to their eager modification to fabricate a wide assortment of lead moieties, which in turn have produced innovative therapeutic candidates for the management of many disorders. In this present review, we have comprehensively reviewed the various anticancer activities of the pyrazoline derivatives, with special reference to cervical, breast, colorectal, prostate, lung, liver and ovarian cancers. Additionally, these compounds could act upon several stages of the cell cycle, induce apoptosis, and decrease cell growth, ATP binding, and caspase activation. They can also inhibit various proteins, enzymes and receptors, including Epidermal growth factor receptors, Vascular endothelial growth factor receptors, Tumor necrosis factor, Estrogen receptors, Human Epidermal receptors, Tyrosine kinase and Janus kinase receptors. Thus, we trust that this paper will be helpful for budding researchers and medicinal chemists seeking their research toward the designing and development of pyrazoline derivatives and developing novel derivatives with different pharmacological properties. Hence, these pyrazoline derivatives will be utilized further in the management of several ailments.

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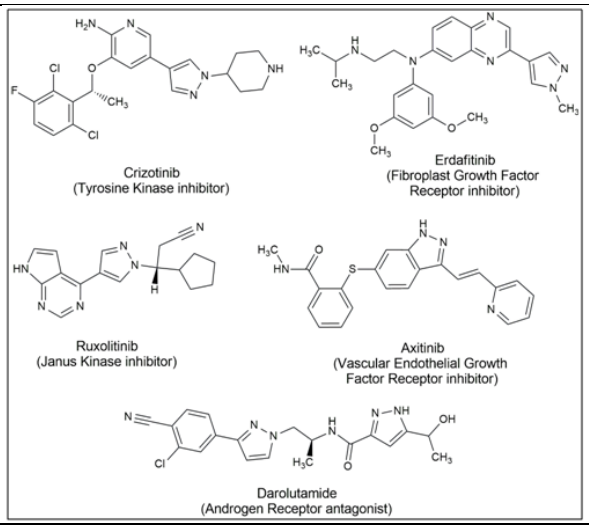


Figure 1: Biologically important FDA-approved anticancer drugs bearing pyrazoline moiety

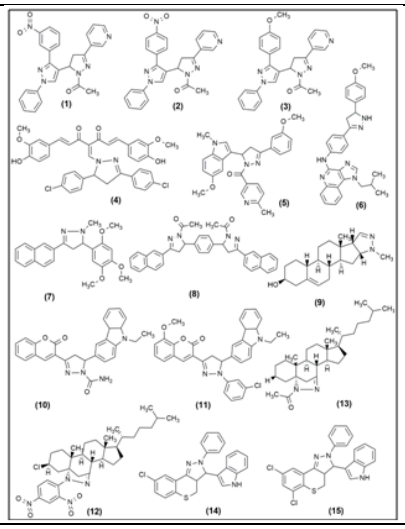


Figure 2: Structures of the compounds against cervical cancer

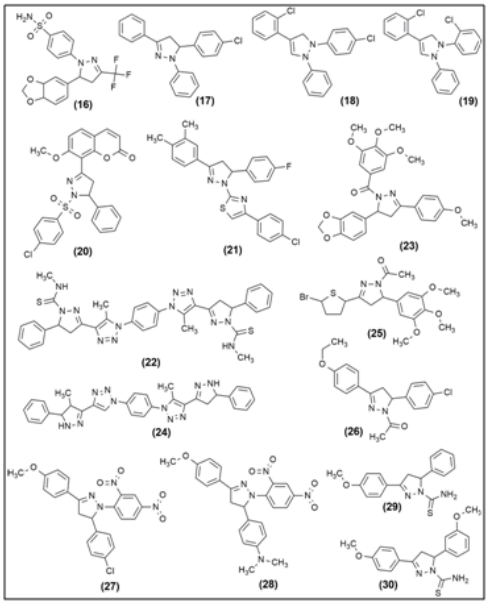


Figure 3: Structures of the compounds against cervical (compound 16) and breast cancer (17-30)

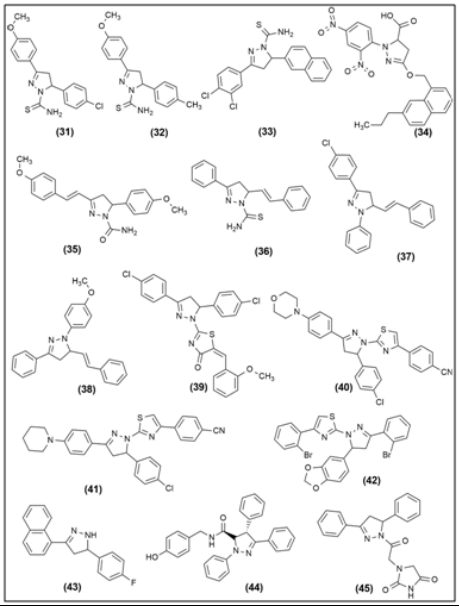


Figure 4: Structures of the compounds against breast cancer

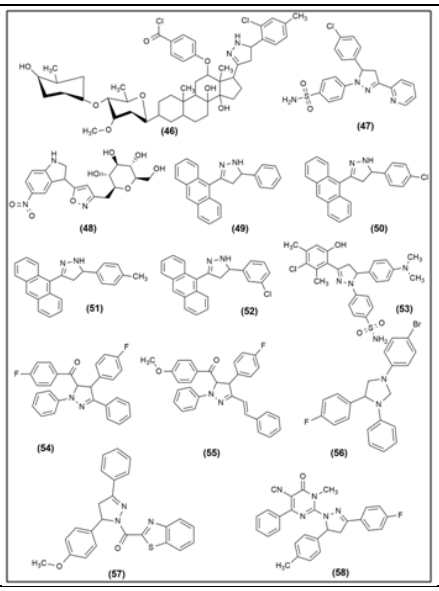


Figure 5: Structures of the compounds against breast adenocarcinoma (compounds 46-48) and colorectal cancer

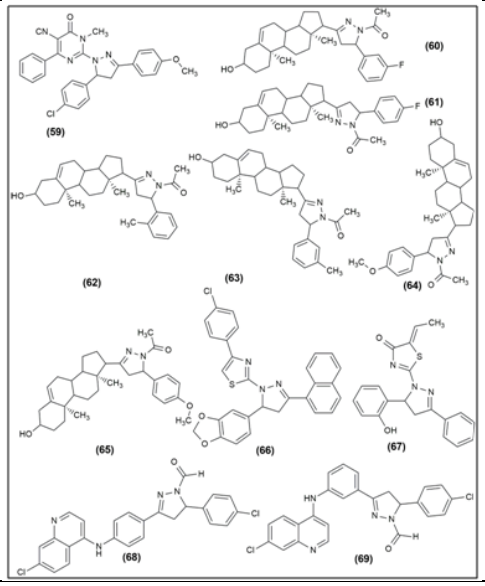


Figure 6: Structures of the compounds against colorectal cancer

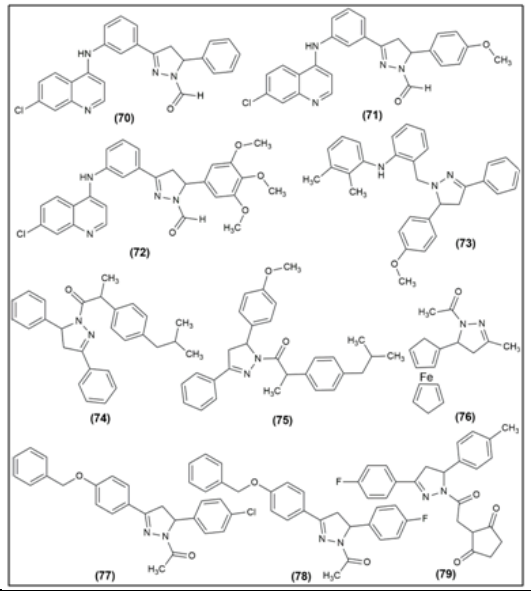


Figure 7: Structures of the compounds against colorectal cancer

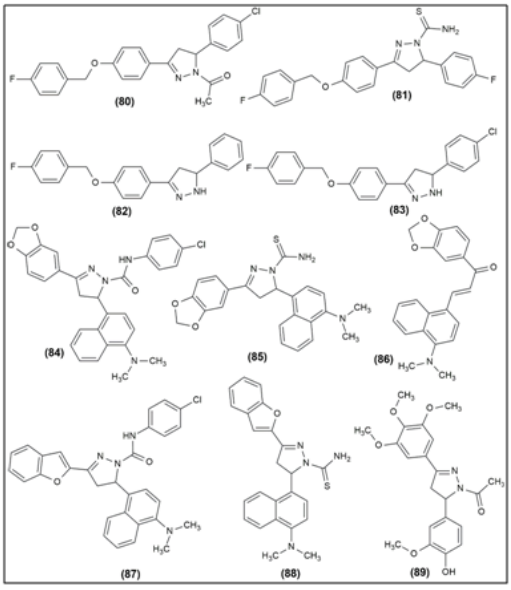


Figure 8: Structures of the compounds against prostate cancer

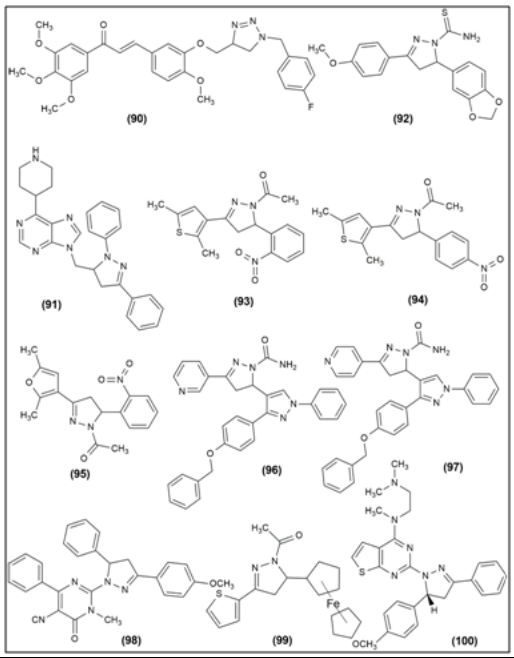


Figure 9: Structures of the compounds against prostate cancer (compounds 90-95) and Lung cancer (compounds 96-100)

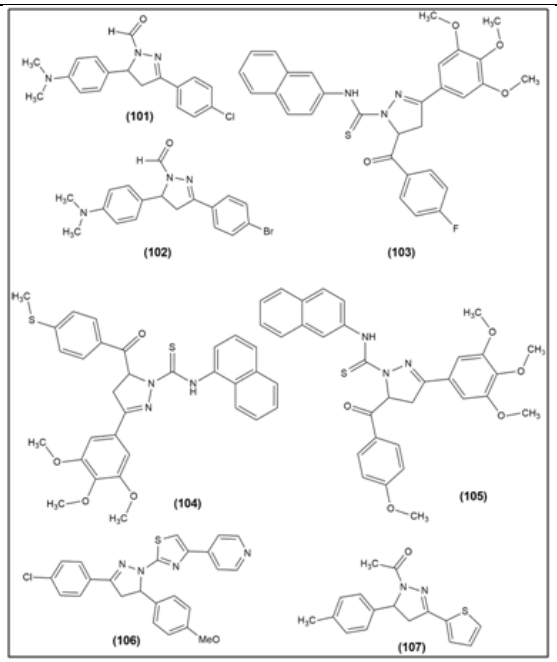


Figure 10: Structures of the compounds against lung cancer

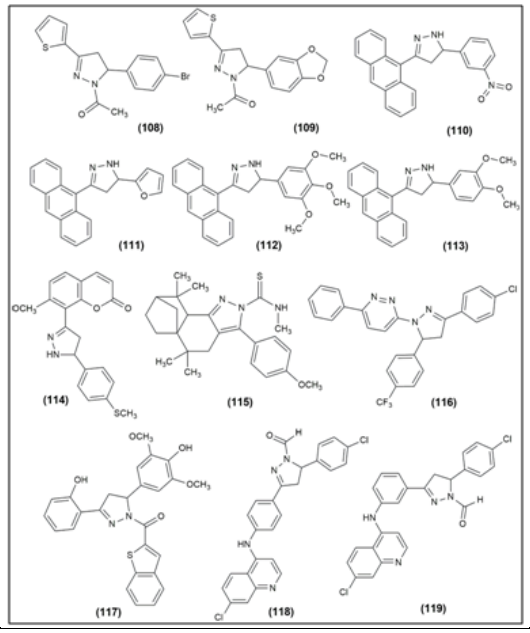


Figure 11: Structures of the compounds against lung cancer (compounds 108 and 109), liver cancer (compounds 110-117) and ovarian cancer (118 and 119)

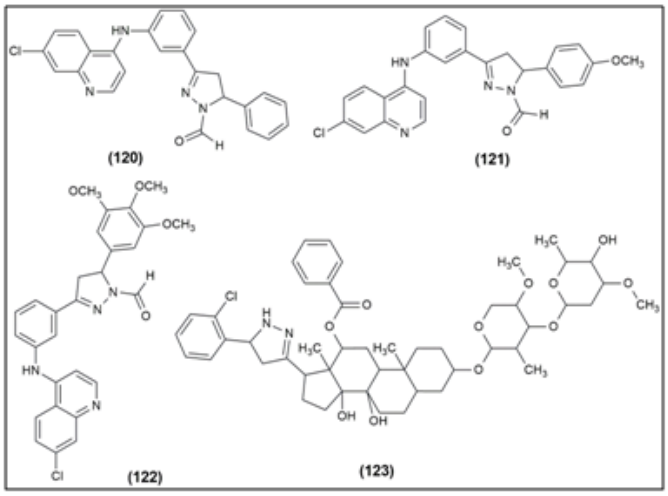


Figure 12: Structures of the compounds against ovarian cancer

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RESEARCH ARTICLE

A Taxonomic Evaluation of Freshwater Cyanobacteria from Berhampore Subdivision of Murshidabad District, West Bengal, India

Tuhin Mallick[1], Prankrishna Debnath[2] and Harisankar Dey[3]*

[1]Research Scholar, Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, (Affiliated to West Bengal State University, Barasat), Kolkata, West Bengal, India.
[2]Guest Lecturer, Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, (Affiliated to West Bengal State University, Barasat), Kolkata, West Bengal, India.
[3]Assistant Professor, P. G. Department of Botany, Ramakrishna Mission Vivekananda Centenary College, Rahara, (Affiliated to West Bengal State University, Barasat), Kolkata, West Bengal, India.

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***Address for Correspondence**
Harisankar Dey
Assistant Professor,
P. G. Department of Botany,
Ramakrishna Mission Vivekananda Centenary College, Rahara,
(Affiliated to West Bengal State University, Barasat),
Kolkata, West Bengal, India.
E. Mail: harisankardey@rkmvccrahara.org

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ABSTRACT

Cyanobacteria are a morphologically diverse group of oxygen-producing phototrophic prokaryotes that appeared about 3. 5 billion years ago, growing in a wide range of environmental conditions where light and moisture are present. The study's primary goal is to investigate the variety of cyanobacteria found in the Berhampore subdivision of the West Bengal, India's Murshidabad region. Berhampore subdivision covers an area of approximately 1195. 57 km² with coordinates of 23˚55' N and 88˚30' E. Samples were collected from different waterlogging areas like rivers, ponds, lakes, paddy fields, etc. In this study 34 cyanobacterial species belonging to 22 genera were documented. Among these, the most dominant genus was *Oscillatoria,* with 5 species, followed by *Aphanocapsa*, *Pseudanabaena*, *Spirulina*, *Lyngbya*, *Phormidium*, *Anabaena*, *Nostoc*, and *Calothrix,* with 2 species each, *Aphanothece*, *Gloeothece*, *Synechocystis, Chroococcus*, *Gloeocapsa*, *Arthospira*, *Tychonema*, *Limnothrix*, *Cylindrospermum*, *Trichormus*, *Macrospermum*, *Rivularia*, and *Hapalosiphon* with 1 species each.

Keywords: Berhampore, Cyanobacteria, Diversity, Freshwater.

INTRODUCTION

Cyanobacteria are morphologically diverse group of pioneering oxygenic phototrophic prokaryotes that appeared about 3. 5 billion years ago, growing in any environment where light and moisture are present. cyanobacteria are renowned for their historical significance in oxygenating Earth's early atmosphere through photosynthesis [1]. Today, they are the key contributors to global oxygen production and nitrogen fixation [2]. Cyanobacteria are also important members of symbiotic relationships with lichens, plants and other phototrophs [3]. Cyanobacteria multiply quickly under favorable conditions, which leads to harmful cyanobacterial blooms. Toxins produced by these cyanobacterial blooms produce toxins that have severe impact on aquatic ecosystems, wildlife, and humans. [4]. The pH range for cyanobacteria growth is neutral to slightly alkaline [5]. pH played an important role in the establishment of cyanobacterial diversity [6]. Systematic surveys of cyanobacteria in West Bengal were done by various workers[7-19]. Very little work has been done on cyanobacterial diversity in Murshidabad district, among these few are, Pal and Santra [20] studied the algal flora of Murshidabad for the first time;They also reported different cyanobacterial species in Berhampore and its adjoining areas [21]. According to recent findings Das [22] studied the variation in thermal traits and plankton assemblage patterns induced by coal power plant discharge in the Ganga River, but they have only reported a few cyanobacterial species from Murshidabad. Therefore, an attempt has been made to study the detailed cyanobacterial diversity in Berhampore subdivision of Murshidabad district.

MATERIALS AND METHODS

Study area

Berhampore subdivision covers an area approximately 1195. 57 km² with coordinates of 23°55' N latitudes and 88°30' E longitudes. The study was conducted in each block of this subdivision (Fig 2b) and the map was constructed using Software (ARC Gis 10. 8. 1). The Bhagirathi River divides the subdivision into two regions, Rarh on the west and Bagri on the east, which give rise to many freshwater bodies suitable for cyanobacterial growth.

Isolation and Characterizations of Samples

Samples were collected from different collection sites; geographical locations of each place were taken by Google Earth Mobile app (Table 1) and pH of each collection sites were measured by (Table 1) a digital pH meter . Collected samples were immediately brought to the lab and temporary slides were prepared from each sample and observed under a trinocular bright-field microscope equipped with a digital camera and a computer running software (Carl Zeiss Primo Star Microscope 4150500-0054-000). All samples were identified based on their morphological features like thallus colour, shape, size of cell, heterocyst, akinetes, and also the colony characteristics, samples were identified using standard monographs of Desikachary T. V[23], Komarek and Anagnostidis [24] [25], Komarek [26].

Purification Preservation and Maintenance of samples

After identification a portion of samples were preserved in 4% Formaldehyde solution and submitted to the Applied Phycology Laboratory of Ramakrishna Mission Vivekananda Centenary College. A portion of sample was then transferred to freshly prepared ± BG-11 medium [27] and culture was maintained under 28±2° C with illumination at 25-30 µmol photon $m^{-2}S^{-2}$ white light and aeration. Pure Cultures were done by several serial dilution and Agar Plate Methods [28].

Diversity Analysis

The alpha diversity is an important measures of species variability within specific ecosystem of habitats which can describe the dominance of different strains among the sampling sites. So, there by four diversity indices are calculated and they are described as follows:

$$\%\ Frequency\ of\ species = \frac{No.\ of\ sampling\ sites\ in\ which\ species\ recorded}{Total\ number\ of\ sampling\ sites\ studied} \times 100$$ [Supplementary table 1]

$$Shannon\text{-}Wiener\ Diversity\ Index\ H' = -\sum_{i=1}^{N} (pi \ln (pi))$$ [29]

Where, *pi* refers to proportion of species *I*, *N*is considered as number of species in total.

Simpson's Index (1-*D*) = 1-$\sum_{i=1}^{N}(pi)^2$ [30]

Where, *pi* refers to relative abundance of recorded species *i*, *N* represents the total number of species.

Dominance Index according to Berger-Parker (*BP*)=$\frac{n_{max}}{N}$ [31]

Where, n_{max} is the number of individuals in the most abundant species, *N* is the total number of individuals in the sample.

Margalef's index (*Dmg*) = $\frac{(S-1)}{\ln (N)}$[32]

Where, *S* is the number of species, *N* is the total number of individuals in the sample.

RESULTS

Total number of 34 cyanobacterial species belongs to 22 genera (Photo plate 1,2,3) were documented from the study area. Among the different cyanobacterial taxa most dominant genera was *Oscillatoria* with 5 species followed by *Aphanocapsa*, *Pseudanabaena*, *Spirulina*, *Lyngbya*, *Phormidium*, *Anabaena*, *Nostoc*, *Calothrix* with 2 species. *Aphanothece*, *Gloeothece*, *Synechocystis*, *Chorococcous*, *Gloeocapsa*, *Arthospira*, *Tychonema*, *Limnothrix*, *Cylindrospermum*, *Trichormus*, *Macrospermum*, *Rivularia*, *Hapalosiphon* with 1 species each. Detailed description of cyanobacterial strains are as follows as per Komarek and Anagnostidis [24] [25], Komarek [26].

Phylum: Cyanobacteria
Class: Cyanophyceae
Order: Chroococcales
Family: Microcystaceae
Genus: *Aphanocapsa*

Aphanocapsa musicola (Meneghni) Wille, Photo plate 3, fig. A
(Komarek 1998, p. 155 fig:181)
Description: Colonies colourless, Yellowish to dark blue-green or olive green, formless, sometimes unite into an amorphous mass with densely packed cells; cells sometimes surrounded by individual gelatinous envelopes. Cells spherical, blue green; 2-4 µm in diam
Habitat: Free floating on shallow stagnant water

Aphanocapsa parietina Nägeli, Photo plate 3, fig. F-G
(Komarek 1998, p. 158, fig. 190)
Description: Cell spherical, 4. 5-5. 5µm wide, many celled colonies, sheath transparent.
Habitat: Attached to the cemented wall
Genus: *Aphanothece*

Aphanothece microscopica Nageli, Photo plate 3, fig. E
(T. V Desikachary1959 p. 142, Pl. 22, Figs-4, 5, 9; Komarek 1998, p. 84 fig. 72c)
Description: Thallus small gelatinous, blue green colour at first rounded later amorphous, cell oblong cylindrical, more or less 4. 5µm broad, cell 2µm as long as broad, individual distinct sheaths.
Habitat: Attached to the mud.
Genus: *Gloeothece*

Gloeothece rupestris (Lyngbye) Bornet in Witterock et Nordestedt, Photo plate 3, fig. D
(**Komarek**1998, p. 98 fig: 181)
Description: Colonies small with 2 cells, enveloped by common Sheaths, more or less oval and mucilaginous, Gelatinous envelopes around cells, colourless, pale blue green to olive green,3. 6-4. 6 µm in diam.
Habitat: Attached to the grass
Genus: *Synechocystis*

Synechocystis pevalekii Ercegovic,Photo plate 3, fig. B
(komarek 1998, p. 143, fig. 163)
Description:cells spherical to hemispherical, cell bredth 3. 5µm wide, blue green in colour.
Habitat: Attached to the mud
Family: Chroococcaceae
Genus: *Chroococcus*

Chroococcus minutus (Kutzing) Nageli, Photo plate 3, fig. H
(T. V Desikachary1959, p. 103 and 105, fig. 5)
Description: Cell width 3-10µm, sheath colourless, envelopes thin.
Habitat: Free floating
Order: Chroococcidiopsidales
Family: Gloeocapsaceae
Genus: *Gloeocapsa*

Gloeocapsa gelatinosa Kutzing, Photo plate 3, fig. C
(T. V Desikachary1959, p. 114 and 139plate 27 fig: 6; Komarek 1998, p. 239, fig. 308)
Description: Cells Without sheath is about 2. 5µm wide and with sheath 6. 2-10µm wide, blue green; colonies about 25µm diam; sheath colourless, seemingly thin when old lamellated.
Habitat: Found on the side of cemented drain
Order : Pseudanabaenales
Family: Pseudanabaenaceae
Genus: *Limnothrix*

Limnothrix obliqueacuminata (Skuja) Meffert, Photo plate 3, fig. L
(Komarek 2005, p. 96 and 99, fig. 83)
Description: Trichomes straight or slightly curved, length of cell 3. 33µm, breadth of trichome 2. 01µm, pale olive green in colour, apical cells ±pointed, without calyptra, sometimes slightly arcuated.
Habitat: Free floating on bill
Genus: *Pseudanabaena*

Pseudanabaena recta Komarek and Cornberg, Photo plate 3, fig. O
(Komarek 2005, p. 92, fig. 75 a-d)
Description: Solitary trichomes, straight or arcuated, with 3-14 cells, yellow green in colour, breadth of cell 1. 5µm, usually small areotopes near the end.
Habitat: Attached to cemented drains.

Pseudanabaena lemnetica (Lemmermann) Komarek, Photo plate 3, fig. P
(Komarek 2005, p. 84, fig. 60)
Description: Trichome straight, 1. 2-1. 5µm wide, cell long cylindrical,4-12µm long, olive blue green in colour, apical cell rounded.
Habitat: Found on mud
Order: Spirulinales
Family: Spirulinaceae
Genus: *Spirulina*

Spirulina subsalasa Oersted ex Gomont, Photo plate 3, fig. I
(Komarek2005, p. 149 and 150, fig. 176 d)

Description: Thallus soft**,** thin, bright blue green, usually in clusters, regularly dense screw-like coiled, 0. 8µm wide, spirals length 3µm.
Habitat: Attached to the submerged stone

Spirulina laxa G. S. West, Photo plate 3, fig. J
(Komarek 2005, p. 140 and 141, fig-159)
Description-Trichomes blue-green 2. 791 µm wide, distance between coils 15. 33µm, coils left-handed, cells shorter than wide, apical cell rounded.
Habitat: Attached to the Mud on Paddy Field
Class: Oscillatoriales
Family: Microcoleaceae
Genus: *Arthospira*

***Arthospira platensis* var. Tenuis** (Rao) Desikachary, Photo plate 3, fig. K
(Komarek 2005, p. 349 and 352, fig. 488,492)
Description: Plant mass greyish brown; trichomes pale blue green of uniform, width 5. 1-6. 4µm, broad in regular spirals, spirals 30-34µm broad not constricted at the joints, cell disc shaped, end cell with rounded apices.
Habitat: Free floating on pond.
Genus: *Lyngbya*

Lyngbya martensiana Meneghini ex Gomont, Photo plate 3, fig. Q
(Komarek 2005, p. 612 and 615, fig. 931)
Description: Thallus pale blue green to dark blue green, filament 8. 19µm wide, sheath hyaline, thick, breadth of trichome 4. 47µm in length, granules visible.
Habitat: Free floating on Bill

Lyngbya spiralis Geitler, Photo plate 3, fig. R
(Komarek 2005, p. 610 and 613, fig. 926b)
Description: Thallus formless, trichome bright blue green, 4. 5-5µm wide, not constricted at cross-wall, cells 1. 5-2. 5µm long, apical cells widely rounded.
Habitat: Attached to grass on paddy field
Genus: *Tychonema*

Tychonema rhodonema (Skuja) Anagnostidis and Komarek, Photo plate 4, fig. E
(Komarek 2005, p. 374 and 375, fig. 528)
Description: Trichome solitary, free floating, straight, cylindrical, not constricted, 4. 9µm wide, cells isodiametric,4-10µm long, slightly violet content, with granules at cross walls.
Habitat: Attached to the grass
Family: Oscillatoriaceae
Genus: *Oscillatoria*

Oscillatoria limosa C. Agardh ex Gomont, Photo plate 3, fig. S
(Komarek 2005, p. 593 and 595, fig. 886)
Description: Trichome bright blue green, breadth of trichome 7. 97µm, length of cell 3. 07µm.
Habitat: Attached to the grass on paddy field

Oscillatoria tenuis C. Agardh ex Gomont, Photo plate 4, fig. A
(Komarek 2005, p. 587 and 588, fig. 878c)
Description: Thallus flat, blue green to olive green, mucilage thin, trichome straight, cells always shorter than wide,2-3. 5µm long, apical cell rounded.

Habitat: Attached to the mud on paddy field

Oscillatoria proboscida Gomont, Photo plate 4, fig. B
(Komarek 2005, p. 596 and 597, fig. 891a)
Description: Trichome bright blue green, rarely olive brown, sporadically irregularly screw like coiled at the end,9-18µm, capitate, cells 1. 5µm long.
Habitat: Free floating in stagnant water

Oscillatoria ornataKutzing ex Gomont, Photo plate 4, fig. C
(Komarek 2005, p. 582 and 583, fig. 663,867)
Description: Thallus blue green in form, at the end screw like coiled, 6-11. 5µm wide, cell shorter than wide 2-5µm long, apical cell rounded without calyptra or thickened cell wall.
Habitat: Attached to the mud

***Oscillatoria* sp.**
Photo plate 4, fig. D
Description: Bright yellow green in mats, cell 3. 02µm broad, length of trichome 31. 16µm.
Habitat: Free floating on Field.
Genus: *Phormidium*

Phormidium animale (Agardh ex Gomont) Anagnostidis et Komarek, Photo plate 3, fig. M
(Komarek 2005, p. 407 and 408, fig. 575)
Description: Both ends slightly bend, breadth of trichome 2. 93µm, cell bredth 2. 19µm, total length of the filament 91. 291µm, blue green in colour.
Habitat: Attached to the mud.

Phormidium breve (Kutzing ex Gomont) Anagnostidis and Komarek, Photo plate 3, fig. N
(Komarek 2005, p. 420 and 421, fig. 599 c, d)
Description: thallus expanded, blue green to olive green in colour, trichome slightly curved, 4-6. 5 µm long, cells 1/2-1/3 as long as wide, 1. 5-3µm long.
Habitat: Free floating on shallow stagnant water
Order: Nostocales
Family:Aphanizomenonaceae
Genus: *Anabaena*

Anabaena sphaerica Bornet *et* Flahault, Photo plate 4, fig. F
(Komarek 2013, p. 796, Fig. 992 a)
Description: Trichomes ± straight or flexuous, cell barrel-shaped up to almost spherical, 5-6µm wide. Heterocyst spherical, intercalary, solitary, 6-7µm in diameter. Akinetes attached to the heterocyst on one or both side, 12-18µm.
Habitat: Attached to mud on bill.

Anabaena torulosa Lagerheim ex Bornet and Flahault, Photo plate 4, fig. G
(Komarek 2013, p. 801, fig. 999)
Description: Trichome flexuous, cylindrical, joined and entangled in the blue green mats to ends sometimes slightly cylindrical, cell barrel shaped 4. 2-5µm wide, heterocyst ellipsoidal or almost spherical,6-10µm wide.
Habitat: Attached to grass on paddy field
Genus: *Cylindrospermum*

Cylindrospermum alatosporum F. E.
Fritsch, Photo plate 5, fig. F
(Komarek 2013, p. 878, fig. 1126)

Description
Mats thin, mucilaginous, bright blue-green. Trichomes long, dense and flexuous. Cell more or less cylindrical, longer than wide 3. 5-4µm wide, apical cells barrel-shaped or slightly narrowed and rounded. Heterocyst is oval, 7. 8µm long and 5. 1µm broad. Akinetes solitary, ellipsoidaland attached with heterocyst, 6. 9µm wide.
Habitat: Attached to the mud.
Genus: *Macrospermum*

Macrospermum fuellebornii (Schmidle) Komarek, Photo plate 4, fig. I
(Komarek 2013, p. 868, fig. 1113 and 1114)
Description:
Cells is more or less isodiametric or slightly barrel-shaped,3. 9µm wide and5µm long; apical cell rounded. Akinetes with granular-dotted.
Habitat: Attached to the grass.
Genus: *Trichormus*

Trichormus khannae (Skuja) Komarek and Anagnostidis, Photo plate 4, fig. H
Komarek 2013, p. 944, fig. 1231)
Description: Filaments creeping on the substrate, ±parallel situated, ±straight or bent, solitary or joined to mats, with up to 7µm wide, colourless or yellowish, diffluent sheaths, 15-20µm wide, cell barrel-shaped,2. 5-4µm wide. Heterocyst terminal or intercalary, barrel-shaped or cylindrical 3. 5-6×3-3. 5µm. Akinates in series, slightly ellipsoidal, elongated or spherical, 6. 5-10µm in diameter.
Habitat: Attached to the mud on Paddy Field.
Family: Nostocaceae
Genus: *Nostoc*

Nostoc paludosum Kutzing ex Bornet and Flahault,Photo plate 5, fig. D
(Komarek 2013, p. 800,801 and 802, fig. 1274-1276)
Colonies microscopic, in cluster, irregular, mucilaginous mass, blue green, colourless gelatinous sheath, Trichome flexuous, irregularly coiled, cell barrel-shaped, blue-green, 3-5µm in diameter. Heterocyst spherical or barrel-shaped, 4-8µm in diameter.
Habitat: Attached to grass on paddy field.

Nostoc zetterstedtii Areschoug ex Bornet and Flahault,Photo plate 5, fig. E
(Komarek 2013, p. 973, fig. 1265)
Description: Colonies macroscopics, gelatinous, ±firm, irregularly spherical, hemispherical, lobate, dark olive green or blue-green Individual sheath around the trichomes, confluent, colourless to yellow. Cell is short, barrel-shaped or slightly elongated,3. 9µm wide. Heterocytes spherical ,6. 9µm in long.
Habitat: Attached to the submersed grass
Family: Rivulariaceae
Genus: *Calothrix*

Calothrix marchica* Var. *Intermedia C. B. Rao,Photo plate 5, fig. A
(Komarek 2013, p. 242, fig. 245)
Description: Filaments always solitary, ±straight or slightly coiled, not branched, 5-6µm wide at the base. Sheath thin, narrow, colourless. Trichomes ± straight, continually narrowed towards ends, blue-green,4-5. 5µm wide at the very slightly widened base. Cell quadratic or shortened up to ¼ as long as wide. Heterocyst basal, single, spherical or subspherical, 4-5. 5µm wide.
Habitat: Attached to the mud on paddy field.

Calothrix brevissima G. S. West,Photo plate 5, fig. B
(Komarek 2013, p. 244, fig. 251d)
Description: Filament epiphytic, usually in groups, filaments up to 94µm long,4. 5-7µm wide, not or very slightly narrowed towards ends, cells rarely quadratic at the base, mostly shorter than wide ,3-5µm wide. Terminal cell rounded. Heterocyst basal, hemispherical up to spherical, 2. 5-4µm wide.
Habitat: Attached to the submersed grass on paddy field
Genus: *Rivularia*

Rivularia aquaticaDe-Wildeman,Photo plate 5, fig. C
(Komarek 2013, p. 308, fig. 354)
Description: Colonies ± spherical, soft, without calcareous precipitations, free-living, dark brownish-green. cells 3-13×5. 5-10. 7µm. Heterocyst spherical.
Habitat: Found on the mud
Family: Hapalosiphonaceae
Genus: *Hapalosiphon*

Hapalosiphon pumilus Kirchner ex Bornet and Flahault, Photo plate 5, fig. G. i-iii
(Komarek 2013, p. 517, fig. 629-634)
Description – Colonies tufty, blue green. Filaments densely entangled 7-25 µm wide. Branches vertically divaricated, sometimes erect slightly narrower than main filaments and 6-12 µm wide. Sheath colourless. Cells barrel shaped 7-10×4-5 µm. Heterocyst dimensions similar to vegetative cells.
Habitat: Attached to the grass.

DISCUSSION

An earlier literature review showed the least amount of diversity was explored in this region. Pal and Santra [20] [21] conducted a systematic study, but along with other groups of algae. In this systematic study, 11 sites (Table 1) of the Berhampore Subdivision were studied. More than 90 samples were examined, and 34 cyanobacterial species belonging to 22 genera were characterized and enumerated. Out of these 7, the coccoid form of cyanobacteria belongs to 6 genera. 16 non-heterocytous forms of cyanobacteria belonging to 8 genera were identified. Out of these 16 species, Oscillatoria was the most dominant. Heterocystous cyanobacteria significantly contribute to nitrogen fixation. In this study, 11 heterocystous cyanobacteria belong to 8 genera, mainly paddy fields and alleged water bodies. In this study, the Shannon-Wiener index (H') is 3. 42 (Table 2), which indicates the subdivision is highly diverse [29]. The Simpson Index (D) quantifies the likelihood of randomly selecting the same species from various ecological habitats [30]. The Simpson Index value in our study is 0. 98 (Table 2), which indicates that species richness is high and the diversity is healthy. The Berger-Parker Dominance Index highlighted the dominance of the most abundant species in this study [31], the value 0. 04 (Table 2), which indicates a moderate level of dominance by a single species. Margalef's Richness Index measures species richness while considering sample size [32]. In this study, the value is 7. 93 (Table 2), which indicates the Berhampore subdivision has high species richness. pH is one of the major components for cyanobacterial diversity [33]. During the study, the highest pH was recorded at 8. 4. *Chroococcus minutus* was recorded at pH 8. 4. Diversity indices provide valuable insights into ecosystem health, species evenness, and richness based on the observed species and individual counts, helping to assess the ecological balance and diversity within the studied environment. Considering the earlier literature review, cyanobacterial diversity of the Murshidabad district is still yet to be explored. From this taxonomic study, one can easily explain the cyanobacterial biodiversity of the Berhampore subdivision. This study will fill the gap in our existing knowledge of the biodiversity of cyanobacteria in the Berhampore subdivision. Further molecular studies along with morphological data will provide the world with new insights.

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Table 1: Cyanobacteria and their collection site

Sl. no	Study Site (SS)	Name of the place	Latitudes and Longitudes	pH	Name of the Organism
1	**SS1**	Berhampore	24° 05' 05'' N 88° 15' 02'' E	8. 4±0. 10	*Aphanocapsa musicola* (Meneghni) Wille; *Pseudanabaena lemnetica* (Lemmermann) Komarek; *Arthospira platensis* var. *tenuis* (Rao) Desikachary; *Limnothrix obliqueacuminata* (Skuja)Meffert *Lyngbya martensiana* Meneghini ex Gomont
2	**SS 2**	Nowda	23° 56' 53'' N 88° 18' 56'' E	7. 9±0. 10	*Arthospira platensis* var. *tenuis* (Rao) Desikachary; *Oscillatoria limosa* C. Agardh ex Gomont; *Gloeocapsa gelatinosa* Kutzing; *Oscillatoria* sp.
3	**SS 3**	Beldanga I	23° 55' 51'' N 88° 15' 21'' E	7. 4±0. 10	*Oscillatoria proboscida* Gomont; *Aphanocapsa parietina* Nägeli; *Aphanothece microscopica* Nageli; *Limnothrix obliqueacuminata* (Skuja)Meffert; *Anabaena sphaerica* Bornet et Flahault; Calothrix *marchica Var. intermedia* C. B. Rao; *Hapalosiphon pumilus* Kirchner ex Bornet and Flahault
4	**SS 4**	Beldanga I	23° 55' 47'' N 88° 15' 18'' E	7. 9±0. 10	*Oscillatoria ornata* Kutzing ex Gomont; *Aphanocapsa musicola* (Meneghni) Wille; *Spirulina laxa* G. S. West; *Lyngbya spiralis* Geitler; *Anabaena torulosa* Lagerheim ex Bornet and Flahault; *Nostoc paludosum* Kutzing ex Bornet and Flahault; *Calothrix brevissima* G. S. West; *Oscillatoria* sp.
5	**SS 5**	Beldanga I	23° 55' 53'' N 88° 15' 35'' E	8. 1±0. 10	*Oscillatoria limosa* C. Agardh ex Gomont; *Synechocystis pevalekii* Ercegovic; Nostocaceae (Agardh ex Gomont) Anagnostidis et Komarek; *Macrospermum fuellebornii* (Schmidle) Komarek *Calothrix marchica* Var. *intermedia* C. B. Rao; *Hapalosiphon pumilus* Kirchner ex Bornet and Flahault
6	**SS 6**	Nowda	23° 53' 42'' N 88° 24' 35'' E	7. 2±0. 10	*Oscillatoria ornata* Kutzing ex Gomont; *Chroococcus minutus (Kutzing)* Nageli; *Anabaena torulosa* Lagerheim ex Bornet and Flahault; C*ylindrospermum alatosporum* F. E. Fritsch; *Trichormus khannae* (Skuja) Komarek and Anagnostidis

7	**SS 7**	Nowda	23° 55' 31'' N 88° 24' 47'' E	7. 4±0. 10	*Oscillatoria tenuis* C. Agardh ex Gomont; *Oscillatoria proboscida* Gomont; *Aphanothece microscopica* Nageli; *Pseudanabaena recta* Komarek and Cornberg; *Anabaena torulosa* Lagerheim ex Bornet and Flahault *Nostoc paludosum* Kutzing ex Bornet and Flahault
8	**SS 8**	Hariharpara	23° 58' 38'' N 88° 24' 53'' E	7. 3±0. 10	*Oscillatoria proboscida* Gomont; *Gloeothece rupestris* (Lyngbye)Bornet in Witterock et Nordestedt *Anabaena sphaerica* Bornet et Flahault; *Trichormus khannae* (Skuja) Komarek and Anagnostidis; *Nostoc zetterstedtii* Areschoug ex Bornet and Flahault; *Hapalosiphon pumilus* Kirchner ex Bornet and Flahault
9	**SS 9**	Hariharpara	24° 01' 42'' N 88° 27' 04'' E	7. 6±0. 10	*Oscillatoria tenuis* C. Agardh ex Gomont; *Pseudanabaena lemnetica* (Lemmermann) Komarek *Arthospira platensis* var. tenuis (Rao) Desikachary; *Spirulina subsalasa* Oersted ex Gomont
10	**SS 10**	Hariharpara	24° 01' 48'' N 88° 26' 44'' E	7. 9±0. 10	*Oscillatoria limosa* C. Agardh ex Gomont; *Aphanocapsa parietina* Nägeli; *Aphanothece microscopica* Nageli; *Pseudanabaena recta* Komarek and Cornberg; *Tychonema rhodonema* (Skuja) Anagnostidis and Komarek; *Rivularia aquatica* De-Wildeman
11	**SS 11**	Berhampore	24° 09' 12'' N 88° 20' 24'' E	8. 1±0. 10	*Aphanocapsa musicola* (Meneghni) Wille; *Pseudanabaena lemnetica* (Lemmermann) Komarek; *Lyngbya martensiana* Meneghini ex Gomont; *Phormidium breve* (Kutzing ex Gomont) Anagnostidis and Komarek: *Anabaena sphaerica* Bornet et Flahault,' *Nostoc zetterstedtii* Areschoug ex Bornet and Flahault; *Oscillatoria* sp.

Table 2: Different diversity indices of Berhampore Subdivision

Shannon Winer Index	Simpson Index	Berger-parker Dominance Index	Margalef's Richness Index
3. 42	0. 98	0. 04	7. 93

Supplementary Table 3: Represents the frequency of Species

Sl. No	Name of the Species	Frequency
1	*Aphanocapsa musicola* (Meneghni) Wille	27. 27
2	*Aphanocapsa parietina* Nägeli	18. 18
3	*Aphanothece microscopica* Nageli	27. 27
4	*Gloeothece rupestris* (Lyngbye) Bornet in Witterock et Nordestedt	9. 09
5	*Synechocystis pevalekii* Ercegovic	9. 09
6	*Chroococcus minutus* (Kutzing) Nageli	9. 09
7	*Gloeocapsa gelatinosa* Kutzing	9. 09
8	*Limnothrix obliqueacuminata* (Skuja) Meffert	18. 18
9	*Pseudanabaena recta* Komarek and Cornberg	18. 18
10	*Pseudanabaena lemnetica* (Lemmermann) Komarek	27. 27
11	*Spirulina subsalasa* Oersted ex Gomont	9. 09
12	*Spirulina laxa* G. S. West	9. 09
13	*Arthospira platensis* var. *tenuis* (Rao) Desikachary	27. 27
14	*Lyngbya martensiana* Meneghini ex Gomont	18. 18

15	*Lyngbya spiralis* Geitler	9. 09
16	*Tychonema rhodonema* (Skuja) Anagnostidis and Komarek	9. 09
17	*Oscillatoria limosa* C. Agardh ex Gomont	27. 27
18	*Oscillatoria tenuis* C. Agardh ex Gomont	27. 27
19	*Oscillatoria proboscida* Gomont	27. 27
20	*Oscillatoria ornata* Kutzing ex Gomont	18. 18
21	*Oscillatoria* sp.	18. 18
22	Phormidium *animale* (Agardh ex Gomont) Anagnostidis et Komarek	9. 09
23	*Phormidium breve* (Kutzing ex Gomont) Anagnostidis and Komarek	9. 09
24	*Anabaena sphaerica* Bornet et Flahault	27. 27
25	*Anabaena torulosa* Lagerheim ex Bornet and Flahault	27. 27
26	*Cylindrospermum alatosporum* F. E. Fritsch	9. 09
27	*Macrospermum fuellebornii* (Schmidle) Komarek	9. 09
28	*Trichormus khannae* (Skuja) Komarek and Anagnostidis	18. 18
29	*Nostoc paludosum* Kutzing ex Bornet and Flahault	18. 18
30	*Nostoc zetterstedtii* Areschoug ex Bornet and Flahault	18. 18
31	*Calothrix marchica* Var. *intermedia* C. B. Rao	18. 18
32	*Calothrix brevissima* G. S. West	9. 09
33	*Rivularia aquatica* De-Wildeman	9. 09
34	*Hapalosiphon pumilus* Kirchner ex Bornet and Flahault	27. 27

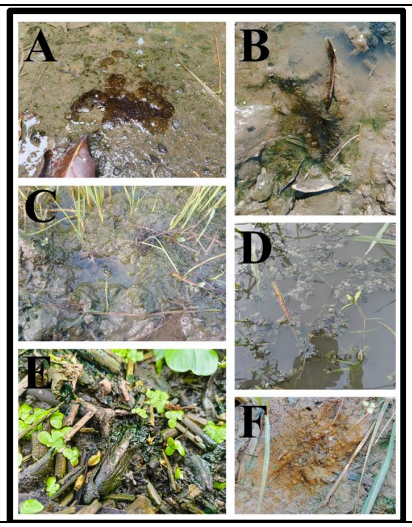


Photo plate 1: (Fig. A-G) Represents photographs of different habitat

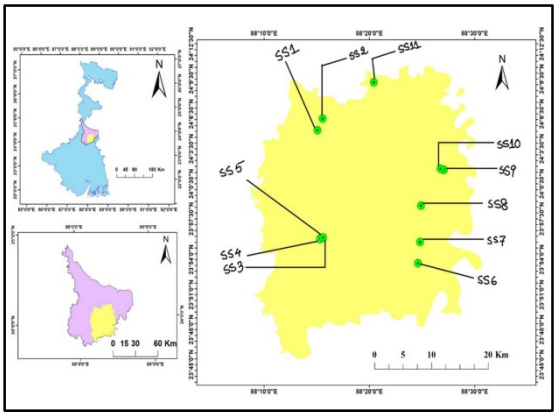


Photo Plate 2: Fig A. represents West Bengal map
Fig. B. represents Murshidabad District map
Fig. C. represents the study area map

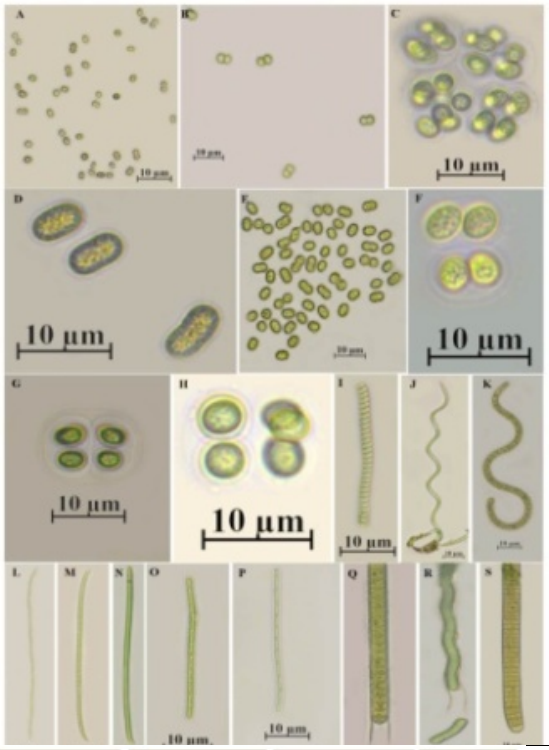


Photo Plate 3: (Fig. A-S) Photomicrograph of cyanobacteria

A. *Aphanocapsa muscicola* (Meneghini) Wille, B. *Synechocystis pevalekii* ErcegovicC. *Gloeocapsa gelatinosa* Kutzing, D. *Gloeothecerupestris* (Lyngbye) Bornet in Witterock et Nordestedt,E. *Aphanothece microscopica* Nageli, F-G. *Aphanocapsa parietina* Nageli, H. *Chroococcus minutus* (Kutzing) Nageli, I. *Spirulina major* Kutzing ex Gomont, J. *Spirulina laxa* G. M. Smith, K. *Arthospira platensis* var. *tenuis* (Rao) Desikachary, L. *Limnothrix obliqueacuminata* (Skuja) Meffert, M. *Phormidium animale* (Agardh ex Gomont) Anagnostidis et Komarek, N. *Phormidium breve* (Kutzing ex Gomont) Anagnostidis and Komarek O. *Pseudanabaena recta* Komarek and Cornberg, P. *Pseudanabaena lemnetica* (Lemmermann) Komarek, Q. *Lyngbya martensiana* Meneghini ex Gomont, R. *Lyngbya spiralis* Geitler S. *Oscillatoria limosa* C. Agardh ex Gomont

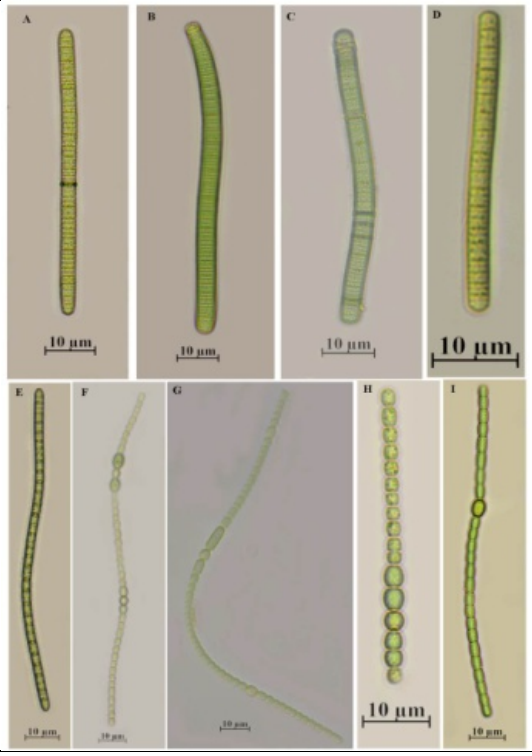


Photo Plate 4: (Fig. A-I) Photomicrograph of cyanobacteria

A. *Oscillatoria tenuis* C. Agardh ex Gomont, B. *Oscillatoria proboscida* Gomont. *Oscillatoria ornata* Kutzing ex Gomont D. *Oscillatoria sp.* ,E. *Tychonema rhodonema* (Skuja) Anagnostidis and Komarek, F. *Anabaena sphaerica* Bornet and Flahault, G. *Anabaena torulosa* Lagerheim ex Bornet and Flahault, H. *Trichormus khannae* (Skuja) Komarek and Anagnostidis, I. *Macrospermum fuellebornii* (Schmidle) Komarek

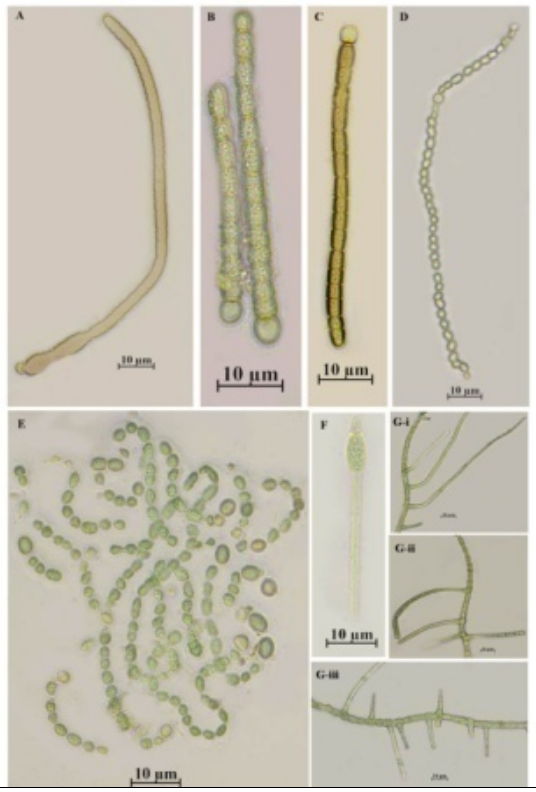


Photo Plate 5: (Fig. A-G) Photomicrograph of cyanobacteria
A. *Calothrix marchica* Var. *intermedia* C. B. Rao, B. *Calothrix brevissima* G. S. West, C. *Rivularia aquatica* De Wildeman, D. *Nostoc paludosum* Kutzing ex Bornet and Flahault,E. *Nostoc zetterstedtii* Areschoug ex Bornet and Flahault, F. *Cylindrospermum alatosporum* F. E. Fritsch,G-i-iii. *Hapalosiphon pumilus* Kirchner ex Bornet and Flahault

RESEARCH ARTICLE

Examining the Impact and Optimization of Large Language Models

Ananjan Maiti[1], Indranil Sarkar[2], Moumita Samanta[3], Subhadip Nandi[4], Shirsendu Dutta[5] and Sandip Roy[6]*

[1]Assistant Professor, Department of Computer Science and Engineering, Guru Nanak Institute of Technology, Panihati, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata, West Bengal, India.
[2]Assistant Professor, Department of Computer Applications, Guru Nanak Institute of Technology, Panihati, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata, West Bengal, India.
[3]Student, Department of Computer Science and Engineering, Guru Nanak Institute of Technology, Panihati, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata, West Bengal, India.
[4]Assistant Professor, Department of Computational Sciences, Brainware University, Barasat, Kolkata, West Bengal, India
[5]Assistant Professor, Department of Computer Applications, Guru Nanak Institute of Technology, Panihati, (Affiliated to Maulana Abul Kalam Azad University of Technology), Kolkata, West Bengal, India.
[6]Professor, Department of Computer Science and Engineering, JIS University, Kolkata, West Bengal, India.

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***Address for Correspondence**
Sandip Roy
Professor,
Department of Computer Science and Engineering,
JIS University, Kolkata, West Bengal, India.
E.Mail: sandiproy86@gmail.com

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ABSTRACT

The intervention of large language models (LLMs) extends beyond areas like natural language processing, autonomous systems, and recommendation systems. Additionally, the growth of LLMs has raised concerns about their performance, productivity, and resource utilization. This comprehensive discussion offers insights into various techniques and measures that can improve the performance and efficiency of LLMs, including methods such as data pruning, mixed precision training, early exit optimization, and resource-efficient architectures. In addition, we address perceived emerging trends in assessing LLMs, including those involving LLMs as judges in constructing domain-specific standards for LLM evaluation. This study aims to lay the foundation for further advancements in designing more resilient and sustainable LLMs closely aligned with actual practice. It has shown how performance-

improving methods, including fine-tuning techniques, retrieval augmentation, and knowledge distillation, have successfully transformed LLMs for downstream tasks and made their performance better. This survey site is intended to be a valuable resource for researchers and practitioners to fine-tune and evaluate large language models.

Keywords: Large Language Models, Performance Optimization, Efficiency, Benchmarking, Resource Management, Evaluation Methodologies.

INTRODUCTION

Overview of Large Language Models [LLMs]

Large language models, or LLM for short, are one of the most influential technologies that have had a large influence on AI's advancement and have changed the progress of natural language processing, recommendations, and several autonomous agents [1]. To understand the weak patterns and produce humanist text, LLMs learn text data with millions of records [2]. Recent works on 4 include various applications like question answering, text summarization, code generation, and more, which can be witnessed in recent LLMs such as GPT-3 [2], BERT [3], and T5, where flexibility is seen to be a prime aspect of LLM's ability**.**

Challenges in LLM Performance and Efficiency

As much as large language models have great abilities, the performance and efficiency of large number computation scales and complexities affect them [4]. As the usage of layers rises in LLMs, the training and deployment cost becomes too expensive because of the humongous parameter size, which could be in billions or trillions [1]; further, owing to the training energy consumption, questions regarding the mental impact and environmental consequence and sustainability of such models are posed [5]. Increasing the use of computational resources and developing efficient computational architectures are now the topics of extensive study to tackle these issues [5].

Motivation and Scope of the Survey

LLM performance and efficiency improvement have led to several methods, such asologies, standards, and optimization tools. This state of affairs has placed researchers and practitioners in a challenging position to identify the appropriate techniques for their specific uses. This survey aims to provide a systematic and more detailed way to identify the methods and standards to improve LLM performance and overall efficiency. Although this survey is versatile, the authors present topics like efficient methods for training with limited resources, assessment measures, methods to improve performance, and optimization approaches for subsequent tasks [Mei *et al*., 2021]. Through synthesizing and integrating the latest research findings, this work aims to offer some methodological and pragmatic observation points to the researchers and practitioners – the developmental and assessing participants in the large language models [6]. In this survey, we will explain modern approaches, which are data pruning [7], mixed precision training [8], early exiting optimization [9], and resource-efficient architecture. So, it will also be investigated how the new perspectives in LLE evaluation, including using LLMs as judges [10] and building domain-specific benchmarks [6], are going to develop. In this way, with the help of the provided detailed introduction to these developments, we would like to encourage the emergence. Robust and economically viable models for operational purposes [11].

Resource - Efficient Training Methodologies

Modeling for large language models is an expensive endeavor that requires a significant amount of computational power and energy consumption [12]. To overcome these challenges, different resource-efficient training approaches have been suggested by the researchers, which were directed towards decreasing the computational cost while at the same time retaining the model performance [5]. This section presents, as Figure 1 shows, data pruning techniques, mixed precision training, early exiting optimization, and efficient architectures and components that lead to resource-efficient LLM training.

Data Pruning Techniques

Data pruning techniques focus on finding and eliminating redundant or less informative samples from the training dataset, thereby reducing the cost of computation with huge data volume [7]. Two popular data pruning techniques are influence score and effort score-based methods and diversity-based coreset selection.

Influence Score and Effort Score

Two types of scores are used to specify the value of each single example of training in learning, known as the Influence score and effort score [13]. Following these scores, A researcher selects samples with high scores, i.e., samples having more influence on the model's performance, and discards the lower-scoring ones [Sui *et al*., 2023], thus increasing the efficiency of computational resource usage during training.

Diversity-based Coreset Selection

To characterize the total diversity of the data, a diversity-based coreset is selected to generate a representative subset of the required training data [14]. The choice of diverse samples reduces the computational load to a minimum by allowing the model to learn different patterns and structures concurrently [9]. There are different algorithms that have been used to find representative and diversified subsets of the training data such as K-means clustering and submodular optimization [15].

Mixed Precision Training

Mixed precision training is the approach that takes advantage of the capabilities of modern hardware to perform calculations on lower precision data types, specifically half-precision floating-point [FP16]. With some operations precision decreased, mixed precision training leads to LLM training inertia and computational cost reduction [8]. This method makes it possible to use much larger batch sizes and, consequently, a faster training time without loss of model performance.

Early Exiting Optimization

Early exit optimization is a method where models terminate computations based on confidence intervals [16]. LLMs, early exiting, can be employed with self-attention layers by LLMs when the model can, at some point, terminate the computation if the attention weights fall below a certain threshold. When early exiting optimization is employed for LLM inference, the model cost and latency will be reduced by dynamically tuning the model depth concerning the complexity of the input.

Efficient Architectures and Components

Creating efficient architectures and components is essential for resource-effective LLM training. Self-attention mechanisms, multi-head attention, and key-value cache optimization are the important aspects under this umbrella.

Self-Attention Mechanisms

The self-attention mechanism is at the heart of the transformer-based LLMs; the architecture allows the model to work with the sequential dependencies in the input [17]. However, the self-attention that involves the computation of the quadratic time is very costly, especially when handling sequences of 20 tokens. To overcome the computational complexity issues, some modified self-attention functions have been introduced, which include Sparse attention [18] and Linear attention [19].

Multi-Head Attention

Multi-head attention enables LLMs to read different parts of a sequence in one set of attention [17]. However, the complexity in the computation of multi-head attention increases with the number of heads in the same proportion of the number of heads. To overcome this issue, researchers have come up with two techniques, namely head pruning [20] and attention head sharing [21], to eliminate the attention heads and achieve the best outcome.

Key-Value Cache Optimization

Key-value cache optimization is a method that reduces memory wastage and computation costs by focusing on redundancy during computation [22]. LLMs can cache keys and value matrices to reuse them at later times, resulting in an increase in efficiency in training. This key-value cache optimization shows improvement in performance in autoregressive language model tasks and reduces memory wastage in large capacity.(Table 1).

Evaluation Methodologies and Benchmarks

For the reader to be able to appreciate the possibilities that are presented by the LLMs as well as the drawbacks, it is important to look at their performance and Efficiency [25]. In the subsequent category, we examine the various assessment methodologies and standards for the LLM-as-a-judge paradigm, domain-specific standards, and assessment criteria metrics and protocols.

LLM-as-a-Judge Paradigm(Fig: 2)

Pairwise Comparison Approaches

In order to assess and rank the quality of different text samples or model outputs, the pairwise comparison approach employs LLMs [6]. Here, the LLM scores the given text samples on the basis of their fluency, coherence, and so on [Mei *et al*., 2021]. It is possible to order the samples or models under consideration in the research. Nevertheless, the LLM judgment can be subjective and irrational in its decision-making if the above-mentioned method is used, which is based on a pairwise comparison approach [10].

Prompt Scoring Techniques

In this kind of scoring approach, the LLMs provide scores or ratings to every text sample individually on the basis of set prompts or queries [9]. It is to highlight language aspects such as grammar, semantics, task-related aspects, etc., thus enabling the LLMs to assign ratings or scores to each sample and therefore quality assessment. The prompt scoring techniques have been seen to perform better than pairwise comparisons and also reveal high congruence with the judgment made by humans [11].

Domain-Specific Benchmarks

Custom-ruled benchmarks are made to evaluate LLMs in particular areas or domains, such as natural language understanding, code intelligence, and recommendation systems. The utilization of these criteria enables LLMs to showcase their capabilities most effectively in authentic contexts [26].

Natural Language Understanding

NLU benchmarks test how well LLMs are with the usage of natural language in the text [27]. Around GLUE benchmark [28], Super GLUE [29], and SQuAD [30] are the most used NLU benchmarks. These milestones deal with a variety of operations like feeling analysis, textual entailment, and answering questions, therefore providing an extensive scale of checking the LLMs 'abilities to understand language.

Code Intelligence

Code intelligence benchmarks are measures of the LLM's ability to generate, understand, and even critically analyze programming code [28]. Marks like CodeXGLUE [29] and HumanEval [30] are considered to judge the efficacy of the LLMs [Language *et al*.] in areas such as code generation, code completion, and error fixing. Such milestones unfold the LLM's ability to assist the developers and automate programming tasks to a certain extent.

Recommendation Systems

The LLM's performance as the recommended system is examined through benchmarks that test the LLMs' ability to generate personalized recommendations according to user specifications and product specifics [31]. Examples include Amazon Product Data [He *et al*., 2016] and Movielens [32], which measure the task performance of LLMs and

report outcomes such as item ranking and item rating prediction. With these benchmarks, the LLMs are analyzed and improved to enhance the recommendation process and make it engaging for users.

Evaluation Metrics and Protocols

Evaluation metrics and protocols are inherently necessary for assessing the performance of LLMs and, subsequently, ensuring the comparability of different models. Evaluation metrics employed widely are perplexity, BLEU, rouge, and f1 score. These indicators have a different dimension, which captures various aspects of language quality, like fluency, coherence, and task-specific ability. Evaluation procedures that are endowed with cross-validation and held-out testing will create the value of LLM evaluations [Xu *et al.*,2022].

Performance Enhancement Techniques

It discusses various techniques that can be applied to improve the performance of LLMs, and the most important area of research in natural language processing is performance. Enhancement of large language models [1] Here, we discuss various techniques that are used to enhance the performance of large language models, among which include fine-tuning approaches, retrieval augmentation, and knowledge distillation. (Table: 2)

Fine-Tuning Strategies

Fine-tuning is a process of adapting a pre-trained LLM for certain target tasks, which is a usual practice [38]. Fine-tuning allows LLMs to adapt to new data during model training for the target task, hence enhancing the performance of the model [3]. Some of the well-known fine-tuning schemes include self-augmented prompting and low-rank adaptation.

Self-Augmented Prompting

Auto-augmented prompting is a fine-tuning method that leverages the generative nature of LLMs to generate additional training data [39]. By providing several seed examples, the self-augmented prompting method is capable of making the LLM come up with new examples and thus expand the training data and improve the model performance in low resource conditions [Ye *et al.*, 2023]. This has been demonstrated to be rather effective, particularly in scenarios where it is challenging or expensive to obtain labeled data.

Low-Rank Adaptation

Low-rank adaptation is a parameter-efficient fine-tuning approach that can re-configure a pre-trained LLM for a specific task by learning a small set of task-specific parameters only [40]. Low-rank adaptation, instead of fine-tuning the whole model, adds a low-rank matrix, which is also learned along with the pre-trained parameters [41]. This approach brings down the trainable parameters count to a considerably low value but, at the same time, maintains the full fine-tuning capability [42]. Low-rank adaption is also applied in several NLP applications, such as text classification and question answering.

Retrieval Augmentation

Retrieval augmentation is a way of enhancing LLMs, which involves incorporating external knowledge sources into the actual generation of responses [43]. In a way, the retrieval augmentation enables LLMs to obtain the right information from large knowledge bases or document collections, which may contain much more information than the model's training corpus [44]. This was because it improved LLMs' performance in knowledge-intensive tasks such as open-domain question answering and fact verification. Retrieval augmentation typically involves a two-stage process: This means that the process involves [1] search for the documents or passages that are relevant to the input query and [2] incorporation of the identified information into the LLM's prediction [45]. Some of the retrieval strategies proposed are dense passage retrieval [46] and sparse vector retrieval [47] to facilitate a quick and precise search and retrieval of information from large-scale knowledge bases. Later on, the obtained information is combined with the LLM's output by employing techniques such as attention mechanisms [48] or knowledge-aware decoding.

Knowledge Distillation

Knowledge distillation is a technique that transfers knowledge of a big, complex model [teacher] to a smaller, more efficient model [student] [49]. In the case of LLMs, knowledge distillation allows the compression of big models in a more practical and usable format and sustains their performance [50]. The student model obtains the knowledge and reasoning abilities of the larger model by imitating the outputs of the teacher model [51]. Such knowledge distillation of LLMs normally involves training the student model to mimic the teacher model on a large set of text. The student model reduces the disparity between its expected outputs and those of the teacher model; thus, in the process, attempts to emulate the teacher model's answers [8]. Some of the distillation targets, like cross-entropy loss and Kullback-Leibler divergence, have been employed to control the learning process [5]. Knowledge distillation has been used in many LLM architectures, including transformer-based models [17] and autoregressive language models [52]. The final models that are derived are as effective as the original models and, at the same time, are computationally efficient and easier to implement in situations where there are resource challenges [53].

Efficiency Optimization in Downstream

So, it is crucial to have adequate and fast inference methods as well as model compression and hardware acceleration techniques for using large language models in small-devices realistic applications [Xu *et al.*, 2022]. In this part, different techniques that are applied to improve the performance of LLMs in downstream tasks are explained.

Efficient Inference Strategies

Framing plays, such as efficiency inference-like learning, are aimed toward decreasing the computational complexity and time of LLM inference while the model performance is kept constant [5]. Dynamic inference is one of the most frequently used techniques and is a method of adjusting the depth of calculations in the model based on the input's complexity [54]. As a result of this, dynamic inference will help to reduce the average inference time significantly by applying early exits on the layers or at the confidence thresholds [55]. Input pruning is another effective strategy for inference, where the irrelevant or redundant parts of the input sequence are identified and pruned [56]. Among the most informative parts of the input, which are the focus of the LLMs, computational is cut, and the speed of inference is improved [8]. Approaches, which include techniques like attention-based pruning [57] [58] and importance sampling [59], have been suggested to detect and, thus, prioritize potential input parts in an effective manner.

Model Compression Techniques

The main task of model compression techniques is to diminish the size and computational complexity of LLMs while still retaining their performance [58]. Quantization represents the common method for compression that degrades the precisions of model weights and activation [60]. Quantization by representing weights and activations with fewer bits can greatly decrease the memory footprint and computational cost of LLMs. Pruning is one of the most efficient compression techniques that eliminates redundant or less significant weights in the model [61]. Pruning is the process of eliminating weights with small magnitudes or low contributions to the model's output, and it can reduce the model size by a substantial amount without pronounced performance degradation [62]. The pruned model should be regular and easy for effective hardware implementation and high performance; therefore, structured pruning methods tend to achieve these objectives, two of them being block pruning [63] and head pruning [20]. The knowledge distillation, touched upon in the previous section, is another important approach for model compression [49]. Through the use of the technique known as distillation, a smaller student model can be taught to replicate the behavior of a larger model. As the teacher and student models can be quite large, knowledge distillation can inherit the knowledge and reasoning capabilities of the teacher and create a smaller and more efficient student model [51].

Hardware Acceleration

Hardware acceleration significantly contributes to the efficiency of LLM inference. The utilization of hardware architectures that have specializations, such as GPUs and TPUs, has been claimed to speed up large-scale neural network computation [64]. The LLM architectures provide huge parallelism and high memory bandwidth, which enables fast and effective implementation of matrix operations, which are the underlying building blocks of LLMs [65].

To improve the performance of LLM inference on hardware accelerators, diverse methods have been suggested. Kernel fusion is a method that merges many small kernel operations to form a considerably larger kernel, hence reducing the overhead of kernel launches and data transfers [66]. Fusion of kernel has been demonstrated to convulsively enhance the performance of transformer-based models in GPUs [67]. The other hardware acceleration approach is mixed-precision training and inference, which exploits the fact that modern hardware is capable of doing calculations at different precision levels [23]. Lowering the precision [e.g., FP16] instead of single-precision [i.e., FP32] at highly computation-sensitive tasks mixed-precision methodologies can lower the memory footprint and computation cost of LLMs without model accuracy degradation [68]. Some customized hardware designs were also suggested to increase the performance of LLM inference. For instance, Efficient Transformer [EFT] architecture [69] is hardware design optimized for computationally efficient execution of self-attention and feedforward layers in transformer-based models. Compared to general-purpose hardware, EFT gains large speed-ups and energy savings with sparsity and regularity of transformer computations.

Emerging Trends and Future Directions

The large language models are a rapidly changing field with new trends and directions emerging to address the challenges and opportunities posed by these powerful models [1]. This subsection deals with some of the recent and future trends in LLM research and development, such as multimodal LLMs, federated learning for privacy, scaling laws and model architectures, and real-world applications and deployment challenges.

Multimodal LLMs Multimodal

LLMs are a new paradigm that intends to improve the performance of the LLMs by including more modes, such as vision or speech, in addition to text [70]. Utilizing the complementing information from different modalities, multimodal LLMs can have a comprehensive perception of the world and perform demanding tasks that need to be conducted within a several-domain framework [71]. Recent progress in multimodal LLMs comprises such models as ViLBERT [72], which, in turn, blends transformer-based architectures for text and vision, and CLIP [73], which learns visual concepts from natural language supervision. These models have shown excellent performance in tasks like visual question answering, image captioning, and text-to-image retrieval [63]. Further research directions of multimodal LLMs would be designing more powerful and scalable architectures to process and combine different modalities [74], seeking new training objectives and alignment strategies for representation across modalities [Tan *et al*., 2022], and extending multimodal LLM to some other domains such as robotics and embodied AI [75](Table: 3).

Federated Learning for Privacy Preservation

Federated learning is a new emergent paradigm enabling the training of LLMs with decentralized data under privacy [76]. Moreover, federated learning is collaborative in that several participants work to train a common model without the exchange of their original data, thus reducing the threat to privacy and ensuring the appropriate level of data secrecy. Federated learning is an option to be considered in the case of LLMs, which permits the use of various sensitive data sources [personal devices, healthcare records, etc. without user privacy violation [5]. Federated language models [LLMs] have been introduced as a solution to privacy-preserving language modeling and downstream tasks such as sentiment analysis and named entity recognition [77]. Further research areas in federated learning for LLMs are designing highly efficient communication protocols and aggregation mechanisms for large-scale federated training [78], handling issues of data heterogeneity and non-IID distributions [79], and guaranteeing the robustness and fairness of federated LLMs [80].

Scaling Laws and Model Architectures

The importance of scaling laws and model architectures for the creation of LLMs lies in the fact that they define the relative sizes and performances of such models [81]. Scaling laws give a description of the relationship between model size, computational resources, and performance, thus allowing one to get an idea of the best model scaling strategies [82]. More recent studies have demonstrated that larger model sizes and more training data indeed benefit the performance of LLMs [2]. Regardless, the effect of computing cost and environment in training and deploying of large LLMs are concerning [12]. To create an efficient and sustainable scaling laws and model architectures strategies

like model compression [58] and neural architecture search [83] are explored. On the other hand, to obtain more performance-efficient trade-offs, new model architectures need to be studied. To model long-term dependencies in language modeling, the Transformer-XL architecture [84] implements segment-level recurrence and relative positional encodings, and to overcome the quadratic complexity of the self-attention and allow more effective training and inference of LLMs, the architecture of the Performer [85] suggests a linearized attention.

Real-World Applications and Deployment Challenges

Constructing models that are practical in real applications like natural language inter- faces, content generation, and decision support systems is the principal goal of LLM research [1], and issues like the robustness of the model, interpretability, and ethical issues appear when LLMs are deployed in real-world applications [86]. The capacity of LLMs to retain their performance and to generalize well to new and unseen data is known as the robustness of a model [87]. Different techniques are used to improve it in LLMs, such as adversarial training [88], data augmentation [15], and domain adaptation [89]. Another key element of LLM implementation is interpretability, which allows users to understand and believe in the model's predictions [90]. To enhance this, methods like restricting attention and probing the classifiers are used. LLMs can have significant societal effects such as biased perpetuating and harmful generating content, so the ethical side is considered to be the principal aspect [4], and management of this issue requires a multidisciplinary approach that should involve the researchers, the policymakers, and the stakeholders who are from various backgrounds. Bias detection and mitigation [91], guaranteeing fairness and accountability [92], and advancing the innovation of responsible practices [93] are some of the feasible approaches.

Discussion and findings

The current methodologies and benchmarks for the improvement of large language model [LLM] performance and efficiency are introduced in this extensive review. We have talked about cost-effective training approaches, evaluation models, performance improvement tactics, and optimization methods for downstream activities. Additionally, we have pointed out the emerging trends and future directions in LLM research, such as multimodal LLMs, federated learning for privacy preservation, scaling laws and model architectures, and real-world applications and deployment challenges.

Summary of Key Findings

Our survey revealed several important findings in the area of LLM performance and efficiency optimization. Some pruning techniques are influence score and diversity-based corset selection, which have shown great potential to reduce com- the computational cost of LLM training while maintaining model performance [7, 9]. Mixed precision training and early exiting optimization are other strategies that have become effective in improving the efficiency of LLM training and inferences [8]. LLM-as-a-Judge paradigm is one of the evaluation methodologies used, where the pairwise comparison methods, as well as the prompt scoring techniques, exhibit a high correlation [6, 11] with human judgments. Specific benchmarks for domains such as natural language understanding, code intelligence, and recommendation systems have been created to assess LLM performance in real-world contexts [28, 30, 31]. The approaches for enhancing the performance, such as self-supplement antici- pation, lower-follower adaptation, retrieval improvement, and knowing boiling, have brought a significant improvement in LLM performance on different downstream tasks [39]. Efficient inference strategies, model compression techniques, and hardware acceleration have also been investigated to improve the efficiency of LLM deployment in resource-constrained environments [54, 60, 67].

Open Research Questions and Opportunities

However, outstanding research questions and opportunities exist in spite of the remark- able progress that has been made in the LLM performance and efficiency optimization. One important direction is to architect better, more efficient, and scalable multimodal LLMs to work with and combine information from different modalities [74]. However, another important area is the federated learning approach of LLMs, which is learning from distributed data to preserve privacy [94]. The question of better scaling strategies, which include model compression and neural architecture search, is also an open research issue [58, 83]. Another interesting solution is the research of new model

architectures, which can offer higher performance-efficiency trade-offs like the Transformer-XL [84] and Performer [85]. Many challenges face the real-world application of LLMs, such as model robustness, interpretability, and ethical issues [86]. Nonetheless, one of the critical issues for further study is the development of techniques for LLM's robustness and interpretability, including adversarial training [88] and attention visualization [95]. Investigation of the ethical challenges connected with LLM deployment, such as bias mitigations and responsible innovation, as a topic also requires a multi-disciplinary approach that unites researchers, policymakers, and stakeholders from various sectors [4,93]. In conclusion, this survey has provided a thorough description of methods, metrics, and optimization techniques that contribute to the improvement in the performance and efficiency of large language models. We hope that by providing a summary of the critical findings and open research questions this will encourage more research and innovation in this exciting area, and hence more powerful, scalable, and ethical LLMs for the practical setting.

CONCLUSION

This survey offers useful information through the analysis of resource-efficient training methods, evaluation paradigms, performance improvement techniques, and optimization strategies for downstream tasks, as well as the best practices in LLM development and deployment. It highlights the gravity of methods like data pruning methods, mixed precision training, early exiting optimization, and efficient architectures in the reduction of cost of computation and consumption of resources of LLM training that have demonstrated a lot of potential by maintaining the model performance while increasing the training speed by a big margin. Also, the LLM-as-a-Judge paradigm and domain-specialized benchmarks for LLM evaluations in practical situations are highlighted in this survey results in providing a complete evaluation in a real-world context of their capacity. Performance-improving methods, including fine-tuning techniques, retrieval augmentation, and knowledge distillation have successfully transformed LLMs for downstream tasks and made their performance better. In addition, the study portrays the importance of effective inference techniques, model compression methods, and hardware acceleration in making LLM deployment efficient in resource-constrained settings. Developing further, the survey points out several emerging trends and future research areas in the development of LLM. Multimodal LLMs that integrate vision and speech modalities have the possibility of making language models more holistic and better. Federated learning methods provide a promising answer to the question of training LLMs on distributed data in a privacy-preserving manner. In this respect, the scaling laws, as well as new model architectures, will lead to improvements in LLM effectiveness and economy. Nevertheless, the survey also notes the difficulties attributed to actual application of LLMs which include model robustness, interpretability, and ethical issues. Solving these challenges needs an interdisciplinary approach with researchers, policymakers and stakeholders from different perspectives. All in all, this survey is a rich source for people who develop and deploy LLMs. Having synthesized the most recent developments and open research questions, it sets the stage for future LLM performance and efficiency optimization innovations. In this dynamic field, efficient and scalable applied LLMs should be of importance while LLMs must be designed to be effectively and ethically applied to real-world situations.

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Table 1: Key-Value Cache Optimization

Methodology	Technique	KeyBenefits	Citation
DataPruning	Influence Score and Effort Score	Reduces computational cost and data volume	[7,13]
DataPruning	Diversity-based Coreset Selection	Maintains data diversity and reduces complexity	[9,14,15]
MixedPrecision Training	Utilizing lower precision data types [FP16]	Faster training and larger batch sizes	[8,23]
Early Exiting Optimization Efficient Architectures	Confidence-based early termination	Reduces inference cost and latency	[9,16]
	Self-Attention Mechanisms [Sparse, Linear]	Improves efficiency while maintaining performance	[17–19,24]
	Multi-Head Attention [Pruning, Sharing]	Lowers computational cost without accuracy loss	[20,21]
	Key-Value Cache Optimization	Saves memory and reduces redundant computations	[22]

Table 2: Performance Enhancement Techniques

Methodology	Technique/Benchmark	KeyBenefits	Citation
LLM-as-a-Judge Paradigm	Pairwise Comparison Approaches	Compares and ranks text samples or model outputs	[6,10]
	Prompt Scoring Techniques	Assign scores to text samples based on prompts	[9,11]
Domain- Specific Benchmarks	Natural Language Understanding [GLUE, SuperGLUE, SQuAD]	Evaluates LLMs' language understanding capabilities	[27,33,34]
	Code Intelligence [CodeXGLUE, HumanEval]	Assesses LLMs' code generation and analysis skills	[28–30]
	Recommendation Systems [Amazon *et al.*]	Measures LLMs' performance in generating recommendations	[31,32]
Evaluation Metrics	Perplexity	Assesses the fluency and coherence of the generated text	[25]
	BLEU, ROUGE, F1 Score	Evaluate the quality and task-specific performance of LLMs	[35–37]

Table 3: Multimodal LLMs Multimodal

Methodology	Technique	KeyBenefits	Citation
Efficient Inference	Dynamic Inference	Reduces computational costand latency	[54,55]
	Input Pruning	Improves inference speed by focusing on informative parts	[56,57,59]
Model Compression	Quantization	Reduces memory footprint and computational cost	[23,60]
	Pruning [Block *et al.*]	Removes redundant weightswithout significant performance loss	[20,61–63]
	Knowledge Distillation	Transfers knowledge from alarger model to a smaller one.	[49–51]
Hardware Acceleration	Specialized Architectures [GPUs, TPUs]	Speeds up computation for large-scale neural networks	[64,65]
	Kernel Fusion	Reduces kernel launch overhead and data transfers	[66,67]
	Mixed-Precision Training and Inference Customized	Lowers memory footprint and computational cost	[23,68]
	Hardware Designs [e.g., Efficient Transformer]	Optimizes hardware for specific LLM architectures	[69]

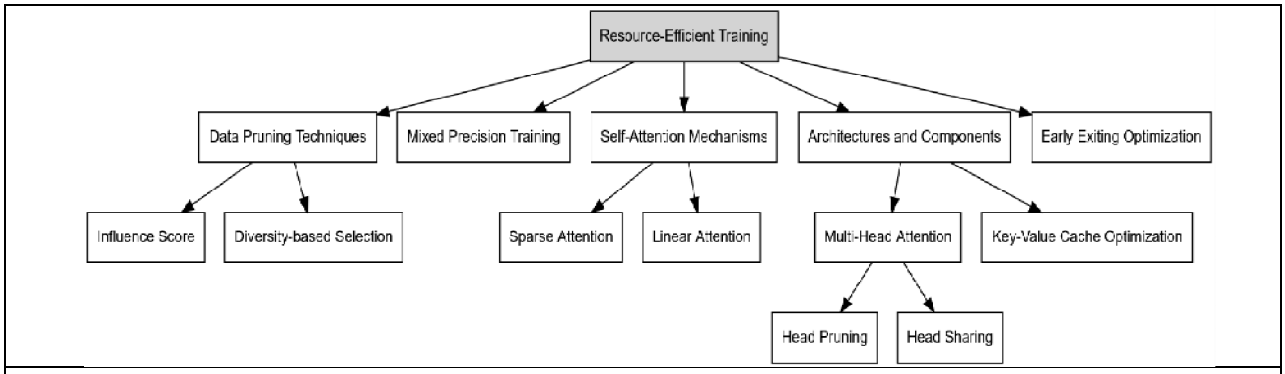


Fig. 1: Overview of Resource-Efficient Training Methodologies in AI Systems

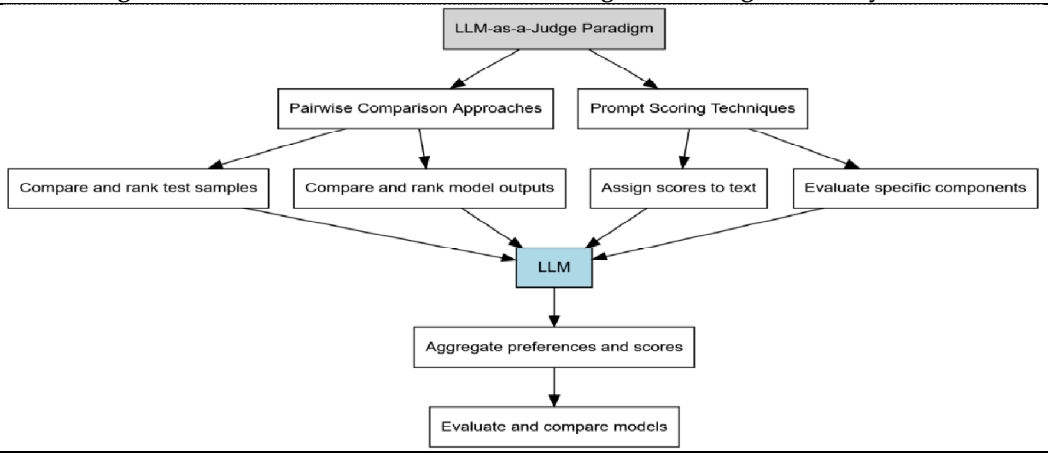


Fig. 2: Exploring the LLM-as-a-Judge Paradigm: A Flowchart of Evaluation Techniques in Language Model Comparisons

RESEARCH ARTICLE

Enhancement of Indomethacin Solubility by Solid Dispersion Technique

Pynskhemlin Syiemlieh[1]*, Chandan Kumar Purbey[2], Durgesh Jyoti[2], Rahul Kumar Sah[2], Samsul Haque Ansari[2], Sunil Sah[2] and Venkatesh D P[3]

[1]Assistant Professor, Department of Pharmaceutics, Acharya and BM Reddy College of Pharmacy, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.
[2]Student, Department of Pharmaceutics, Acharya and BM Reddy College of Pharmacy, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.
[3]Professor and HOD, Department of Pharmaceutics, Acharya and BM Reddy College of Pharmacy, (Affiliated to Rajiv Gandhi University of Health Sciences), Bengaluru, Karnataka, India.

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***Address for Correspondence**
Pynskhemlin Syiemlieh
Assistant Professor,
Department of Pharmaceutics,
Acharya and BM Reddy College of Pharmacy,
(Affiliated to Rajiv Gandhi University of Health Sciences),
Bengaluru, Karnataka, India.
E.Mail: pynskhemlinsyiemlieh@gmail.com

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ABSTRACT

Indomethacin is an NSAID utilized for treatment of slight to severe intense pain and soothe indications of arthritis (osteoarthritis and rheumatoid arthritis) or gout such as joint discomfort, stiffness, edema and irritation. Indomethacin is having less solubility resulting in low bioavailability. Hence in order to increase the bioavailability solubility has to be increased. In this study, Indomethacin solubility was increased by using solid dispersion method. Four formulations of Solid dispersions of Indomethacin are formulated utilizing physical and kneading method using ratio of 1:1 and 1:2 with HPMC respectively. Pre-formulation studies of Indomethacin were studies including organoleptic characteristics, solubility, melting point, determination of λmax, and compatibility study by using FT-IR spectroscopy. Formulated solid dispersions are then encapsulated to hard gelatin capsule shells and subjected for evaluation like weight difference, disintegration, drug presence and in-vitro drug discharge studies. Pre-formulation studies show results within the limit and compatibility test proof no reaction among Indomethacin and polymer. All evaluation parameters show results within the limit. F1 formulation containing 97.22% of drug presence and shown maximum drug discharge of 94.93% hence it is considered the best formulation where it was subjected for comparison study with marketed formulation in which it shows higher

dissolution rate as that of marketed formulation. It can be concluded that solubility of Indomethacin was enhanced by solid dispersion method.

Keywords: Indomethacin, solid dispersion, HPMC, physical mixture, kneading method, solubility enhancement.

INTRODUCTION

The pharmaceutical industry faces challenges in developing water-insoluble drug formulations because of limited solubility. Solid dispersions have been proven effective in improving drug bioavailability [1]. Formulating badly water-soluble medications is a challenge for the pharmaceutical sector. With several manufacturing techniques including solvent evaporation, spray-drying, and fusing being extensively researched, solid dispersions (SDs) have shown to be successful in enhancing medication solubility and bioavailability. Although knowledge of drug release mechanisms and optimization of these approaches have advanced, cheap and scalable continuous processing options for commercial usage still few [2]. Used in more than 15 marketed goods, the solid dispersion technique was among the most effective remedies of drug sector for raising the solvability of the insoluble medications. Among solid dispersion technology' benefits, are drug particle size reduction, enhanced wettability, and crystalline conversion to amorphous state [3]. Solid dispersions are the preparations made up of two parts, water-loving matrix and water-hating drug. The drug will be dispersed in the matrix where it can be amorphous or crystalline in nature. Some of the materials that can be used are sugars (dextrose, sucrose, sorbitol, lactose), acids (citric acid, succinic acid), polymeric materials (cellulose derivatives, PVP, PEG), insoluble or enteric polymer (Eudragit), surfactants (spans, tweens) and others. Solid dispersion offers range of application in pharmaceutical field besides solubility and absorption enhancement they also offer uniform distribution of drug, dispensing of liquid or gas substances in solid form and formulation of sustained release dosage forms by using polymers [4].

MATERIALS AND METHODOLOGY

Materials

Indomethacin, HPMC are obtained from Research lab fine chem industry, Mumbai and all ingredients used are good quality.

Methodology

Pre-formulation tests

The pre-formulation tests carried out for the pure drug Indomethacin are as follows

Solubility studies

Solubility of Indomethacin was checked in different solvents like water, alcohol, HCl, Phosphate buffer pH 7.4 [5].

Detection of melting temperature

Indomethacin melting temperature is detected by Thiele's tube method where a capillary tube was sealed in one side and Indomethacin was inserted on it. The tube is attached to a thermometer, attached to a cork fitted in the Thiele's tube contained liquid paraffin and heated [6].

Determination of λmax

λmax of Indomethacin was determined by using ethanol in the concentration of 100 µg/ ml by using UV spectrophotometer in the range of 200 – 400nm.

Standard curve

Standard calibration curve of Indomethacin was plotted as the concentration (µg/ml) v/s the absorbances of the aliquots of 5, 10, 15, 20, 25µg/ml prepared from the stock solution of Indomethacin [7].

Compatibility study

Compatibility study of Indomethacin and HPMC was carried out by subject Indomethacin and combination of Indomethacin with HPMC for FTIR at 4 cm^{-1} resolution and scanning range of 400 - 4000 cm^{-1}. Spectra of Indomethacin and HPMC was compared to that of Indomethacin [5].

Preparation of solid dispersion of Indomethacin

Solid dispersions of Indomethacin were formulated using HPMC as a polymer by physical mixture and kneading methods in different ratios of 1:1 and 1:2. The formulation of solid dispersions can be seen in Table 1.

Physical mixture

Indomethacin and HPMC were mixed in the ratio of 1:1 and 1:2 by triturating in mortar and pestle for 3-5 minutes. The powder was passed through sieve # 60 then filled into the hard gelatin capsules (Size 0) [8].

Kneading method

Solid dispersions of Indomethacin and HPMC by kneading method were prepared in the ratio of 1:1 and 1:2 by triturating in a mortar and pestle then mixture of alcohol and water is added and a coherent mass is formed which is then passed through sieve #22 to obtain granules. The granules are dried for 20 minutes at 60°C and filled into the capsule shell (Size 0) [9].

Evaluation of Solid dispersion of Indomethacin

The encapsulated solid dispersions are tested for the parameters as followed

Weight difference

Individually 20 capsules were weighed and calculate the average weight. Weight variation was determined by following formula [10].

Weight difference =(Weight of capsule-Average weight)/(Average weight) ×100

Disintegration

The capsules were place in the basket rack of dissolution test apparatus contained a medium adjusted at 37°C. duration needed for the capsule to fully disintegrate into a soft mass was noted [11].

Drug content

Randomly 10 capsules were selected and emptied, 50ml of alcohol was added, filtered, the volume was made up to 100ml, diluted, then subjected for absorbance at λmax and the drug presence was calculated [12].

***In-vitro* drug release tests**

In-vitro drug discharge tests are performed in USP Type II equipment (Paddle method) utilizing phosphate buffer pH 7.4 as a dissolution fluid, adjusted at 37 ± 0.5°C and rate of 50 rpm. Solutions are withdrawn at 10 minutes interval for 60 minutes and substituted the same by buffer. The solutions are then examined for % drug released [13].

Comparison study

Comparison study was carried out by comparing the *in-vitro* drug discharge tests of encapsulated Indomethacin solid dispersion and marketed formulation (Indomethacin capsule IP manufactured by DRUGFARM) [14].

RESULTS AND DISCUSSION

Solubility profile
Indomethacin is soluble in alcohol, phosphate buffer pH 7.4 and insoluble in water and HCl.

Melting temperature
The melting temperature on Indomethacin was determined as 160 ºC.

Determination of λmax of Indomethacin
λmax of Indomethacin was determined at 229 nm.

Standard graph
The standard graph of Indomethacin using ethanol was plotted as Concentration (µg/ml) v/s absorbance using Table 2. Regression coefficient was determined by utilizing a regression equation. Value of regression coefficient was used to calculate %CDR and the curve obeys Beer's Law. The calibration curve of Indomethacin is given in Figure 1.

Compatibility study of drug with polymers by FT-IR spectroscopy
The FT IR Spectra of Indomethacin and combination of Indomethacin and HPMC K100 shows characteristic peaks of C-N stretch amine at 1186 cm^{-1}, -C=O- (alpha and beta unsaturated) at 1713 cm^{-1} and 1712 cm^{-1}, C-H stretch alkane at 2966 cm^{-1}, C-Cl stretching at 802 cm^{-1} and Aromatic C=C stretch at 1587 cm^{-1}. The FT IR spectra prove no reaction among Indomethacin and HPMC K100 because the combination of Indomethacin and HMPC K100 shows characteristic peak of Indomethacin as shown in Figure 2, 3 and 4. Therefore, Indomethacin and HPMC K100 used are suitable with one another.

Evaluation
Post formulation evaluation of solid dispersion encapsulated hard gelatin capsule
The prepared solid dispersions were encapsulated into the capsule shell of Size 0 and tested for weight difference where the % weight difference of the dosage forms is within the limit, breakdown duration is detected around 2 min 3 secs- 3mins 14 secs and drug content was determined and it is in the range of 95.94-97.22 %, where F1 shows the maximum drug presence. The result is given in Table 3.

***In-vitro* drug discharge tests**
In-vitro drug discharge tests are carried out for 1 hour in USP Type II equipment utilizing phosphate buffer pH 7.4. The solid dispersion encapsulated in capsules shows the drug release in the range of 90.07-94.93%. The results specify that F1 shows maximum drug release of 94.93%. The result is given in Table 4 and Figure 5.

Comparison study
By comparing the *in-vitro* drug discharge of F1 and marketed formulation, F1 leads the marketed formulation. Hence, study indicates that optimized formulation proves greater dissolution as that of marketed formulation. The result is shown in Figure 6 and Table 5.

CONCLUSION

Solid dispersions of Indomethacin were formulated and evaluated for the enhancement of solubility of Indomethacin. Solid dispersions are formulated by physical mixture and kneading techniques using HPMC in the ratio of 1:1 and 1:2. Pre-formulation studies of the drug were performed, and the results were within the limit. By using the FT-IR study, compatibility was checked between the drug and the polymer, and it was shown no reaction among Indomethacin and the polymer and suitable for the formulation. Four batches are formulated, and the formulation are encapsulated in capsule shell and these capsules are tested for weight difference, disintegration and

drug presence. All parameters are in the limits. The in-vitro drug discharge tests are performed for 1 hours where the F1 with Indomethacin and HPMC in the proportion of 1:1 prepared by physical mixture method shows maximum solubility and maximum drug content and was considered the best formulation. The optimized formulation F1 was subjected for comparison study with marketed formulation where F1 shows higher dissolution rates compared to marketed formulation. Hence, it can be concluded that solid dispersion of Indomethacin has been successfully formulated and can be considered an approach to enhance the solubility of Indomethacin.

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Table 1: Formulation of Indomethacin Solid Dispersion

Formulation code	Solid dispersion	Drug and carrier ratio	Method employed
F1	IND:HPMC	1:1	Physical mixture
F2	IND:HPMC	1:2	
F3	IND:HPMC	1:1	Kneading method
F4	IND:HPMC	1:2	

Table 2: Calibration Curve of Indomethacin

Concentration (µg/ml)	Absorbance
0	0
5	0.0702
10	0.1567
15	0.2169
20	0.2873
25	0.3474

Table 3: Evaluation parameters F1-F4

Formulation code	Weight variation (gm)	Disintegration test (mins)	Drug content (%)
F1	0.115	3min:14sec	97.22
F2	0.139	2min:12sec	96.71
F3	0.112	2min:30sec	95.94
F4	0.137	2min:3sec	96.02

Table 4: *In-vitro* drug discharge studies for batches F1-F4

Duration (min)	F1 (%)	F2 (%)	F3 (%)	F4 (%)
0	0	0	0	0
10	27.5142	29.3142	29.8285	31.1142
20	39.1162	40.9182	45.2902	42.4631
30	55.3597	56.9065	67.1977	51.7674
40	70.8497	68.5411	73.1865	66.4820
50	82.2425	83.2742	80.2105	76.8414
60	94.9337	93.9094	90.0708	91.3265

Table 5: Comparison study between F1 & Marketed Formulation

Time (Min)	F1 (%)	Marketed Formulation (%)
0	0	0
10	27.5142	15.9428
20	39.1162	33.9605
30	55.3597	51.9982
40	70.8497	63.8845
50	82.2425	76.0411
60	94.9337	88.9825

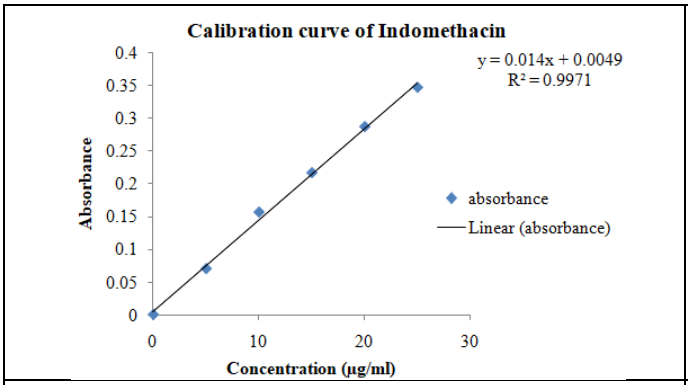


Figure 1: Calibration Curve of Indomethacin

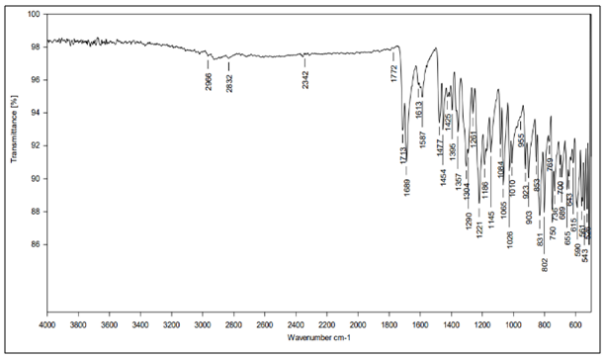


Figure 2:Indomethacin's FT-IR Spectra

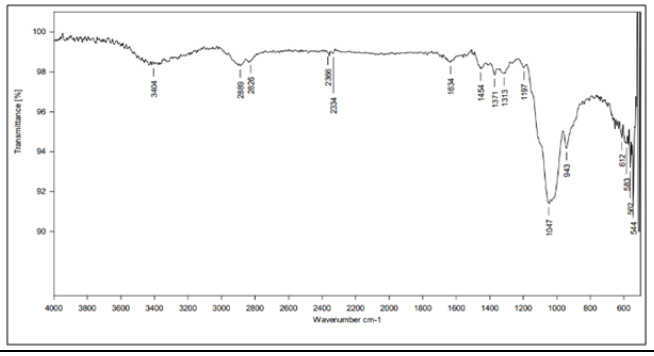


Figure 3:HPMC K100's FT-IR Spectra

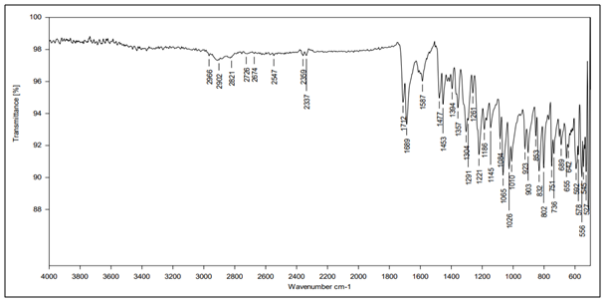


Figure 4: Indomethacin + HPMC K100's FT-IR Spectra

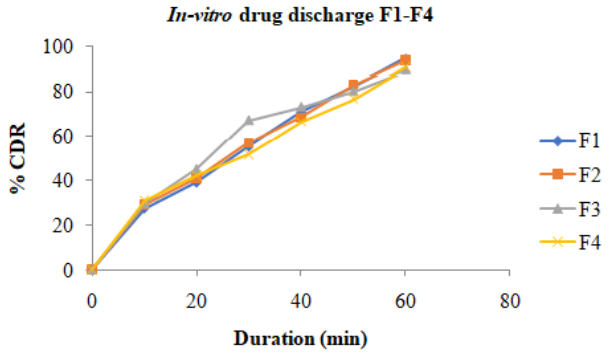


Figure 5: *In-vitro* drug discharge tests of Formulations F1-F4

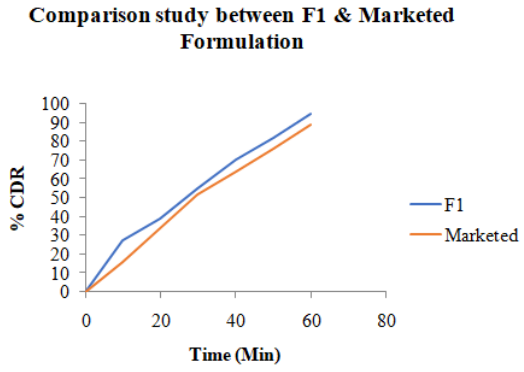


Figure 6: Comparison study between F1 and Marketed Formulation

RESEARCH ARTICLE

Diagnosis of Immuno Deficiency by using Similarity Matrix Together with Interval - Valued Fuzzy Soft Matrices

R. Hema[1]* and S. Prabha[1]

Assistant Professor, Department of Mathematics, A.V.V.M. Sri Pushpam College (Autonomous), Poondi, (Affiliated to Bharathidasan University, Tiruchirappalli), Thanjavur, Tamil Nadu, India.

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***Address for Correspondence**
R. Hema
Assistant Professor,
Department of Mathematics,
A.V.V.M. Sri Pushpam College (Autonomous), Poondi,
(Affiliated to Bharathidasan University, Tiruchirappalli),
Thanjavur, Tamil Nadu, India.
E.Mail: rhema2450@gmail.com

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ABSTRACT

We encounter many health problems in the modernization environment, which is governed by artificial intelligence. Non-communicable diseases account for the majority of mortality nowadays, and they are caused by immunological deficiencies.The majority of the serious NCDs are caused by immune deficiencies. We experience mortality as well as long-term health issues as a result. Thus, the immune system is mostly responsible for our health issues. Many primary immunodeficiency problems can be treated to strengthen the immune system.We want to diagnose the most affected person by immunodeficiency in this study. We employ an interval-valued fuzzy soft matrix alongside with a similarity matrix to give priority the immunological deficiencies severity.

Keywords: Fuzzy set, Fuzzy soft set, fuzzy soft matrix, Interval-valued fuzzy soft set, Interval-valued fuzzy soft matrix, Similarity matrix.

INTRODUCTION

Most of our real life problems often involve data which are not necessarily crisp, precise and deterministic in character due to various uncertainties associated with these problems. Fuzzy mathematics is a branch of mathematics which captures uncertainty in real life and tries to extract precise meaning from the given vague information. It is a powerful tool for decision-making systems.In 1965 Zadeh introduce the notion of fuzzy set theory. In many complicated problems arising in the fields of engineering, social sciences, economics, medical sciences, healthcare etc., involving uncertainties. Classical methods are found to be inadequate in recent times. Molodtsov pointed out

that the important existing theories viz. probability theory, fuzzy set theory, Intuitionistic Fuzzy set theory, Rough set theory etc, which can be considered as mathematical tool for dealing with uncertainties , have their own difficulties. In 1999 he initiated the novel concept of Soft Set as a new mathematical tool for dealing with uncertainties. Soft set theory, Initiated by Molodtsov is free of the difficulties present in these theories. Though all these techniques do not consider parameterization of the tools hence could not be applied truly in handling problems of uncertainties. In 2003 Maji put forward a hybrid model known as fuzzy soft set, which is a combination of soft set and fuzzy set. Matrices play an important role in the broad area. The definition of soft matrices by Cagman in 2010, and the proposal of weighted fuzzy soft set by Feng in the same year. Yong's successful application of the notion of fuzzy soft matrices in certain decision-making problems in 2011 and the extension made by Neog, Bora and Sut in 2012 further enriched this field of study. The application of fuzzy soft matrices in medical diagnosis has also been demonstrated by Lavanya M and Akila S. Mondal *et al*. and Shyamal *et al*. initiated a matrix representation of soft set and interval valued fuzzy set respectively. In Yong *et al* initiated a matrix representation of a fuzzy soft set and applied it in certain decision making problems. Borah *et al* extended fuzzy soft matrix theory and its application. In Chetia *et al* and in Rajarajeswari *et al*. defined intuitionistic fuzzy soft matrix and interval valued intuitionistic fuzzy soft matrix and its types. Also extended some operations and an algorithm for medical diagnosis. In this paper, we extended the concept of interval valued fuzzy soft matrix together with the similarity matrix.

METHODOLOGY

Step 1 : Determine the disease's symptoms
Step 2 : Form the model Interval-Valued Fuzzy Soft Matrix
Step 3: Create a set of criteria for every person based on the symptoms of the disease.
Step 4: The similarity between the model Interval-Valued Fuzzy Soft Matrixand each person's Interval-Valued Fuzzy Soft Matrix is assessed.
Step 5: The significantly similar Interval-Valued Fuzzy Soft Matrix is then obtained.
Step 6: Higher the similarity value, greater is the chance for mostly disease affected.

RESULTS AND DISCUSSION

We observe the most common symptom for immune deficiency with the help of healthcare professionals,

S_1 -Slow wound healing
S_2 - Frequent infections
S_3 -Skin issues
S_4 - Digestive issues
S_5 - High stress level
S_6 - Cold hand and feet
S_7 - Chronic fatigue
S_8 - Frequent allergies

This symptoms are relate to four persons

χ_1– John
χ_2 – Peter
χ_3- Adam
χ_4-chinchung

Let us the symptom occurrence and non-occurrence level in model interval-valued fuzzy soft matrices by the help of professional

(δ,S)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(0.2,0.5)	(0.7,0.8)	(0.3,0.5)	(0.3,0.4)	(0.4,0.6)	(0.3,0.5)	(0.7,0.9)	(0.6,0.8)
Δ_2	(0.5,0.8)	(0.2,0.3)	(0.5,0.7)	(0.6,0.7)	(0.4,0.6)	(0.5,0.7)	(0.1,0.3)	(0.2,0.4)

Symptoms - occurrence and non-occurrence for $\chi_1, \chi_2, \chi_3, \chi_4$

Interval valued fuzzy soft matrix forχ_1

(δ_1,S)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(0.4,0.6)	(0.6,0.8)	(0.4,0.6)	(0.7,0.9)	(0.2,0.4)	(0.5,0.7)	(0.5,0.8)	(0.2,0.4)
Δ_2	(0.2,0.5)	(0.7,0.9)	(0.6,0.9)	(0.3,0.5)	(0.5,0.7)	(0.7,0.8)	(0.3,0.5)	(0.3,0.6)

Interval valued fuzzy soft matrix for χ_2

(δ_2,S)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(0.2,0.4)	(0.2,0.4)	(0.3,0.7)	(0.5,0.6)	(0.5,0.7)	(0.6,0.8)	(0.2,0.4)	(0.2,0.3)
Δ_2	(0.6,0.7)	(0.4,0.6)	(0.7,0.9)	(0.6,0.8)	(0.3,0.5)	(0.3,0.4)	(0.5,0.6)	(0.3,0.5)

Interval valued fuzzy soft matrix for χ_3

(δ_3,S)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(0.5,0.6)	(0.6,0.9)	(0.7,0.9)	(0.3,0.4)	(0.5,0.6)	(0.4,0.5)	(0.5,0.6)	(0.7,0.8)
Δ_2	(0.7,0.8)	(0.6,0.8)	(0.5,0.6)	(0.5,0.7)	(0.7,0.8)	(0.6,0.7)	(0.6,0.8)	(0.3,0.5)

Interval valued fuzzy soft matrix for χ_4

(δ_4,S)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(0.8,0.9)	(0.3,0.6)	(0.4,0.6)	(0.8,0.9)	(0.5,0.8)	(0.5,0.7)	(0.3,0.6)	(0.2,0.3)
Δ_2	(0.4,0.7)	(0.5,0.8)	(0.4,0.8)	(0.5,0.7)	(0.4,0.5)	(0.3,0.4)	(0.6,0.8)	(0.2,0.5)

Case (i) Similarity between (δ ,S) and (δ_1,S)

$(\delta ,S) \bigcup' (\delta_1,S) = (\zeta_1,\gamma) = \mu_1$

(ζ_1,γ)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(.52,.80)	(.88,.96)	(.58,.80)	(.79,.94)	(.52,.76)	(.65,.85)	(.85,.98)	(.68,.88)
Δ_2	(.60,.90)	(.76,.93)	(.80,.97)	(.72,.85)	(.70,.88)	(.85,.94)	(.37,.65)	(.44,.76)

$|(\zeta_1,\gamma)| = [(.60,.90) \ (.88,.96) \ (.80,.97) \ (.79,.94) \ (.70,.88) \ (.85,.94) \ (.85,.98) \ (.68,.88)]$

$= [0.75 \quad 0.92 \quad 0.89 \quad 0.87 \quad 0.79 \quad 0.90 \quad 0.92 \quad 0.78]$

$|\mu_1| = 6.82$

$(\delta ,S) \bigcap' (\delta_1,S) = (\eta_1,\gamma) = \mu_2$

(η_1,γ)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(.08,.30)	(.42,.64)	(.12,.30)	(.21,.36)	(.08,.24)	(.15,.35)	(.35,.72)	(.12,.32)
Δ_2	(.10,.40)	(.14,.27)	(.30,.63)	(.18,.35)	(.20,.42)	(.35,.56)	(.03,.15)	(.12,.32)

$|(\eta_1,\gamma)| = [(.10,.40) \ (.42,.64) \ (.30,.63) \ (.21,.36) \ (.20,.42) \ (.35,.56) \ (.35,.72) (.12,.32)]$

$[0.25 \quad 0.53 \quad 0.47 \quad 0.29 \quad 0.31 \quad 0.46 \quad 0.54 \quad 0.22]$

$|\mu_2| = 3.07$

$\Gamma (\delta ,S),(\delta_1,S) = \frac{(\delta ,S)}{(\delta 1,S)} = \frac{3.07}{6.82} = 0.45$

Case(ii)Similarity between (δ ,S) and (δ_2,S)

$(\delta ,S) \bigcup' (\delta_2,S) = (\zeta_2,\gamma) = \mu_3$

(ζ_2,γ) S_1 S_2 S_3 S_4 S_5 S_6 S_7 S_8

Δ_1 (.36,.70) (.79,.90) (.51,.85) (.65,.76) (.82,.92) (.72,.90) (.76,.94) (.80,.92)

Δ_2 (.80,.94) (.52,.72) (.85,.97) (.84,.94) (.88,.96) (.65,.82) (.55,.72) (.76,.88)

$|(\zeta_2,\gamma)|=$ (.80,.94) (.79,.90) (.85,.97) (.84,.94) (.88,.96) (.72,.90) (.76,.94) (.80,.92)

0.87 0.85 0.91 0.89 0.92 0.81 0.85 0.86

$|\mu_3| = 6.96$

$(\delta,S) \cap' (\delta_2,S) = (\eta_2,\gamma) = \mu_4$

(η_2,γ) S_1 S_2 S_3 S_4 S_5 S_6 S_7 S_8

Δ_1 (.04,.20) (.14,.32) (.09,.35) (.15,.24) (.20,.42) (.18,.40) (.14,.36) (.12,.24)

Δ_2 (.30,.56) (.08,.18) (.35,.63) (.36,.56) (.12,.30) (.15,.28) (.05,.18) (.06,.20)

$|(\eta_2,\gamma)|=$ (.30,.56) (.14,.32) (.35,.63) (.36,.56) (.20,.42) (.18,.40) (.14,.36) (.12,.24)

0.43 0.230.490.46 0.31 0.290.25 0.18

$|\mu_4| = 2.64$

$\Gamma(\delta,S),(\delta_2,S) = \frac{(\delta,S)}{(\delta 2,S)} = \frac{2.64}{6.96} = 0.39$

Case (iii) Similarity between (δ,S) and (δ_3,S)

$(\delta,S) \cup' (\delta_3,S) = (\zeta_3,\gamma)$

(ζ_3,γ) S_1 S_2 S_3 S_4 S_5 S_6 S_7 S_8

Δ_1 (.85,.94) (.88,.98) (.79,.95) (.51,.64) (.7,.84) (.58,.75) (.85,.96) (.88,.96)

Δ_2 (.85,.96) (.68,.86) (.75,.88) (.80,.91) (.88,.92) (.8,.91) (.64,.86) (.44,.7)

$|(\zeta_3,\gamma)| =$ (.85,.96) (.88,.98) (.79,.95) (.80,.91) (88,.92) (.8,.91) (.85,.96) (.88,.96)

0.91 0.93 0.87 0.86 0.90 0.86 0.91 0.92

$|\mu_5|= 7.16$

$(\delta,S) \cap' (\delta_3,S) = (\eta_3,\gamma)$

(η_3,γ) S_1 S_2 S_3 S_4 S_5 S_6 S_7 S_8

Δ_1 (.10,.30) (.42,.72) (.21,.42) (.09,.16) (.20,.36) (.12,.25) (.35,.54) (.42,.64)

Δ_2 (.35,.64) (.12,.24) (.25,.45) (.30,.49) (.28,.48) (.30,.49) (.06,.24) (.06,.20)

$\mu_6=$ (.35,.64) (.42,.72) (.25,.45) (.30,.49) (.28,.48) (.30,.49) (.35,.54) (.42,.64)

0.50 0.57 0.35 0.40 0.38 0.40 0.45 0.53

$|\mu_6|= 3.58$

$\Gamma(\delta,S),(\delta_3,S) = \frac{(\delta,S)}{(\delta 3,S)} = \frac{3.58}{7.16} = 0.5$

Case (iv) Similarity between (δ,S) and (δ_4,S)

$(\delta,S) \bigcup' (\delta_4,S) = (\zeta_4,\gamma)$

(ζ_4,γ)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(.84,.95)	(.79,.92)	(.58,.80)	(.86,.94)	(.70,.92)	(.72,.90)	(.79,.96)	(.80,.98)
Δ_2	(.70,.94)	(.60,.86)	(.70,.94)	(.80,.91)	(.64,.80)	(.65,.82)	(.64,.86)	(.36,.70)

$|(\zeta_4,\gamma)| =$

(.84,.95)	(.79,.92)	(.70,.94)	(.86,.94)	(70,.92)	(.72,.90)	(.79,.96)	(.80,.98)
0.90	0.86	0.82	0.900.81	0.75	0.88	0.77	

$|\mu_7| = 7.57$

$(\delta,S) \bigcap' (\delta_4,S) = (\eta_4,\gamma)$

(η_4,γ)	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8
Δ_1	(.16,.45)	(.21,.48)	(.12,.30)	(.21,.36)	(.20,.48)	(.15,.35)	(.21,.54)	(.12,.24)
Δ_2	(.20,.56)	(.10,.24)	(.20,.56)	(.30,.49)	(.16,.30)	(.15,.28)	(.06,.24)	(.04,.20)

$\mu_6 =$

(.20,.56)	(.21,.48)	(.20,.56)	(.30,.49)	(.20,.48)	(.15,.35)	(.21,.54)	(.12,.24)
0.38	0.350.38	0.40	0.34	0.25	0.38	0.51	

$|\mu_8| = 2.66$

$\Gamma(\delta,S),(\delta_4,S) = \frac{(\delta,S)}{(\delta 4,S)} = \frac{2.66}{7.57} = 0.35$

CONCLUSION

We encounter many persons in our everyday lives these days who are frequently afflicted by ailments. In this study we choose and discuss about the immunodeficiency disease. Immunodeficiency diseases are conditions in which the immune system's ability to fight infections and diseases is compromised or entirely absent. Immune deficiencies and a lack of knowledge on how to protect oneself from such issues are the causes When fuzzy soft matrices are used in immunodeficiency impact analysis, predictive modeling and risk assessment are improved, which results in more leading treatments and improved patient healthcare. People with immunological deficiencies are most susceptible to illness than the average individual. Our study examines the disease in order to provide protection. Using the Similarity relation together with Interval-Valued Fuzzy Soft Matrices from the calculation and depicts of the figure, Adam highly affected by the immune deficiency disease. Future studies can improve these models by combining machine learning methods with actual clinical datasets to get even more accurate results. To diagnosis at earlier stage and find out disease severity level to protect from that disease is better for our healthcare. To concentrate highly disease affected person to give the exact extra caring to protect and choose the appropriate medicine. This study can be an increase understanding of the immune deficiencies among the public and health care. It would be better to have access to prompt and appropriate immunodeficit treatment. In our calculation diagnosis we find out the highly

disease affected person. In this method very effectively diagnose the disease affected person. Adam highly affected by the immunodeficiency disease.

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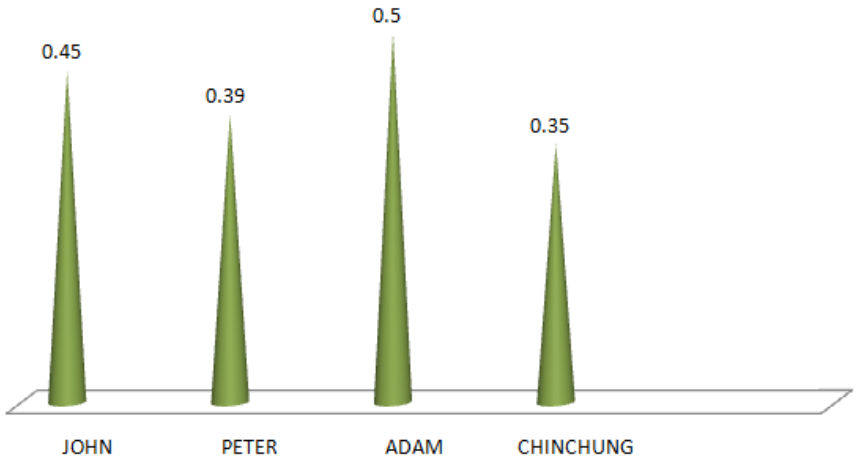


Fig.1: Diagnosis of Immuno Deficiency

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RESEARCH ARTICLE

Ethnobotanical Survey of Wild Edible Plants Sold in the Market of Dimapur, Nagaland

Temsutola[1]*, Jichule Seb[1] and Masakala[1]

Assistant Professor, Department of Botany, Immanuel College, Dimapur, (Affiliated to Nagaland University), Nagaland, India.

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***Address for Correspondence**
Temsutola
Assistant Professor,
Department of Botany,
Immanuel College, Dimapur,
(Affiliated to Nagaland University),
Nagaland, India.
E.Mail: temsutolawalling@immanuelcollege.in

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ABSTRACT

A market-based survey on Wild Edible Plants (WEPs) was conducted in the local markets of Dimapur from January 2024 to March 2025. Wild edible plants (WEPs) hold significant importance for rural communities across numerous developing nations. The emphasis on WEPs has significantly increased in recent decades, particularly within rural and tribal communities. This study aim to record the WEPs found in local markets, including their seasonal availability, market price and the conservation status. Market survey, semi-structured questionnaire and interview were conducted for the data collection. A total of 59 WEPs representing 53 genera and 40 families were recorded during the present survey. Among these, 21 species were found to be available year round, while *Canarium strictum* was the only species classified under Endangered Category (EN).

Keywords: Market survey, Ethnobotany, Wild Edible Plants, Dimapur

INTRODUCTION

Nagaland, nestled in India's north-eastern corridor, is recognized as a critical biogeographic zone with exceptional ethnobotanical wealth. Nagaland is inhabited by more than 17 major tribes, with a diverse spectrum of traditional ecological knowledge (TEK) rooted in the use of plant-based resources. Wild edible plants (WEPs) serve as indispensable components of daily diets, traditional medicine, seasonal foraging practices, and local economies. This ethnobotanical tradition not only sustains nutritional security but also fosters cultural identity and intergenerational knowledge transmission (Mao, 2014). Jigme and Yangchen (2022) defined WEPs as uncultivated plant species gathered from natural habitats. WEPs are an important source of food in India and have a significant place in the

dietary habits of small and marginal farmer's families and forest dwelling communities during the periods of food scarcity (Beluhan and Ranogajei, 2010). The food habits of tribal communities are typically shaped by the seasonal availability of food and its nutritional content, resulting in a traditional reliance on their own foraged resources. Dimapur, the state's largest urban hub of Nagaland, presents a unique ethnobotanical interface. Unlike the most homogenized tribal districts, Dimapur functions as a socio-ecological crossroad where rural harvests meet urban consumption. The city's bustling local markets, such as Super Market and New Market, act as vital points of aggregation for WEPs collected from forest areas across districts like Peren, Chümoukedima, Mokokchung and Wokha. Furthermore, selling WEPs in markets like Dimapur offers an important avenue for livelihood generation, particularly for women and economically weaker section. Despite this strategic role, comprehensive documentation of WEPs and the market price remains scarce. Documenting wild edible plants utilized by indigenous groups of this region offers valuable insights for planning, conservation, and further research. This study intends to perform an ethnobotanical survey of wild edible plants found in the market area of Dimapur, Nagaland, concentrating on their identification, uses, importance to local communities and the global conservation status. It seeks to record this knowledge as a means of protecting cultural heritage and promoting the sustainable utilization of indigenous plant resources. Furthermore, the findings will highlight the role of WEPs in local diets and their valuable role in contributing to both food security and primary healthcare needs.

MATERIALS AND METHODS

The market survey for documenting wild edible plants through ethnobotanical methods was conducted over the period from January 2024 to March 2025. The markets under study area includes Tuesday market at Lengrijan (Lat. 25.904573, Long. 93.690006) Wednesday market at Supermarket (Lat. 25.905888, Long. 93.739879), New market (Lat. 25.908116, Long. 93.722589) Monday Market at Padampukhuri (25.901349, Long. 93.771217) and City tower junction (Lat. 25.911360, Long. 93.720541) . The survey engaged 98 shopkeepers, serving as key informants for the study. The data were collected through one-on-one interaction, semi-structured questionnaire, and personal observation. Before commencing the survey ,Prior informed consent was obtained from each informants. The plant species collected during the study were identified using reference materials, including the Medicinal plant repertoire: A perspective of Biogeographical gateway of India, Nagaland Bio Resource Mission, Conservation status was updated from IUCN Red List of threatened Species and the botanical names were updated through POWO and Eflora of India. All the plants were photographed using PocoF1 phone and DSLR D5200 Nikon camera.

RESULT

The market survey results in the documentation of 59 wild edible plants sold in the main local markets of Dimapur. The documented 59 WEPs belong to 40 families and 53 genera (Table 1). Among the families, Phyllanthaceae is the dominant family with 4 species, followed by Anacardiaceae, Fabaceae, Moraceae, and Solanaceae with 3 species each, Amaranthaceae, Apiaceae, Araceae, Asteraceae, Cucurbitaceae, Lauraceae, Poaceae, and Zingiberaceae with 2 species each and the rest of the families were represented by one species (Figure 1). The plant belongs to different habits where tree occupies the highest with 22 species, 19 were herbs, 8 were shrubs, 4 were climbers, 3 fungi and 2 grasses (Figure 2). Out of 59 species, 51 were under the division Angiosperms with 42 species under the clade eudicots, 9 species under monocots, and 3 species under Magnoliids, 3 species were fungi, and 1 species each belongs to Pteridophytes and gymnosperms respectively (Table 1 and 2). Amongst the edible parts sold in the market, fruits were the highest which is followed by leaves (Table 1). All 59 wild edible plants were categorised with details including their botanical names, common names, families, divisions, growth habits and traditional uses (Table 3). Of the 59 WEPs recorded from market surveys, 21 plant species were found to be available throughout the year and the remaining were seasonal (Table 4). The price ranges between Rs.10-Rs.30 for leafy vegetables, Rs.20 – Rs.100 for fruits and Rs.100- Rs.150 for wild mushroom (Table 4). Based on the IUCN Red List of Threatened Species, one species fell under the Endangered Category (EN), 26 species were listed as Least Concern (LC), one was categorised under Data

Deficient (DD) and the rest were not evaluated (NE) [Table 4]. Table 5 displays photographs of several WEPs documented from Dimapur local market.

DISCUSSION

The identification of 59 wild edible plants (WEPs) sold in the market of Dimapur highlights the significant role of wild flora in the daily diet of local population. The traditional culinary practices of the Nagas in Dimapur are deeply shaped by the availability of wild edible plants found in the region. It not only add to the delicacy of Naga cuisine but also helps the local to earn their daily livelihood. The present market survey revealed women playing the leading role in marketing wild edible plants in Dimapur, while men were mainly involved in foraging and packaging activities. Among the recorded plant species, trees were most prevalent (38%), followed by herbs (33%), shrubs (14%), Climbers (7%), fungi (5%) and grasses (3%). This observation supports the findings of Gogoi *et. al.,* (2023) which showed the predominance of trees in their study. The dominance of trees may be attributed to their abundance in nature as trees has greater resilience and adaptability to different environmental conditions. Angiospermic plants were the most dominant, comprising 54 of the documented species. Kar and Borthakur (2007) also reported the dominant of angiospermic plants in their study on the market survey of Karbi Anglong, Assam. The incredible diversity of angiospermic plants must have attributed to their global dominance. The different parts of the plants are consumed, out of which fruits were the most frequently used parts sold in the market. Ripe fruits are primarily eaten raw, though some species are prepared through cooking and consumed as part of vegetable dishes. Similar findings were demonstrated by Asfaw *et al* (2023), and Pai*et. al*., (2019) in their survey on wild edible plants. The findings of this study promotes the high consumption and utilization of fruits locally. The commonly available fruits were primarily utilised for preparing fermented juices, traditional beverages and pickles. Deb et.al., (2013) reported the use of several wild edible species, including *Musa acuminata, Solanum torvum, Bambusa tulda, Diplazium esculentum, Colocasia esculenta, Centella asiatica, Ipomoea aquatic, Lasiaspinosa* among the ethnic communities of Tripura. Comparable results were also reported by Doni and Gajurel (2020) in their studies on diversity of wild edible plants used by Galo tribe of Arunachal Pradesh. Plants such as *Amaranthus viridis, Amomum dealbatum, Artocarpus heterophyllus, Centella asiatica, Clerodendrum glandulosum, Dioscorea esculenta, Diplazium esculentum, Houttuyniacordata,* and *Zanthoxylum rhetsa* were found to use as vegetables in both the states. The pseudostem of wild banana eaten as vegetable in the local markets of Dimapur was also observed in the study of Meitei *et. al*., (2022). This demonstrates the intercultural exchange of knowledge among the different community through selling in the markets of different states and also exhibit harmonial coexistence of different ethnic groups The highly consumed plants were *Alpinianigra, Bambusa tulda, Bambusa balcooa, Centella asiatica, Clerodendrum glandulosum, Colocasia esculenta, Diplazium esculentum, Gnetum gnemon, Livistonajen kinsiana, Moringa oleifera, Musa acuminata, Parkia timoriana, Rhus chinensis, Phyllanthus emblica, Solanum violacum, and Zanthoxy lumrhetsa* and therefore showed high market demand. The different parts of the plants were usually sold in the form of bunch, group or bundles. Each bunch of leafy vegetables were sold by rs 30 to rs 50. Among the wild vegetables sold in the market, mushroom priced the highest as it is rarely available and also difficult to collect from the forest. The price of wild edible plants differ from market to market, depending on the supply and demand. 29 WEPs sold in the market were available throughout the year, with the remaining species being seasonally available based on favourable growing seasons. As per the IUCN red list of threatened species, it is found that only 1 species namely *Canarium strictum* were under endangered category. *Canarium strictum*were commonly eaten by many ethnic groups in dried form after boiling. Although only one species is classified as threatened, it is important to conserved and used sustainably for the future generation.

CONCLUSION

Through this study, the biodiversity richness of the region is evident in the variety of wild edible plants available in the local markets of Dimapur. Wild-collected plants from natural habitats hold significant importance for rural populations. Beyond addressing food security challenges, it also fosters income opportunities and encourages

sustainable resource utilization. Additionally, the present study plays a role in preserving the traditional knowledge of the aboriginal population.

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Table 1: Categorization of the identifies plants according to their taxonomic divisions

Plant division	Family	Genera	Species
Angiosperms	25	48	54
Gymnosperms	1	1	1
Pteridophytes	1	1	1
Fungi	3	3	3
Total	40	53	59

Table 2: Classification of documented Angiospermic plants into different clade

Angiospermic plants	Magnoliids	Eudicots	Monocots
	3	42	9

Table 3: List of Botanical name, family, local name, habit and uses

Sl. No.	Botanical Name	Common Name	Family	Clade/ Division & Habit	Part used and Uses
1.	*Acmella oleracea* (L.) R.K.Jansen	Toothache plant	Asteraceae	Eudicots Herb	Leaves Vegetable
2.	*Alpinia nigra* (Gaertn.) B.L. Burtt.	Black galangal	Zingiberaceae	Monocots Herb	Shoot Vegetable
3.	*Amaranthus viridis* L.	Green amaranth	Amaranthaceae	Eudicots Herb	Leaves Vegetable
4.	*Amomum dealbatum* Roxb.	East Himalayan Cardamom	Zingiberaceae	Monocots Herb	Young inflorescence Vegetable
5.	*Antidesma acidum* Retz.	Sour Currant Shrub	Phyllanthaceae	Eudicots Shrub	Leaves Vegetable
6.	*Artocarpus heterophyllus* Lam.	Jack fruit	Moraceae	Eudicots Tree	Fruit, Seed Eaten raw, Vegetable
7.	*Artocarpus lacucha* Buch.-Ham.	Monkey jack	Moraceae	Eudicots Tree	Fruit, seeds Eaten raw, Vegetable
8.	*Auricularia auricular-judae (Bull.) J.Schrot.*	Jelly ear	Auriculariaceae	Fungi	Entire fruiting body, Vegetable
9.	*Baccaurea ramiflora* Lour.	Burmese grape	Phyllanthaceae	Eudicots Tree	Fruits Eaten raw
10.	*Bambusa tulda* Roxb.	Indian timber bamboo	Poaceae	Monocots Grass	Young shoots vegetable
11.	*Bambusa balcooa* Roxb.	Balcooa bamboo	Poaceae	Monocots Grass	Young shoots vegetable,
12.	*Begonia roxburghii* A. DC.	East Himalayan Begonia	Begoniaceae	Eudicots Herb	Leaves, stem Vegetable
13.	*Blumea lanceolaria* (Roxb.) Druce	Lanceleaf Blumea	Asteraceae	Eudicots Herb	Leaves Vegetable
14.	*Canarium strictum* Roxb.	Black dammar	Burseraceae	Eudicots Tree	Fruit Eaten raw

15.	*Centella asiatica* (L.) Urban	Indian pennywort	Apiaceae	Eudicots Herb	Entire plant Vegetable
16.	*Chenopodium album* L.	Wild spinach	Amaranthaceae	Eudicots Herb	Leaves Vegetable
17.	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & C.H.Eberm.	Indian bay leaf	Lauraceae	Magnoliids Tree	Leaves Spices, beverages
18.	*Clerodendrum glandulosum* Lindl.	East Indian glory bower	Lamiaceae	Eudicots Shrub	Leaves Vegetable
19.	*Colocasia esculenta* (L.) Schott	Taro	Araceae	Monocots Herb	Leaves, Stem Vegetable
20.	*Crotalaria spectabilis* Roth	Showy rattlebox	Fabaceae	Eudicots Herb	Flowers Vegetable
21.	*Dillenia indica* L.	Elephant apple	Dilleniaceae	Eudicots Tree	Fruits Eaten raw
22.	*Dioscorea esculenta* (Lour.) Burkill	Lesser yam	Droscoreaceae	Monocots Climber	Tubers Vegetable
23.	*Diplazium esculentum* (Retz.) Sw.	Vegetable fern	Athyriaceae	Pteridophytes Herb	Leaves, young shoot Vegetable
24.	*Docynia indica* (Wall.) Decne	Wild apple	Rosaceae	Eudicots Tree	Fruits Eaten raw, fermented juice
25.	*Elatostema sesquifolium* (Reinw. ex Blume) Hassk	Entire leaf Elatostema	Urticaceae	Eudicots Herb	Leaves Vegetable
26.	*Ficus religiosa* L.	Sacred fig	Moraceae	Eudicots Tree	Young shoots Vegetable
27.	*Garcinia lanceifolia* Roxb.	Lanceleaf garcinia	Clusiaceae	Eudicots Shrub	Fruits Eaten raw, pickle
28.	*Gnetum gnemon* L.	Gnetum/ joint fir	Gnetaceae	Gymnospermae Tree	Leaves, fruits Vegetable
29.	*Hodgsonia macrocarpa (Blume) Cogn.*	Lard seed	Cucurbitaceae	Eudicots Climber	Nuts Vegetable
30.	*Houttuynia cordata Thunb.*	Fish mint	Saururaceae	Magnoliids Herb	Leaves, roots Vegetable
31.	*Ipomoea aquatica* Forssk.	Water spinach	Convolvulaceae	Eudicots Herb	Young shoots Vegetable
32.	*Lasia spinosa* (L.) Thwaites	Spiny lasia	Araceae	Monocots Herb	Young shoots Vegetable
33.	*Litsea citrata Blume*	Mountain pepper	Lauraceae	Magnoliids Tree	Fruits Vegetable
34.	*Livistona jenkinsiana* Griff.	Fan palm	Arecaceae	Monocots Palm	Fruits Eaten raw
35.	*Momordica charantia* Linn. var. abbreviata Ser.	Wild bitter gourd	Cucurbitaceae	Eudicots Climber	Fruits Vegetable
36.	*Moringa oleifera* Lam.	Drumstick tree	Moringaceae	Eudicots	Fruits, flowers

				Tree	Vegetable
37.	*Musa acuminata* Colla	Banana	Musaceae	Monocots Herb	Inflorescence, Pseudostem Vegetable
38.	*Oenanthe javanica* (Blume) DC.	Water celery	Apiaceae	Eudicots Herb	Young shoots Vegetable
39.	*Olax imbricata*	South Asian olax	Olacaceae	Eudicots Shrub	Leaves Vegetable
40.	*Parabaena sagittata* Miers ex Hook.f. & Thomson	Arrowhead vine	Menispermaceae	Eudicots Climber	Leaves Vegetable
41.	*Parkia timoriana* (DC.) Merr.	Tree bean	Fabaceae	Eudicots Tree	Seeds Vegetable
42.	*Phlogacanthus thyrsiformis* (Roxb. ex Hardw.) Mabb.	Red flame Acanthus	Acanthaceae	Eudicots Shrub	Inflorescences Vegetable
43.	*Phyllanthus acidus* (Linn.) Skeels	Star gooseberry	Phyllanthaceae	Eudicots Tree	Fruits Eaten raw
44.	*Phyllanthus emblica* Linn.	Indian gooseberry	Phyllanthaceae	Eudicots Tree	Fruits Eaten raw
45.	*Plantago major L.*	Broadleaf plantain	Plantaginaceae	Eudicots Herb	Leaves Vegetable
46.	*Rhus chinensis* Mill.	Nutgall tree	Anacardiaceae	Eudicots Tree	Seeds Eaten raw
47.	*Rhynchotechum ellipticum* (Wall. ex Dietrich) A.DC.	Elliptic Rhynchotechum	Gesneriaceae	Eudicots Shrub	Leaves Vegetable
48.	*Schizophyllum* commune *Fr*	Split-gill mushroom	Schizophyllaceae	Fungi	Entire fruiting body Vegetable
49.	*Solanum nigrum* L.	Black nightshade	Solanceae	Eudicots Herb	Leaves Vegetable
50.	*Solanum torvum* Sw.	Devil's fig	Solanaceae	Eudicots Shrub	Fruits Vegetable
51.	*Solanum violacum* Ortega	Indian nightshade	Solanaceae	Eudicots Shrub	Fruits Vegetable, pickle
52.	*Spondias dulcis* L.	April Plum	Anacardiaceae	Eudicots Tree	Fruits Eaten raw, pickle
53.	*Spondias pinnata* (L.f.) Kurz.	Hog plum	Anacardiaceae	Eudicots Tree	Fruits Eaten raw, beverages
54.	*Tamarindus indica* L.	Tamarind tree	Fabaceae	Eudicots Tree	Fruits Eaten raw, pickle
55.	*Terminalia chebula* Retz.	Chebulic myrobalan	Combretaceae	Eudicots Tree	Fruits Eaten raw
56.	*Termitomyces heimii* Natarajan	Termites mushroom	Lyophyllaceae	Fungi	Entire fruiting body Vegetable
57.	*Trevesia palmata* (Roxb. ex	Snowflake tree	Araliaceae	Eudicots	Inflorescences

	Lindl.) Vis.			Tree	Vegetable
58.	*Zanthoxylum rhetsa* (Roxb.) DC.	Indian prickly ash	Rutaceae	Eudicots Tree	Leaves Vegetable
59.	*Ziziphus mauritiana* Lam.	Indian jujube	Rhamnaceae	Eudicots Tree	Fruits Eaten raw, pickle

Table 4: List of WEPs, price, seasonal availability and conservation status

Sl. No.	Botanical name	Price range in rupees	Availability	Conservation Status
1.	*Acmella oleracea* (L.) R.K.Jansen	10 (per bundle)	Throughout the year	NE
2.	*Alpinia nigra* (Gaertn.) B.L. Burtt.	20-30 (per bundle)	Throughout the year	LC
3.	*Amaranthus viridis* L.	10-20	February- April	NE
4.	*Amomum dealbatum* Roxb.	50-100	February- May	DD
5.	*Antidesma acidum* Retz.	20-30	Throughout the year	LC
6.	*Artocarpus heterophyllus* Lam.	20-50 (per piece)	March- July	NE
7.	*Artocarpus lacucha* Buch.-Ham.	20-50 (Per piece)	March- July	NE
8.	*Auricularia auricular-judae (Bull.) J.Schrot.*	100-150	April- June	NE
9.	*Baccaurea ramiflora* Lour.	30-50	June - August	LC
10.	*Bambusa tulda* Roxb.	50-100	April – July Fermented Bamboo – Throughout the year	NE
11.	*Bambusa balcooa* Roxb.	50-100	April – July Fermented Bamboo – Throughout the year	NE
12.	*Begonia roxburghii* A. DC.	20-50	Throughout the year	NE
13.	*Blumea lanceolaria* (Roxb.) Druce	30-50	Throughout the year	NE
14.	*Canarium strictum* Roxb.	100 per	March- May	EN
15.	*Centella asiatica* (L.) Urban	30-50	January- May	LC
16.	*Chenopodium album* L.	20-50	March- May	NE
17.	*Cinnamomum tamala* (Buch.-Ham.) T.Nees & C.H.Eberm.	10	Throughout the year	LC
18.	*Clerodendrum glandulosum* Lindl.	20-50	February-April	NE
19.	*Colocasia esculenta* (L.) Schott	10-20 (for young shoots) 100 (for tubers)	Throughout the year	LC
20.	*Crotalaria spectabilis* Roth	50	October to March Dried flower – Throughout the year	NE
21.	*Dillenia indica* L.	20-50	September-December	LC
22.	*Dioscorea esculenta* (Lour.) Burkill	30-50	November - March	NE
23.	*Diplazium esculentum* (Retz.) Sw.	20-50	Throughout the year	LC
24.	*Docynia indica* (Wall.) Decne	10-50	Throughout the year	NE
25.	*Elatostema sesquifolium* (Reinw. ex Blume) Hassk	30-30	Throughout the year	NE

26.	*Ficus religiosa* L.	30-50	February-March	LC
27.	*Garcinia lanceifolia* Roxb.	30-50	April-May	LC
28.	*Gnetum gnemon* L.	30-50	April - July	LC
29.	*Hodgsonia macrocarpa (Blume) Cogn.*	100 (4 nuts)	February- May	NE
30.	*Houttuynia cordata Thunb.*	20-50	Throughout the year	NE
31.	*Ipomoea aquatica* Forssk.	30-50	Throughout the year	LC
32.	*Lasia spinosa* (L.) Thwaites	20-50	March- July	LC
33.	*Litsea citrata Blume*	50	March- June	NE
34.	*Livistona jenkinsiana* Griff.	50-100	September - January	NE
35.	*Momordica charantia* Linn. var. abbreviata Ser.	30-50	February-	NE
36.	*Moringa oleifera* Lam.	30-50	February-April	LC
37.	*Musa acuminata* Colla	50-100	January- June	LC
38.	*Oenanthe javanica* (Blume) DC.	20-50	Throughout the year	LC
39.	*Olax imbricata*	20-30	January-April	LC
40.	*Parabaena sagittata* Miers ex Hook.f. & Thomson	20-30	March- May	NE
41.	*Parkia timoriana* (DC.) Merr.	50 (for 15 piece)	January- May	NE
42.	*Phlogacanthus thyrsiformis* (Roxb. ex Hardw.) Mabb.	20-50	February- March	NE
43.	*Phyllanthus acidus* (Linn.) Skeels	20-50	June- August	NE
44.	*Phyllanthus emblica* Linn.	25 Per kg	June- August	LC
45.	*Plantago major L.*	20-30	March-May	LC
46.	*Rhus chinensis* Mill.	50-100	Throughout the year	LC
47.	*Rhynchotechum ellipticum* (Wall. ex Dietrich) A.DC.	50-100	October - December	NE
48.	*Schizophyllum* commune *Fr*	100-150	May - June	NE
49.	*Solanum nigrum* L.	20-50	March- May	NE
50.	*Solanum torvum* Sw.	20-50	Throughout the year	NE
51.	*Solanum violacum* Ortega	30	Throughout the year	NE
52.	*Spondias dulcis* L.	50-100	August - March	LC
53.	*Spondias pinnata* (L.f.) Kurz.	50-100	August - March	LC
54.	*Tamarindus indica* L.	100 Per kg	March-April	NE
55.	*Terminalia chebula* Retz.	50	Throughout the year	LC
56.	*Termitomyces heimii* Natarajan	100-150	May- June	NE
57.	*Trevesia palmata* (Roxb. ex Lindl.) Vis.	30-50	February- March	LC
58.	*Zanthoxylum rhetsa* (Roxb.) DC.	30-50	Throughout the year	LC
59.	*Ziziphus mauritiana* Lam.	20-50	December –February	LC

Table 5: Photograph A to I- Some WEPs sold in different markets of Dimapur

 A	 B	 C
 D	 E	 F
 G	 H	 I

RESEARCH ARTICLE

Next-Generation Approaches to Bone Fracture Classification

S.Hareesh[1]* and S.Rethinavalli[2]

[1]Research Scholar, PG and Research Department of Computer Science, Shrimati Indira Gandhi College, (Affiliated to Bharathidasan University) Tiruchirappalli, Tamil Nadu, India.
[2]Research Supervisor, PG and Research Department of Computer Science, Shrimati Indira Gandhi College, (Affiliated to Bharathidasan University) Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
S.Hareesh
Research Scholar,
PG and Research Department of Computer Science,
Shrimati Indira Gandhi College,
(Affiliated to Bharathidasan University)
Tiruchirappalli, Tamil Nadu, India.
E.Mail: hareeshphd0@gmail.com

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ABSTRACT

This compendium paper offers a thorough examination of the most recent developments in bone fracture detection and classification methodologies, using technologies in AI and medical imaging. This paper conducts a systematic review and analysis of cutting-edge techniques, such as computer-aided diagnosis systems, deep learning algorithms, and innovative image processing methods, in response to the growing need for accurate fracture diagnosis. This survey examines the strengths and limitations of various approaches, highlighting their adaptability to different imaging sources, including Magnetic Resonance Imaging, Computed Tomography, and X-rays. Furthermore, the paper addresses potential future orientations, challenges, and emerging trends in the field, with the objective of assisting researchers, healthcare professionals, and technologists in the creation of more dependable and durable tools for bone fracture detection and classification.

Keywords: Convolutional Neural Network (CNN), Image Processing, Classification, Machine Learning, Bone Fracture.

INTRODUCTION

Bone fractures are a substantial global health concern, resulting in substantial healthcare costs and the annual impact on millions of individuals. Effective treatment planning and patient outcomes necessitate accurate and timely fracture diagnosis. Advanced technologies help to improve efficiency and precision in detecting the bone fracture and its classification. The conventional diagnostic methods usually use medical images, which have transformed to a

new dimension using AI which has shown significant potential for diagnosing fractures. The process of fracture assessment is subjective and potentially susceptible to human error due to the significant dependence on the expertise of radiologists in conventional methods. In order to confront these obstacles, researchers have progressively implemented diagnosis using DL algorithms. These technologies have exhibited extraordinary capabilities in identification and classification of bone fractures. For example, Sharma *et al*. (2019) [1] have demonstrated the efficacy of CNNs in the precise identification of fracture patterns in X-ray images, thereby expediting the diagnostic workflow and enhancing diagnostic accuracy. Despite the extraordinary advancements made in this field, there are still opportunities and challenges that require further investigation. The integration of these technologies into clinical practice, the necessity of large and diverse datasets for the training of robust models, and the variability in fracture patterns are among the critical aspects that necessitate your attention. The purpose of this survey is to critically evaluate the current literature and illuminate the potential opportunities for future research and development, while also providing a comprehensive overview of the most recent developments in bone fracture detection and classification. This survey enhances the collective comprehension of how technology can further improve fracture diagnosis, thereby enhancing patient care and outcomes, by synthesising the current state of the field.

Bone Fracture Types

Fractures are classified according to a variety of criteria to describe their characteristics, which is essential for comprehending and treating bone injuries. The severity of the injury, the location within the body, the morphology or shape of the fracture, the presence of an external wound, and the stability of the fracture after aligning the fractured bone fragments are all included in these criteria[2]. The primary distinction in fracture classification is between closed fractures, which are characterised by the preservation of the skin, and open fractures, which are characterised by the rupture of the skin. Due to the bone's exposure to the external environment, open fractures have high risk of infections and complications. Long bone fractures, such as those in the femur, humerus, and tibia, can be categorised according to the forces that produce them [3]. Stress fractures are caused by repetitive loading or overuse, whereas simple and comminuted fractures are indicative of varying degrees of bone fragmentation that result from a singular injury. Comprehending these classifications is essential for the precise diagnosis of fractures and the development of treatment strategies that are tailored to the unique characteristics of each injury. The description suggests that Figure 1 demonstrates the characteristics of bone fractures, identifying the nature of the break in a variety of fracture types [4].

Image Processing in Bone Fracture Images

For the detection of bone fracture and classification, image processing is essential to get critical information from images and the improvement of diagnostic accuracy. Image processing techniques are implemented to resolve obstacles such as noise reduction, feature extraction, and image enhancement in the context of bone fracture images, which are typically acquired through X-rays, CT scans, or MRIs [5]. Image pre-processing is an essential step that is designed to improve the image and prepare them for further analysis. Noise reduction and the elimination of irrelevant or undesirable data are the two primary components of pre-processing that were addressed. The signal-to-noise ratio in the images is enhanced by noise reduction, which ensures that the pertinent information is more easily discernible [6].

Image Noise

Image Noise is the term used to describe the unintended random fluctuations in the luminance or colour of an image. It is frequently regarded as unfavourable due to its potential to obscure the actual details and structures of the image. Image noise may result from a variety of factors, such as environmental conditions, sensor limitations, electronic interference, or imperfections in the imaging process [7][8][9]. The quality of data and, as a result, the accuracy of research analysis can be substantially determined by the types of noise present in the images. The specific forms of noise that are observed in images may differ depending on the imaging process, environmental conditions, and the imaging equipment. Researchers frequently encounter the following categories of noise.

Gaussian Noise

Gaussian noise, which is characterised by random fluctuations in pixel intensity that adhere to a Gaussian distribution, can be the result of a variety of sources, such as electronic interference and sensor limitations [10].

Salt-and-Pepper Noise

This is characterised by the presence of bright and dark pixels in the image that occur at random, resembling grains of salt and pepper. It may be the result of transmission errors or deficiencies in the imaging apparatus [11].

Shot Noise

Shot noise is an inherent characteristic of images that are captured with low levels of illumination, as it is a result of the discrete nature of light. In low-light conditions, it can be especially apparent [12].

Speckle noise

This type is distinguished by granular interference patterns that manifest as brilliant and dark spots or specks in an image. It is frequently detected in images obtained through techniques such as ultrasound imaging, synthetic aperture radar (SAR), and specific forms of laser imaging [13].

Feature Extraction

The process of capturing and representing the key characteristics or patterns within an image that are pertinent to a specific analysis is referred to as feature extraction in image processing. The objective is to decrease the dimensionality but preserve the critical data for subsequent processing or recognition tasks. Feature extraction is important for a variety of applications, including recognition of pattern, computer vision, and machine learning. The selection of features in image processing is determined by the intended system. Spectral, geometric, and texture features are the three primary categories of features that address distinct aspects of image analysis.

Spectral features

Colour information can be extracted through colour histograms. Insights into the distribution of pixel intensities for each colour channel (e.g., red, green, and blue) in the image are provided by these histograms. Fourier transform coefficients, which are spectral features derived from the frequency domain, are capable of capturing patterns and variations in bone fracture images at various spatial frequencies [14].

Shape Features

The fractured bone's perimeter and area can be determined. The extent and magnitude of the fracture are quantified by these characteristics. The degree to which the fracture's geometry resembles a perfect circle is determined by circularity, which is calculated as 4π times the ratio of area to perimeter squared. Eccentricity is a metric that quantifies the elongation of a fracture, thereby revealing its orientation and shape [15].

Texture features

The spatial relationships between pixel intensities can be quantified and textural patterns in the fissure can be highlighted by texture features extracted from the co-occurrence matrix, such as contrast, energy, and entropy. Local Binary Patterns (LBP) are a texture descriptor that is highly effective in identifying the local patterns within an image. It is capable of capturing the fine details and textures that are present in bone fracture images. By analysing local spatial frequency content in various directions, Gabor filters are effective in extracting texture features when applied at various orientations and scales [16].

MATERIALS AND METHODS

The process of medical image classification entails the classification of images that have been acquired through imaging diagnosis including X-rays, CT scans, MRIs, and ultrasounds. For diagnostic purposes, treatment planning, and disease monitoring, it is essential to ensure that medical images are accurately classified. There are numerous methodologies that are frequently implemented for the classification of medical images

Conventional machine learning methodologies

Traditional machine learning techniques are essential for medical image classification, as they provide effective solutions that are interpretable and well-suited for scenarios with limited labelled data. In binary and multiclass classification tasks, Support Vector Machines are frequently employed to recognise the most effective hyperplanes for distinguishing between distinct classes based on feature representations of the data. A robust classification result with reduced susceptibility to overfitting is achieved by constructing multiple decision trees and amalgamating their predictions in Random Forest, an ensemble learning method.

Deep learning

By utilising neural networks, especially CNNs, was used to get intricate patterns and features from complex medical images, deep learning has revolutionised medical image classification. The precision of tasks such as disease detection and diagnosis has been substantially enhanced by the capacity of CNNs to detect subtle details. Transfer learning, which utilizes pre-trained models on extensive datasets, effectively addresses the challenges posed by the scarcity of labelled medical data by integrating knowledge from diverse domains. In addition to CNNs, RNNs are used to analyse temporal patterns in medical image time-series data. Although deep learning models demonstrate unparalleled accuracy, they are confronted with the necessity of substantial annotated datasets, computational resources, and potential interpretability issues. Nevertheless, the growing prominence of deep learning architectures and explainability tools is a direct result of their continuous development, which provides sophisticated and precise solutions for medical image classification in the healthcare sector.

Transfer learning

Transfer learning is a powerful technique in the domains of deep learning and machine learning, which entails leveraging the cognition acquired from training a model on one task to enhance performance on a related yet distinct task. This approach has proven particularly advantageous in the realm of medical image classification, especially when confronted with limited labelled datasets. Pre-trained models, which are frequently trained on extensive and diverse datasets like ImageNet, function as feature extractors that have acquired hierarchical representations of general patterns and features from a diverse array of images.

Classification Techniques for medical images (Table: 1)

RESULT AND DISCUSSION

Recent techniques have been employed by researchers to demonstrate augmented accuracy, good prediction, sensitivity and overall strong classification in a classification system for medical image melodies, as illustrated in TABLE II. Nevertheless, the text underscores the necessity of a sufficient quantity of datasets to achieve high sensitivity and specificity values in research. This is essential for guaranteeing the viability of implementing these methods in computer-aided diagnosis. X-ray, CT, MR, and US imaging are frequently employed by medical image analysis researchers. These images provide information that aids in the determination of the presence or absence of lesions in relation to a patient's medical history. Additionally, it is noted that Magnetic Resonance Imaging (MRI) is distinct from other modalities in that it does not employ X-rays. The text emphasises the critical role of AI in image classification, which is capable of both supervised and unsupervised techniques, with an eye towards future work. Special emphasis is placed on the challenge of attaining superior classification results, with a target accuracy of over

95%. This suggests that there is a persistent emphasis on enhancing the performance of classification algorithms for medical image analysis.

Classification Framework for Medical Image Categorization Across Various Image Classification Techniques (Table: 2)

Visual representation of the melodies employed in the images (Fig 2).

Performance Analysis With High Accuracy

The objective of this discussion is to analyze the various algorithms and the enhanced accuracy achieved by the techniques presented in Table 3. Analysis of Performance Metrics for Achieving High Accuracy Levels of 95% and Above (Table: 3).

Utilising a Graphical Chart to Enhance Precision (Fig 3).

CONCLUSION AND FUTURE RESEARCH

In summary, the exhaustive review of the existing literature on the detection and classification of medical bone fracture images reveals a landscape that is distinguished by significant contributions to the field of healthcare and rapid advancements.The accuracy and efficacy of medical image analysis have been significantly enhanced by the use of AI and ML algorithms, which has shown potential in a host of diagnostic applications. However, the necessity for ongoing research and development is underscored by the persistence of challenges such as the interpretability of models, the scarcity of annotated datasets, and ethical considerations. In order to enhance the generalisation & robustness of classification models, future research should prioritise the expansion and diversification of annotated medical image datasets. Furthermore, endeavours should be made to create interpretable models in order to foster trust and transparency within the medical community. Investigating transfer learning techniques is crucial for adapting pre-trained models to specific medical imaging tasks, especially in contexts where labeled data is limited. The development and implementation of these technologies in real-world healthcare settings should be guided by ethical considerations surrounding patient privacy and responsible AI deployment, which should remain at the vanguard of research endeavours. The medical image curation and classification is on the brink of further expansion as a result of these avenues of investigation, which will ultimately enhance patient outcomes and enhance the capabilities of diagnostic medicine.

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Table 1. Classification Techniques for medical images

Year	Author	Methodology Used	Image Sources	Precision	Benefits& Drawbacks
2023	Damith Senanayake [17]	DeepDXA (Deep Learning for DXA-based Fracture Risk Prediction)	X-Ray	74.3%.	to more precise predictions of fracture risk. A modest sample size results in limited generalisability.
2023	Rania Mostafa Almolla [18]	Meyers and McKeever (MM) classification.	MRI	The accuracy of tissuedamage detection for MRI-based ranged from 91.2 to 94.1%.	resulting in enhanced detection efficacy and the potential to influence treatment strategy. necessitates meticulous evaluation in clinical decision-making.
2024	Li-Wei Cheng	A machine	X-ray images	99%, 74%, and 94%	Orthopaedists or

	[19]	learning model that utilises ResUNet and YOLOv4 is developed for the purpose of X-ray images		precision	radiologists are required to possess extensive expertise. Its applicability may be restricted by the presence of X-ray data.
2023	Yan Gao [20]	DCNN	X-Ray images	AUROC: 0.0990 AUPRC:0.948 Sensitivity:94.2% Specificity: 96.3%, Negative Predictive value: 99.2%	has a high negative predictive value, specificity, and sensitivity, which results in strong performance. The study does not offer any insights into the potential limitations.
2024	Jeong [21]	Deep Learning	CT images	A specificity of 77% and a sensitivity of 100%	the algorithm to facilitate the accurate and expeditious diagnosis. datasets that are significantly larger.
2023	Jörg Schilcher [22]	CNN	radiographs	ROC curve (AUC): 0.987 sensitivity 0.903.	The AUC of the ROC curve elevated from 0.966 to 0.987, and the sensitivity elevated from 0.796 to 0.903. To evaluate the robustness and generalisability of the proposed method.
2023	Cato Pauling, [23]	commercial artificial intelligence (AI) tools	radiographs	The AI algorithm demonstrated a sensitivity of 54.9% (95% CI, 45.7-63.9), a specificity of 94.1% (95% CI, 92.9-95.1), a positive predictive value (PPV) of 38.7% (95% CI, 33.1-44.7), and a negative predictive value (NPV) of 96.8% (95% CI, 96.2-97.4).	The use of AI tools in radiology workflows has enhanced efficacy. Risk of overreliance on artificial intelligence (AI) instruments and healthcare
2023	Young-Dae Jeon [24]	deep learning	CT Image	accuracy of 95%, sensitivity of 96.2%	The high average precision values (>0.60) underscore its reliability and usability in 3D visualization.
2023	Deshmukh, P. K. [25]	Deep Convolutional Neural Networks	CT Image	CHMNIST dataset (99.71%), ISIC dataset (98.67%)	Enhanced diagnostic precision and adaptability to a wide

		(CNNs)			range of tasks. Data Privacy and Security Issues.
2021	Hela Yahyaoui [26]	DenseNet and 3D-CNN models.	MRI image	DenseNet Model:92.06% 3D-CNN Model: 85% Fusion Level: 100%	Comprehensive Three-Level Framework, High Accuracy Rates Validation on a Single Test Set, Computational Complexity.
2020	Tanzi [27]	CNN	X-ray images	Accuracy level 94%	The results achieved were comparable to those obtained by humans in the classification of bone fractures. Additionally, the number of incorrect diagnoses was reduced.
2020	Yadav *et al*. [28]	DeepNN	X-ray image	The precision of the classification model percentage is 92.44%.	Improved Performance and High Classification Accuracy in Comparison to Previous Models Limited Generalisability, Requirement for Comparative Analysis
2019	Marcus A. *et al*. [29]	CNNs	radiographic	Precision: 95%	Enhances the precision of fracture prediction. Difficulty in Interpretation
2020	Feng Yang *et al*. [30]	SVM, CNN, SVM, ELM, RF	radiographic	The Accuracy level was 78.63%.	Innovative method of feature extraction, with the potential for future applications. Limited Model Comparison:
2018	Kim and MacKinnon [31]	pre-trained CNNs	radiographs.	The values of sensitivity were (0.9) and specificity (0.88)	The augmented images were employed for computation, allowing for easy application with a modest dataset size.
2019	Nisha V M *et al*. [32]	Radius Boundary Cellular Automata	X-Ray images	-	Algorithm not employed; constant time complexity.
2016	K. Seetharaman and S. Sathiamoorthy [33]	Full Range Autoregressive Model (FRAR)" and a "Bayesian Approach (BA)	CT, MRI, Microscopy, Mammogram, Ultrasound, X-ray and Endoscopy images	The Average retrieval rate is 84.87%.	unified learning framework for the retrieval of heterogeneous medical images. It does not provide a comprehensive

					comparison of performance metrics with specific baseline methodologies.
2014	S. Yazdani, R. Yusof, A. Riazi, and A. Karimian [34]	Support Vector Machine	MRI	Precision: 95%	Magnetism is desirable. It did not considersubcortical structures and 3 T images which reduced the error rate 10% from 30%.

Table 2: Classification Framework for Medical Image Categorization Across Various Image Classification Techniques

	Image Sources			
	X-Rays	CT	MRI	Ultra sound
Damith Senanayake[17]	✔			
Li-Wei Cheng [19]	✔			
Yan Gao [20]	✔			
Young-Dae Jeon [24]		✔		
Deshmukh, P. K. [25]		✔		
K. Seetharaman and S.Sathiamoorthy [45]	✔	✔	✔	✔
Rania Mostafa Almolla[18]			✔	
Hela Yahyaoui [26]			✔	

Table 3: Analysis of Performance Metrics for Achieving High Accuracy Levels of 95% and Above

S.NO.	Used Algorithm	Precision
1	AI	97.2%
2	CNN	95%
3	DeppCNN	99.2%
4	ANN	96.8%
5	SVM	95%

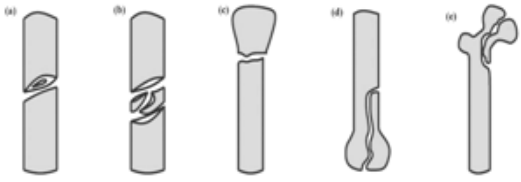


Fig. 1: Types of fractures: (a) diaphyseal (simple), (b) diaphyseal (complex), (c) proximal (extra-articular), (d) distal (partial articular), and (e) proximal (partial articular).

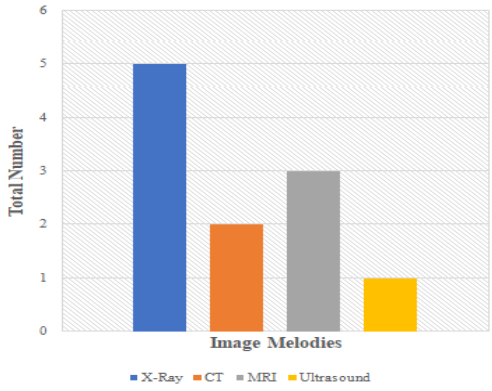


Fig 2: Visual representation of the melodies employed in the images.

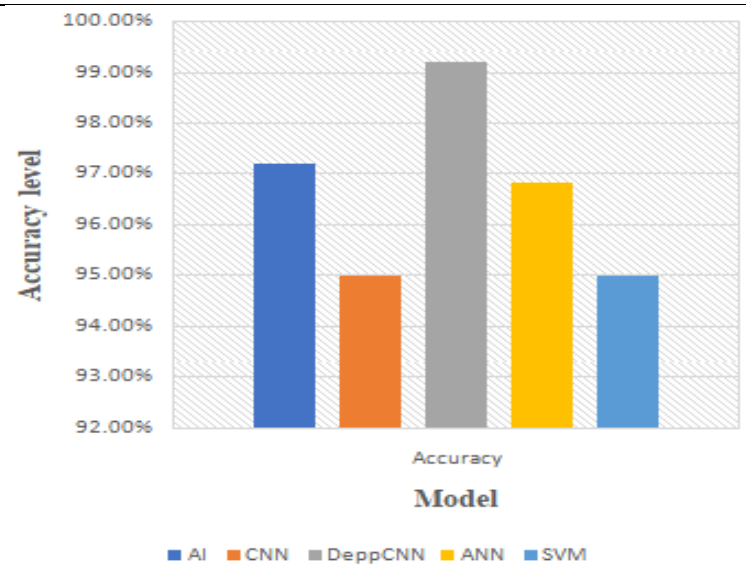


Fig 3: Utilising a Graphical Chart to Enhance Precision

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RESEARCH ARTICLE

Seed Desiccation Studies in *Carica papaya* L.

Suju Skaria .C[1]*, Rejitha L.R.[1] and Anupama Praveen[2]

[1]Assistant Professor, Department of Botany, Mar Ivanios College, Nalanchira, (Affiliated to University of Kerala, Palayam), Thiruvananthapuram, Kerala, India.
[2]Student, Department of Botany, Mar Ivanios College, Nalanchira, (Affiliated to University of Kerala, Palayam), Thiruvananthapuram, Kerala, India.

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***Address for Correspondence**
Suju Skaria .C
Assistant Professor,
Department of Botany,
Mar Ivanios College, Nalanchira,
(Affiliated to University of Kerala, Palayam),
Thiruvananthapuram, Kerala, India.
E.Mail: suju.skaria@mic.ac.in

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ABSTRACT

Carica papaya L., belonging to the family Caricaceaea is a fruit crop known for its immense nutritional and medicinal applications. Although papaya is a common fruit tree, it is not widely cultivated due to a lack of awareness about its nutritional and medicinal benefits. However, *Carica papaya* has the potential for large-scale commercialization because of its short maturation period and year-round fruit production. To encourage the cultivation of *Carica papaya*, comprehensive studies on seed behaviour and associated properties are necessary. Understanding seed storability, response to desiccation, moisture content patterns, effective priming methods for improved germination, and other key seed characteristics is crucial for proper seed storage and successful cultivation. Therefore, before introducing papaya as a commercial crop, an in-depth investigation of seed desiccation and physiological traits is required. This study was conducted to assess the desiccation and germination parameters of *C. papaya.*

Keywords: *Carica papaya*, Seed germination, Desiccation, Physiological traits

INTRODUCTION

With the seed, the independence of the next generation of plant begins. Over evolutionary period, seed plants dominate biological niches on land, from forests to grasslands both in hot and cold climates. Seed production in natural plant populations varies widely from year to year in response to weather variabilities, insects, diseases and the plants' internal cycles. A seed is an embryonic plant enclosed in a protective outer covering. The end product of the flowering process is a fruit with one or many seeds (Bhatnagar *et al*., 1972). Seed formation is one of the

significant events in the post-reproductive stages in plants. Seeds are ripened or fertilized ovules. Seeds play an essential role in reproduction and existence of gymnosperms and angiosperms, relative to the primitive plant forms. Seed germination is usually the growth of a plant from a seed. During germination, the completely functional metabolic machinery of the seed results in the emergence of radicle and plumule. A fully developed seed contains an embryo, endosperm and a seed coat. Seed germination depends on both internal and external conditions. The most important external factors include different variables for successful seed germination. Often this depends on the individual seed variety and is closely linked to the ecological conditions of a plant's natural habitat. Seed dormancy is a factor that affects germination. Seed germination and dormancy are complementary adaptive traits of higher plants that are influenced by many genes and environmental factors (Bewley, 1997). *Carica papaya*, a fruit crop cultivated in tropical to subtropical regions, is known for its nutritional benefits and medicinal applications (Ming *et al*., 2008). Its origin is in the tropical region of America. *Carica papaya* is one of the 22 accepted species in the genus *Carica* of the family Caricaceae. There was a time when papaya was considered an exotic fruit because of its wide occurrence in Mexico and Central America. Today, however, it is one of the most common crops across the globe, which is cultivated for its fruits. The fruits of *Carica papaya* are soft, fleshy and are used in a wide variety of culinary ways. They are highly nutritious and possess a wide range of health benefits. Zeaxanthin, an antioxidant found in papaya, helps in filtering out harmful blue light rays and protects the eyes. The presence of β-carotene, an antioxidant pigment, lowers the risks of cancer, asthma and diabetes. Papayas are rich in vitamin K, which improves calcium absorption, which in turn helps in strengthening and rebuilding of bones. They possess an enzyme called papain, which aids in digestion. They are high in water and fibre content, both of which help to prevent constipation and promote regularity and a healthy digestive tract. The presence of fibre, potassium and vitamins help to ward off heart diseases. Papaya is a tree that is seen everywhere irrespective of the season. Despite being a common fruit tree, it is not cultivated for any purpose, due to the lack of knowledge about the nutritional and medicinal values of this tree. *C. papaya* can be commercialized on a large scale due to its short maturation period and due to the occurrence of fruits throughout the season. It grows abundantly in the tropical region. Hence, if cultivated commercially, papaya could emerge as a potential tropical fruit crop. To promote the cultivation of *Carica papaya*, proper studies should be conducted to know the seed behaviour and associated properties. It is essential to know about the seed storability, response of seeds to desiccation, moisture content pattern, better priming methodology with improved germination and other characteristics of seeds as they play an important role in the storage of seeds. Prior to mass cultivation it is essential to know these features. Study of *C. papaya* seed physiology is relevant in the present scenario. As mentioned earlier, data related to seed desiccation, germination and priming are essential. Hence, prior to introducing Papaya as a crop plant, a detailed seed desiccation and physiological study needs be conducted. The present study was conducted to determine the desiccation and germination parameters in *C. papaya*.

MATERIALS AND METHODS

Collection of mature seeds of *Carica papaya*

Mature seeds of *Carica papaya* were collected from different parts of Thiruvananthapuram. The collected seeds were cleaned, de-pulped and then utilized for further studies.

Evaluation of morphological parameters

The morphological parameters such as length and breadth of the seeds were determined using a digital verniercaliper. The weight of the seeds was taken using a digital weighing balance. All these morphological parameters were done on randomly selected ten seeds of each desiccation stages. The morphological parameters of seeds after hormonal priming and water uptake was also noted, prior to germination trials.

Determination of dry matter accumulation and moisture content

The chopped seed, taken in weighing bottles, were weighed in an electronic balance and dried in a hot air oven at 130 °C for 17 hours. For each time, three replicas of 10 seeds each were use and the moisture content was calculated as; Moisture content (%) = ($F1-F2$)/ $F1$ × 100; Where F1 is the initial fresh weight and F2 is the oven-dried weight

Determination of biochemical parameters

Estimation of metabolites like starch, sugars, total protein, amino acids, phenolics, lipids, tetrazolium chloride reduction and enzyme activities of peroxidase, polyphenol oxidase and catalase were carried out side by side during seed desiccation.

Determination of ideal medium for *Carica papaya* seed germination

For the present study, river sand: cow dung: coir pith (1:1:1), acid-free germination paper and cotton were used to identify the better medium for seed germination among them. Three replicas of 10 seeds were used for the germination trials.

Determination of tetrazolium reduction activity

The modified method of Kittock *et al*., (1968) was used for the estimation of tetrazolium reduction activity. The slightly broken seeds were incubated in 0.1% 2,3,5-triphenyl tetrazolium chloride in darkness for two hours. After this, tetrazolium solution was removed, and the seeds were collected and transferred into petri plates for the determination of viability based on the pink colour developed. After three washes in distilled water, 5ml acetone was added and incubated at room temperature in darkness for 16-18 hours to elute the formed formazan. The supernatant was filtered through Whatmann No1 filter paper and the colour was measured at 480nm in UV- VIS spectrophotometer (PharmaSpec UV-1700, Shimadzu, Japan) Treated extracts from seeds incubated in the absence of tetrazolium served as the blank.

Determination of leachate conductivity

For the estimation of electrolyte leakage, three replications of ten *Carica papaya* seeds from different desiccation periods were taken. The seeds were placed in a glass beaker containing 25-50 ml distilled water and covered to reduce evaporation and contamination by dust and were kept in open laboratory condition. After 24 hours, the seeds were removed and the electrical conductivity of the seed leachate was measured using a digital conductivity meter. Specific conductance was expressed as μ/m S $cm^{-1}g^{-1}$.

Determination of enzyme activity

About one gram fresh seeds were homogenised in a pre-chilled mortar and pestle with 0.1M cold sodium phosphate buffer (pH 7.0). The homogenate was centrifuged at 5000 rpm for 20 minutes and the supernatant was collected. Proteins from the supernatant was precipitated in cold acetone (1:3 ratio) and kept in an ice bath for 30 minutes. The pellet was washed twice with acetone and re-suspended in the same buffer after the complete evaporation of acetone. The combined supernatant served as the enzyme source for both peroxidase, polyphenol oxidase and catalase. All enzyme extractions were carried out at 4ºC using standard procedures.

Determination of water uptake capacity

Each set of seeds were soaked in distilled water at 27°C for 6, 12, 24 and 48 hours respectively. After each duration, water uptake was determined by recording the difference between the original weight of the seeds and the weight of the imbibed seeds. All experiments were conducted using the randomised block design (CRBP). For each treatment, 10 seeds were used with three replications.

Germination pattern of *C. papaya* seeds in response to desiccation

The seeds of *C. papaya* were undergone germination trials in different desiccation stages. The germination percentage and germination parameters were noted at all those desiccation stages.

Effect of seed priming on germination

Hydro-priming and hormonal priming were studied to observe the effect of priming on germination.

Biochemical comparison of pulped and de-pulped seeds

All the above mentioned biochemical parameters were also studied for pulped seeds. Then, comparisons were made between pulped and de-pulped seeds based on these parameters.

RESULTS

Carica papaya contains numerous seeds with woody testa enclosed within the pulp. Papaya seed is composed of endosperm and embryo. Completely mature *C. papaya* seeds are shed from the mother plant with 60.05% moisture content. 25% of the seeds constitute the pulp.

Morphological variation of *Carica papaya* seeds during desiccation

On morphological observation, it was seen that seeds show a rapid decrease in their weight from 0^{th} to 4^{th} stage of desiccation. Slight variations was also observed for length and breadth of the seeds during successive stages of desiccation. This shows the transformation of the seeds from a stage of turgidity to the stage of flaccidity, as given below in Table 1.

Dry matter accumulation and moisture content of seeds during desiccation

Mature seeds of *C. papaya* possessed 60.05% high initial moisture content that declined rapidly within a week of open desiccation (Table 2). The fastest drying rate was observed and the seeds were dehydrated to 23.08, 18.04, 11.15 and 10.43% at different stages of open laboratory desiccation. Nearly 49.62% loss of moisture content was recorded within 72 hours of desiccation. The lowest safe moisture below which the seed become non-viable was noted to be 23.08% by the 2nd day of desiccation. The dry matter accumulation increases with the desiccation.

Biochemical estimations

The biochemical estimations were conducted to determine lipid, total soluble sugar (TSS), starch, phenol, sucrose, protein and amino acid. It was observed that, mature papaya seeds possessed 61.07 mg/g dwt of lipid content. The lipid content was observed to increase to a maximum of 205.70 mg/g dwt on the second day of desiccation. Thereafter a steep decline in lipid content was noticed. A ready decrease in total soluble sugar content can be seen in C. papaya seeds from 52.16 mg/g dwt to 12.47 mg/g dwt. Mature seeds possessed higher starch content than the TSS and they were almost stable up to the first desiccation stage. The aging of seeds also causes a decline in the starch content. But the end of the desiccation marks 90.31 mg/g dwt starch content in *C. papaya* seeds in the fourth stage. *C. papaya* are less phenolic in nature. Initially, the seed possessed 4.2 mg/g dwt of phenolic content, but drying of seeds caused an increase in phenolic content. Sucrose content was maximum on the first day of desiccation. The rapid decline in moisture content made a steep increase in sucrose accumulation of 105.94 mg/g dwt. Thereafter, the sucrose content of desiccated seeds decreased. Total protein content was decreased throughout the desiccation of *C. papaya* seeds and a maximum protein content of 54.69 mg/g dwt was observed in the fresh mature seeds. Amino acid content in papaya seeds are very feeble. Along with the decreasing protein content, amino acid content was also decreased. Maximum amino acid content of 1.03 mg/g dwt could be seen at initial stage seeds. The results of the same were depicted in Table 3.

Tetrazolium reduction activity of *C. papaya* seeds during desiccation

The tetrazolium reduction activity in the seeds of *C. papaya* showed a gradual decrease. The pink colour in the seeds, indicating formazan formation, is found to gradually decrease from the 0^{th} stage to the 4^{th} stage of desiccation. The values of absorption of respective days ranged from 0.30 to 0.12, which showed a decrease in the viability of seeds. The results of the same were summarized in Table 4.

Electrolyte leakage conductivity of *C. papaya* seeds during desiccation

The electrolyte leakage conductivity of *C. papaya* seeds showed a gradual increase from the 0th stage to 4th stage of desiccation. The leachate conductivity value of the 1st day was 30.34 US/cm/g which increases to 33.06, 37.00, 38.45

μS/cm/g. During the 6th day, leachate conductivity reached a maximum of 38.58 μS/cm/g. The results obtained are tabulated below in Table 5.

Antioxidant enzyme activity during desiccation of *C. papaya* seeds

Carica papaya seeds had a significant membrane peroxidation reaction during desiccation. Fresh seeds with moisture content 60.05% showed 54.69 min / mg/ protein activity. When moisture content was dropped, peroxidase activity decreased on the 1st day of desiccation. Thereafter, the activation of peroxidase enzyme can be seen and the activity increased and reached maximum on the 3rd stage of desiccation. The most active antioxidant enzyme is polyphenol oxidase. Polyphenol oxidase activity trend was almost similar to that of peroxidase activity. Maximum activity was expressed at the end of desiccation 30 min/mg/protein. Catalase enzyme activity was found to be very low in comparison with other enzymes. Fresh seeds showed very low activity, but with the reduction in moisture, antioxidant system activates the catalase enzyme activity and becomes maximum on the 3rd stage of desiccation. The tabulated results are given below in Table 6.

Water uptake capacity of *C. papaya* seeds

Results of the water uptake capacity of seeds showed that weight of the seeds increased from the initial weight of 0.055g to 0.056g, 0.062g, 0.064g and 0.066g respectively during 6 hours, 12 hours, 24 hours and 48 hours of imbibition.

Germination pattern of *C. papaya* seeds in response to desiccation

The germination pattern of seeds occurred at the 0^{th} stage of desiccation, when the moisture content was 60.05%. Interestingly, no germination was observed further. This implied the necessity in conducting priming for enhanced germination. Thus, hydropriming via water soaking and hormonal priming via treatment with solutions of GA3, Salicylic acid and Cytokinins were conducted. The results of the same were summarized in Table 7 and 8. The results of hydro priming failed to produce germination. On the other hand, hormonal priming showed surprising results. It was observed that, treatment of seeds in 100 ppm GA3 at 48^{th} hour showed maximum germination (60%). Whereas, 500 ppm GA3 treatment showed complete germination at 72hrs, thereby indicating the efficiency of seeds to germinate under hormonal control.

Biochemical comparison of pulped and de-pulped seeds

Biomolecules like lipid, TSS, starch, phenol, sucrose, protein, and amino acid were compared in both pulped and de-pulped seeds. Pulped seeds showed all biochemical parameters at a maximum when compared to de-pulped seeds. Maximum lipid content of 101.62 mg/g dwt was seen in pulped seeds and de- pulped seeds showed 91.01 mg/g dwt suggesting that more lipids are accumulating in seeds than the pulp. TSS were also maximum in pulped seeds (61.71± 0.30 mg/g/dwt) than the de- pulped seeds (52.16 ± 0.44 mg/g/dwt). Pulped seeds possessed maximum starch content of 264.54 mg/g dwt whereas, the de-pulped seeds possess 105.34 mg/g dwt. Pulp of the seeds contain maximum amount starch than the seeds. Phenolic content were also high in pulped seeds. But the protein and amino acid content were almost similar in both pulped and de-pulped seeds, but a slighter increase in pulped seeds than the de-pulped seeds. The results are given below in Table 9.

DISCUSSION

Carica papaya is one of the most common species of Caricaceae family, grown widely in the tropical regions. The seeds of *C. papaya* are slightly thick, covered by a pulpy coating. They are recalcitrant in nature, and cannot survive in drying and freezing during *ex-situ* conservation. These seeds cannot resist the effects of drying or temperature less than 10°C. Thus, they cannot be stored for more extended periods like orthodox seeds, because they may lose their viability. The seed size, dry matter accumulation, moisture content, colour changes etc. of the seeds act as markers for detecting maturity of seeds. According to Ren *et.al.*,(1998), seed development and germination can be studied based on seed colour, seed morphology, fresh weight and dry weight. Hence, the present study employed these techniques to determine seed desiccation in *C. papaya* seeds. Evaluation of morphological parameters of the seeds exhibited a

length of 5.82mm, width of 3.78mm and weighed 0.08g on the initial day of desiccation and subsequently showed a progressive decline in the measured parameters. The observed decrease in length and breadth may occur as a result of desiccation. Seed desiccation in *C. papaya* gauged by changes in fresh weight pertaining to viability followed a pattern typical to recalcitrant nature (Kamarudeen kunju, 2003). Moisture content is a good indicator of seed development. *Carica papaya* contains high moisture content at the time of shedding which was found to be the characteristic feature of recalcitrant seed. According to Berjak *et al*. (2013), the maturation drying phase is absent in recalcitrant seeds and possess a high percentage of water at the time of shedding. The fresh seeds of *C. papaya* possess an initial moisture content of 60.05%. The initial moisture content rapidly fell to 23.08% at the second stage of desiccation. But,in the successive stages, a gradual decrease in moisture content was observed. Initially, the cells of the seeds contained high water content but as desiccation proceeds, the seeds lose the water content which results in the accumulation of dry matter. When papaya seeds are compared with *Lycopersicon esculentum* Mill., both possess an initial moisture content between 53-72% (Demir *et al*., 1992). The estimation of biochemical parameters revealed that, during *C. papaya* seed desiccation, lipid content increases from 61.07 mg/g/dwt to a maximum of 205.7 mg/g/dwt in the mid- desiccation and then decreases gradually in the late desiccation phase. The obtained data support the desiccation induced-dormancy state in *C. papaya* seeds (Wood *et al*., 2000). Desiccated seeds with increased lipid content might be viable in the dormant condition even though they generated zero germination percentage during the limited period of the study. According to Gasulla *et al*., (2013), dehydration results in an increased quantity of lipid content in the plant, ensuring the membrane stability. The TSS content during desiccation of *C. papaya* seeds showed a higher concentration initially and an almost stable lower concentration throughout the desiccation treatment. Near the point of transition from viability to non- viability, the level of TSS is maximum and during those days of desiccation and germination percentage was maximum. The reduction in sugar content might be due to hydrolysis followed by Madori Maillard reactions which might cause protein and nucleic acid damage, thus threatening the viability of seeds. The high amount of soluble sugar content provides a protective mechanism during the cell membrane destruction process. The result obtained thus indicates the direct correlation of TSS with the viability of seeds. During the initial stages of desiccation, the amount of starch content decreases from the peak value to a minimum value at the point of transition from viability to non-viability (Roberts, 1972). The seeds of *C. papaya* exhibited an increasing phenolic content during the desiccation, reaching a maximum value at the 4th stage of desiccation (7.2 mg/g/dwt). High phenolic content might be attributed to the stress during desiccation (Aalen., 1999), and this high phenolic content might get lowered down during the germination by oxidation of phenolic compounds. During dessication, papaya seeds exhibited a gradual reduction in sucrose concentration except in the second day of desiccation. This in turn may be associated with moisture content as, the moisture decreased rapidly, to cope up with the change. Such a response involves the production of sucrose whose accumulation rapidly increased to 105.94 mg/g/dwt from 80.08 mg/g/dwt. Sucrose is an active membrane protectant. As the water content in the seed decreases during desiccation, the hydroxyl group of sucrose may replace water by hydrogen bonding to the phospholipid head groups to the cell membrane. The total protein content of *C.papaya* seeds showed a significant decline from 54.69mg/g/dwt during the 4th stage which is in accordance with the protein content of *Hopea parviflora* seeds during desiccation (Kamarudeen kunju, 2003). The decrease in protein content might be due to the activity of protease during desiccation. The data obtained indicate that with the reduction in moisture, amino acids exhibit a decreasing pattern from 1.03 mg/g/dwt to a minimal value of 0.0143 mg/g/dwt (Shemy, 2018). The reduction in amino acids during desiccation might be due to increased production of antioxidant enzymes during desiccation stages, which was not quantified in the lower rpm extraction protocol of total protein. The tetrazolium test indirectly determines the respiratory activity in the cells that make up the seed tissues. The time required for tetrazolium staining and the intensity of staining might be the reflection of the loss of viability due to desiccation (Kumar *et al*., 1997). When tetrazolium is reduced to triphenyl formazan in the tissue, it indicates that respiratory activity in the mitochondria of seed tissue cells which are considered alive. The resulting red colour in the seed tissue is a positive indication of viability, an indirect method of detecting respiratory activity at the cellular level. The non-viable seed tissues do not react with tetrazolium and consequently do not stain. In the present study, the fresh seeds of papaya with 60.05% MC showed the formazan formation of 0.30. This gradually decreased to 0.12 on the 4th desiccation stage. Desiccation, thus, affects patterns of staining too. Electrolytic leakage of *C. papaya* seeds during desiccation depicts the recalcitrant behaviour of the seeds (Bewley, 1986). Cellular membranes are most vulnerable to damage

caused by desiccation. In the present study on the 0th stage of desiccation, the leachate conductivity was 30.34 µS/cm/g and it increased to 38.58 µS/cm/g on the 4th stage of desiccation. The relationship between desiccation tolerance and the stability of the seed membrane was reported by Bailly *et al*., (2004). The increase in the electrolyte leakage observed in *C. papaya* seed indicated the desiccation-induced damage to the cell membrane. According to Simon, (1978) and Bewley, (1986), an increase in electrolyte leakage is an indication of increased membrane destabilization. The linear increase in leachate conductivity during prolonged desiccation may be due to the loss of membrane integrity (Kamarudeen kunju, 2003). Desiccating seeds build-up free radicals during the metabolism which largely affects the seed viability. To counteract the radicals produced, seeds are best endowed with antioxidant molecules and scavenging systems (Puntarulo *et al*., 1991). Most prominent ones among the antioxidant molecules are the antioxidant enzymes like polyphenol oxidase, catalase and peroxidase. Polyphenol oxidase is a copper-containing antioxidant enzyme which metabolises the excess phenolics in the system. Both peroxidase and catalase metabolise the toxic hydrogen peroxide generated during the desiccation stress. In *C. papaya* seeds, polyphenol oxidase exhibits an enhanced activity initially which then increases to a maximum activity at the late desiccation stage. During desiccation, peroxidase activity gradually increases to a maximum towards the late desiccation stages. Catalase activity in *C. papaya* gradually increased to a maximum activity during the mid-desiccation stage followed by a steep reduction. Beyond a specific concentration of hydrogen peroxide, catalase cannot reduce hydrogen peroxide, its activity gets saturated and declines. The pattern of polyphenol oxidase, peroxidase and catalase activity obtained in *C. papaya* seeds (up to 6 days of desiccation) was similar to the enzyme activity of 0 to 8 days desiccation in *Vatteria indica* and *Hopea parviflora* (Kamarudeenkunju, 2003) and 0 to 24 hours of desiccation in *Knema attenuata* (Shemy, 2018). Data obtained from the antioxidant enzyme activity during desiccation of *C. papaya* seeds indicates its recalcitrant nature. Data obtained from the germination pattern of *C. papaya* seeds in response to desiccation revealed that only fresh seeds germinated (20%) and the rest of the desiccated seeds exhibited zero percentage germination. The absence of germination in the desiccated stages during the period of study might not be due to the non-viability of desiccated seeds, but might be due to the desiccation- induced dormancy (Wood *et al*., 2000).In the hormonal priming treatment, 500 pm gibberellic acid for 72 hours yielded maximum germination percentage (100%). While in the case of 250ppm and 100ppm gibberellic acid priming, 24 hours (30%) and 48 hours (60%) exhibited the maximum germination percentage. Least time required for first seedling emergence was noted in 100ppm gibberellic acid for 6 hours (7 days) but the germination percentage concerning it was least (10%). Concerning 500 ppm gibberellic acid treatment for 72 hours, FDG was observed on the 10th day while the LDG was 14th day, yielding four days of TSG. Maximum time spread of germination (7 days) was observed in 500 ppm Gibberellic acid for 24 hours. Maximum mean daily germination (7 days) was observed in the best priming treatment, 500 ppm gibberellic acid for 72 hours. The maximum peak value obtained was 100% in 500 ppm gibberellic acid treatment for 72 hours, which also yielded the maximum germination value in the lot. In concern with the germination energy, the maximum value was in the 500 ppm gibberellic acid treatment for 72 hours. All these germination characters of control and primed seeds indicate that 500 ppm gibberellic acid treatment for 72 hours yielding 100% germination is the best priming method among all the methods employed. The result obtained in the hormonal priming treatment is in accordance with the findings of Pourazar *et al*., (2015) i.e., seed priming with higher doses of gibberellic acid might improve the germination and vigorous performance. Comparing the biochemical composition of pulped and depulped seeds of *C. papaya*, all the biochemical parameters are present in comparatively higher quantities in the pulped seeds. Starch and phenolic content are approximately two fold higher in the pulped seeds. This might be due to the presence of sclerotesta in the pulped seeds. The sclerotesta provides an additional physical barrier to the rapid desiccation when abscissed from the mother plant or when exposed to the external environment. The presence of antioxidant molecule like phenol and the higher water content in the pulp, to some extent, could act as an external shield for the desiccating seeds.

CONCLUSION

Carica papaya could be introduced as a promising crop since it has established nutritional and medicinal features with its broad spectrum of phytochemicals. Since the study conducted on *C. papaya* seeds encompassed only a limited

number of aspects, there is still a lot more left to understand and study in detail regarding this topic. Data obtained in the present study only form the stepping stone, further detailed studies have to be conducted to come across more ideas regarding the seed physiology of papaya seeds prior to introducing it as a safe crop. A detailed analysis should be conducted on seed development, seed germination, phytohormones, antioxidant molecules, storage properties etc. such that papaya could be introduced as a crop with least expense and care but yielding a promising harvest.

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TABLE 1: Morphological Variation of *Carica papaya* L. Seeds During Desiccation

Stages of desiccation	Days of desiccation	Weight of seeds(g)	Length(mm)	Width(mm)
0	1	0.08±0.03	5.82±0.04	3.78±0.08
1	2	0.05±0.02	5.42±0.04	3.53±0.01
2	3	0.03±0.05	5.20±0.03	3.46±0.017

3	5	0.02±0.01	5.03±0.03	3.38±0.025
4	6	0.01±0.02	4.83±0.10	3.34±0.056
d(n-1)=4	d(n-1)=4	d(n-1)=4	d(n-1)=4	d(n-1)=4
		F=0.13	F=0.017	F= 0.839

TABLE 2: Dry Matter Accumulation and Moisture Content of Seeds During Desiccation

Stages of desiccation	Days of desiccation	Fresh weight(g)	Dry weight(g)	Moisture content(%)
0	1	0.43± 0.008	0.17±0.01	60.05± 1.25
1	2	0.17± 0.005	0.13±0.003	23.08± 0.43
2	3	0.20±0.00	0.16±0.002	18.04±1.5
3	5	0.208±0.005	0.18±0.00	11.15± 0.16
4	6	0.204±0.009	0.18±0.005	10.43± 0.31
d(n-1)=4	d(n-1)=4	d(n-1)=4	d(n-1)=4	d(n-1)=4
		F= 0.008	F= 0.076	F= 0.030

TABLE 3: Results of Biochemical Parameters Evaluated In *C. Papaya* L. Seeds

Stages	Days	Lipid content(mg/g/dwt)	TSS (mg/g/dwt)	Starch(mg/g/dwt)	Phenol(mg/g dwt)	Sucrose(mg/g dwt)	Protein(mg/g dwt)	Aminoacid(mg/gdwt)
0	1	61.07±0.07	52.16± 0.44	105.34±0.52	4.2±0.08	80.08±0.67	54.69±0.41	1.03±0.08
1	2	151.17±0.10	22.85± 1.36	102.35±0.48	4.9±0.05	105.94±0.78	47.75±0.61	1.01±0.05
2	3	205.70± 0.72	16.4±0.42	52.49±0.57	5.2±0.03	67.70±0.29	46.23±0.40	0.96±0.05
3	5	102.81±0.41	14.5±0.27	68.26±0.52	6.9±0.2	42.03±0.93	18.77±0.78	0.021±0.02
4	6	57.85±0.56	12.47± 0.29	90.31±0.48	7.2±0.018	21.43±0.65	14.18±0.06	0.014±0.03
d(n-1)=4	d(n-1)=4	d(n-1)=4 f=0.034	d(n-1)=4 f=0.04	d(n-1)=4 f=0.99	d(n-1)=4 f=0.017	d(n-1)=4 f=0.287	d(n-1)=4 f=0.148	d(n-1)=4 f=0.124

TABLE 4: Results of Tetrazolium Reduction Assay in the seeds of *C. papaya* L.

Stages of desiccation	Days of desiccation	Formazan formation
0	1	0.30± 0.004
1	2	0.21± 0.002
2	3	0.18± 0.003
3	5	0.124±0.001
4	6	0.12±0.001
d(n-1)=4	d(n-1)=4	d(n-1)=4
		F= 0.008

TABLE 5: Table Showing Electrolytic Leakage Conductivity of *C. papaya* L. Seeds During Desiccation

Stages of desiccation	Days of desiccation	Leachate conductivity(µS/cm/g)
0	1	30.34±0.31
1	2	33.06±0.50
2	3	37.00±0.04
3	5	38.45±0.06
4	6	38.58±0.26
d(n-1)=4	d(n-1)=4	d(n-1)=4
		F=0.067

TABLE 6: Table Showing Antioxidant Enzyme Activity oF *C. papaya* L. During Seed Desiccation

Stages of Desiccation	Moisture content(%)	Polyphenoloxidase(min^{-1} $mgprotein^{-1}$)	Peroxidase(min^{-1} $mgprotein^{-1}$)	Catalase(min^{-1} $mgprotein^{-1}$)
0	60.05±1.25	26.82±0.25	0.039±0.008	0.015±0.005
1	23.08±0.43	23.96±0.467	0.036±0.005	0.020±0.0061
2	18.04±1.5	26.88±0.16	0.049±0.005	0.29±0.004
3	11.15±0.16	28.71±0.31	0.053±0.001	0.22±0.001
4	10.43±0.31	30.00±0.50	0.044±0.001	0.21±0.003

TABLE 7: Table Showing The Results of Hydropriming in *C. papaya* L. Seeds

Initial weight of seeds(g)	Timeperiod of imbibition(hr)	Weight of seeds after imbibition(g)	Germination
0.055	6	0.056	0
	12	0.062	0
	24	0.064	0
	48	0.066	0

TABLE 8: Table Showing the Results of Hormonal Priming in *C. papaya* L. Seeds

Treatment	Duration(hr)	Concentration(ppm)	Initial seed wt(g)	After priming(g)	Germination percentage
Control					20
Gibberellic acid	6	100	0.0498	0.050	10
	24			0.052	10
	48			0.054	60
	72			0.055	0
Treatment	**Duration(hr)**	**Concentration(ppm)**	**Initial seed wt(g)**	**After priming(g)**	**Germination percentage**
Gibberellic acid	6	250	0.055	0.056	10
	24			0.058	20
	48			0.060	10
	72			0.062	30
Treatment	**Duration(hr)**	**Concentration(ppm)**	**Initial seed wt(g)**	**After priming(g)**	**Germination percentage**
Gibberellic acid	6	500	0.06	0.061	30%
	24			0.063	60%

	48			0.065	50%
	72			0.067	100%

TABLE 9: Table Showing the Results of Biochemical Comparison of Pulped and De-Pulped Seeds

Biochemical comparison	Pulped seeds	De-pulped seeds
Lipid	101.62±0.46	91.07±0.078
TSS	61.71±0.30	52.16±0.44
Starch	264.54±1.90	105.34±0.52
Phenol	9.8±0.04	4.28± 0.08
Sucrose	81.83±0.36	80.08± 0.67
Protein	56.21±0.55	54.69± 0.41
Aminoacid	1.38±0.01	1.03± 0.008

RESEARCH ARTICLE

Phytochemical Evaluation and Diuretic Activity of Hydroalcoholic Extract of *Nephelium lappaceum* Fruits in Albino Rats

J. Jeya Sheely[1] * and X. Kanirajan[2]

[1]Assistant Professor, Department of Pharmacology, Bethlahem College of Pharmaceutical Sciences, Karungal, Kanyakumari District, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.
[2]Scholar, Department of Pharmacology, Sankaralingam Bhuvaneswari College of Pharmacy, Anaikuttam, Sivakasi, (Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai), Tamil Nadu, India.

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***Address for Correspondence**
J. Jeya Sheely
Assistant Professor,
Department of Pharmacology,
Bethlahem College of Pharmaceutical Sciences,
Karungal, Kanyakumari District,
(Affiliated to The Tamil Nadu Dr. M.G.R. Medical University, Chennai),
Tamil Nadu, India.
E.Mail: jeyasheely26@gmail.com

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ABSTRACT

Diuretics play a vital role in the regulation of fluid and electrolyte balance and are widely used in the management of hypertension, edema, and renal disorders. *Nephelium lappaceum* L. (rambutan), a tropical fruit rich in polyphenols, flavonoids, and other phytoconstituents, has been traditionally used for various medicinal purposes. This study aimed to evaluate the phytochemical profile and diuretic activity of the hydroalcoholic extract of *N. lappaceum* fruit rind in albino rats. The fruit rind was extracted using ethanol and water (70:30) by maceration and screened for phytochemicals using standard qualitative tests. GC-MS analysis was performed on both ethanol (polar) and chloroform (non-polar) fractions to identify active constituents. The diuretic activity was assessed in Wistar albino rats (150–200 g), divided into four groups: normal control, standard (furosemide 20 mg/kg), and two test groups receiving 250 mg/kg and 500 mg/kg of the extract, respectively. Urine output was recorded hourly for 5 hours in metabolic cages. Statistical analysis was conducted using one-way ANOVA followed by Dunnett's test. Phytochemical screening confirmed the presence of flavonoids, saponins, alkaloids, tannins, phenolics, carbohydrates, and proteins. GC-MS analysis revealed a variety of bioactive compounds such as 9,12-octadecadienoic acid, ricinoleic acid, and dibenzofuranamine. The 500 mg/kg dose of the extract significantly increased urine output at all time points compared to the control ($p < 0.0001$), showing comparable efficacy to furosemide. The 250 mg/kg dose produced only a mild, statistically nonsignificant increase in urine

volume. The hydroalcoholic extract of *N. lappaceum* fruit rind demonstrated significant dose-dependent diuretic activity in rats, particularly at the 500 mg/kg dose. These findings support the ethnopharmacological use of *N. lappaceum* rind as a natural diuretic and warrant further studies for the isolation of active constituents and exploration of their mechanisms of action.

Keywords: Diuretic activity, Hydroalcoholic extract, GC-MS analysis, Albino rats, Herbal medicine, Urine output, Plant-based diuretics

INTRODUCTION

Background of Study

The physiological processes by which the body maintains fluid balance play a crucial role in overall health, influencing a variety of clinical conditions. One such process, diuresis, involves the increased production and excretion of urine, serving as a key mechanism for regulating blood volume, electrolyte balance, and waste elimination. Advances in medical imaging and diagnostic techniques have enhanced the understanding of renal functions related to diuresis, enabling improved identification and treatment of renal masses through novel ultrasound applications that provide non-invasive insights into kidney health Since medicinal plants are historically good sources of several therapeutic compounds, research into them is necessary to find safe and efficient diuretics[1]. *Nephelium lappaceum* L., also known as rambutan, is a tropical fruit native to Southeast Asia. It is closely related to lychee, longan, pulasan, and guinep[2]. The fruit is a round to oval drupe, 3-6 cm long and 3-4 cm broad, with a reddish leathery skin and fleshy, pliable spines. The fruit flesh is translucent, whitish, or pale pink, with a sweet, mildly acidic flavor. Rambutans are rich in potassium, minerals, and vitamin C[3]. And are used in medicine for heartbeat, kidney function, and muscle contraction. Polyphenols, flavonoids, and tannins, including quercetin, ellagic acid, geraniin, and corilagin, are all abundant in rambutan peel. With intriguing uses in the treatment of metabolic disorders and illnesses linked to oxidative stress, these chemicals demonstrate potent antioxidant, antibacterial, anti-inflammatory, antidiabetic, and anticancer properties[4]. They are also used as astringent, stomachic, vermifuge, febrifuge, and a remedy for diarrhea and dysentery[5]. The seeds have antihyperglycemic, antioxidant, and antihypertensive properties due to their high concentrations of oleic and arachidic acids, triterpenoids, and polyphenols. While roots are used as a febrifuge and to cure tongue infections, other plant components, such as leaves, have long been used to alleviate headaches and are currently being researched for their coloring agents and possible cosmetic uses. Oral thrush is treated with the bark in traditional medicine[6]. *N. lappaceum* is a significant, untapped resource with potential for the creation of natural therapies, especially in light of the growing interest in phytomedicine across the world. In order to promote the use of certain plant components of *Nephelium lappaceum* in both conventional and contemporary healthcare systems, this study aims to investigate and scientifically evaluate their antidiuretic qualities[7,8].

MATERIALS AND METHODS

Collection and Authentication of Plant Material

Fresh *Nephelium lappaceum* (rambutan) fruits were collected locally from Sivakasi, Tamil Nadu. The plant material was authenticated by Dr. N. Senthilkumar, Head, Dept. of Botany, Ayya Nadar Janaki Ammal College, Sivakasi.

Preparation of Crude Extract

The fruit rind (220 g) was separated and macerated in a hydroalcoholic solvent (ethanol: water = 420:180 mL) with 5 mL of chloroform to prevent microbial growth. After 96 hours of soaking with occasional shaking, the mixture was heated at 30°C for 60 minutes. Solvents were evaporated on a hot plate, and the concentrated extract was stored at −20°C [9].

Qualitative screening of phytochemicals present in of *Nephelium lappaceum* fruit extract

The hydroalcoholic extract of *Nephelium lappaceum* underwent qualitative phytochemical screening to detect various phytoconstituents. The procedure followed standard methods as described by Harborne (1973). The ethanolic extract was dissolved in dilute hydrochloric acid, filtered, and subjected to tests for alkaloids, carbohydrates, glycosides, steroids, sterols, tannins, phenolic compounds, proteins, amino acids, gums and mucilage, flavonoids, saponins, foam tests, and triterpenoids. The tests included Dragendorff's, Wagner's, Mayer's, Molsch's, Fehling's, Benedict's, Keller-Kiliani's, and Borntrager's tests. The extract was also tested for tannins, phenolic compounds, proteins and amino acids, ruthenium red, Shinoda, foam, and triterpenoids. The results showed that the extract was soluble in water, filtered, and treated with various solvents, resulting in color changes and changes in various compounds. The extract was also tested for proteins and amino acids, ruthenium red, shinoda, foam, and triterpenoids[10].

Preparation of a fraction

One gram of *Nephelium lappaceum fruit extract was* dissolved in 50 mL of ethanol and 50 mL of chloroform (50:50) and allowed for 10mins to mix. The sample solution was separated into polar solution (ethanol fraction of fruit extract) and nonpolar solution (chloroform fraction of fruit extract). Both samples were analysed for GC-MS to identify the phytoconstituents present in the fruit extract[11].

GC-MS Analysis[12]

GC-MS was used to identify major phytoconstituents. Two fractions (ethanol = polar, chloroform = non-polar) were analyzed using an Agilent 7820A GC system with a DB-5 column and a 5977E MS detector.

Pharmacological Activity: Diuretic Study[13]

Animals

Male Wistar albino rats (150–200 g) were used. They were acclimatized under standard laboratory conditions. The experiment was approved by the Institutional Animal Ethics Committee (IAEC NO: SBCP/2021 – 2022/ CPCSEA/IAEC/I (1)F/F16/142).

Experimental Design

In the experimental study, a total of 12 healthy Wistar albino rats were randomly divided into four groups, each consisting of three animals[2].

- **Group 1** served as the normal control and received 2.5 mL/kg of saline orally.
- **Group 2** was designated as the standard treatment group and was administered furosemide at a dose of 20 mg/kg orally.
- **Group 3**, labeled as Test 1, received 250 mg/kg of the hydroalcoholic extract of *Nephelium lappaceum* fruit rind via the oral route.
- **Group 4**, designated as Test 2, was treated with a higher dose of 500 mg/kg of the same extract, also administered orally
- Urine was collected hourly for 5 hours using metabolic cages.

Statistical Analysis

Data expressed as mean ± SD. Analyzed using one-way ANOVA followed by Dunnett's test ($p< 0.05$ considered significant).

RESULTS

Preliminary phytochemical studies

The preliminary phytochemical screening of the hydroalcoholic extract of *Nephelium lappaceum* fruit rind revealed the presence of several important bioactive compounds. The extract tested positive for flavonoids, saponins, tannins, carbohydrates, alkaloids, gum and mucilage, phenolic compounds, and proteins and amino acids. However, it tested

negative for glycosides and steroids. These results suggest that the extract contains a diverse range of phytoconstituents that may contribute to its observed pharmacological activity, particularly its diuretic potential.

GC-MS Findings

The GC-MS analysis of the ethanol and chloroform fractions of the hydroalcoholic extract derived from the *Nephelium lappaceum* (rambutan) fruit rind revealed the presence of several naturally occurring phytochemical compounds. The ethanol fraction showed a rich profile of bioactive constituents, including Xylitol, N-Acetyl-D-glucosamine, Aziridine, Maltose, Diethyl glutaconate, Sucrose, Octadecanoic acid, Ricinoleic acid, and Lactose. In contrast, the chloroform fraction contained fewer phytochemicals, with the primary compounds identified being Furan, 2-(1-pentenyl)-, (E), n-Hexadecanoic acid, Octadecanoic acid, 2-Ethylacridine, 3-Hydroxy-2-methylbenzaldehyde, n-Decanoic acid, and cis-Vaccenic acid. These findings suggest that the ethanol fraction may possess a broader spectrum of phytochemicals with potential pharmacological activities.

Chromatogram of Ethanol Fraction (polar)

GC-MS chromatogram of the ethanol fraction (polar) of *Nephelium lappaceum fruit rind extract* (Figure 1). The chromatogram displays multiple peaks at various retention times, indicating the presence of diverse phytochemical constituents. Major peaks were observed at retention times 13.333, 15.051, 18.131, 18.806, and 19.534 minutes, corresponding to significant compounds such as Xylitol, N-Acetyl-D-glucosamine, and Octadecanoic acid. fraction.

Phytoconstituents present in the ethanol fraction (polar) of *Nephelium lappaceum* fruit extract

This table 2 presents 21 major compounds detected in the ethanol fraction, along with their retention times, and relative area percentages. Prominent phytochemicals include 9, 12-Octadecadienoic acid (5.22%), Octadecanoic acid (1.72%), Ricinoleic acid (3.61%), and 3-Dibenzofuranamine (17.22%), indicating the presence of various bioactive fatty acids, sugars, alcohols, and amines that may contribute to the extract's pharmacological activity.

Chromatogram of Chloroform fraction (non-polar)

GC-MS chromatogram showing phytoconstituents in the chloroform (non-polar) fraction of *Nephelium lappaceum* fruit rind extract (Figure 2.). The chromatogram displays several prominent peaks at different retention times, indicating the presence of various non-polar bioactive compounds. Major peaks were observed at retention times 6.931, 13.718, 15.842, and 16.713 minutes, representing the dominant constituents in this fraction. The high abundance at these peaks suggests a significant concentration of lipophilic phytochemicals, which may contribute to the therapeutic potential of the extract.

Phytoconstituents present in the chloroform fraction (non-polar) of *Nephelium lappaceum* fruit extract

The table 3 lists ten major compounds detected in the chloroform extract, along with their molecular formula, molecular weight, retention time, and relative abundance (area%). Notable compounds include 2-Ethylacridine, 1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester, and 2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl, each contributing the highest area percentages (12.02%) at around 16.714 minutes. These compounds suggest the presence of biologically active non-polar phytochemicals with potential therapeutic relevance.

Diuretic activity

Table 4 : The study evaluated the diuretic effect of *Nephelium lappaceum* fruit rind hydroalcoholic extract in Wistar albino rats over a 5-hour period. The results showed that the extract induces diuresis in a dose-dependent manner. The 500 mg/kg dose (Group IV) produced a significant increase in urine volume compared to the control and was nearly comparable to furosemide. In contrast, the 250 mg/kg dose showed only a moderate effect. The results suggest that the active phytoconstituents in the extract, possibly flavonoids, tannins, and phenolic compounds, may be responsible for the observed diuretic activity. The extract's potent diuretic effect was confirmed by the results.

Effect of Hydroalcoholic Extract of *Nephelium lappaceum* (HAENL) Fruit Rind on Urine Output in Wistar Albino Rats (1st to 5th Hour)

- Std – Standard
- HAENL – Hydroalcoholic extract of *Nephelium lappaceum*
- Group 2,3 and 4 was compared to group 1.

Figure 3 illustrates the time-dependent urine output (mL/100g) across five hours following oral administration of saline (control), standard diuretic (furosemide 20 mg/kg), and HAENL at two dose levels (250 mg/kg and 500 mg/kg). A significant increase ($p < 0.0001$) in urine volume was observed in both the standard group and the high-dose HAENL group (500 mg/kg) at all time points compared to the control. In contrast, the 250 mg/kg HAENL group exhibited a mild but statistically nonsignificant effect.

DISCUSSION

This study evaluated the diuretic activity of fruit extract from *N. lappaceum* using albino rats at two different doses. The phytochemical investigation revealed the presence of flavonoids, alkaloids, tannins, and phenolic compounds. The GC-MS analysis confirmed the presence of many naturally occurring compounds. The increased urinary excretion may be due to the presence of various phytoconstituents in the crude extract. The lower test dose (250 mg/kg) did not show a significant diuretic effect compared to the control group, possibly due to the low concentration of the active principle(s) present in the extract. However, the higher dose (500 mg/kg) showed a significant diuretic effect, suggesting that the high dose of crude hydroalcoholic extract of *N. lappaceum* fruit rind possesses substantial diuretic activity.

CONCLUSION

In traditional medicine, the plant is used to treat many ailments. The present study results showed that single dose administration of hydroalcoholic extract of fruit rinds of *N. lappaceum 250* and 500 mg/Kg, and standard Furosemide (20 mg/kg) has increased the urinary output. The diuretic effect of the crude extract of *N. lappaceum* at a dose of 250 mg/kg was statistically not significant. However, at the dose of 500 mg/kg, the crude extract of *N. lappaceum exhibited* a significant diuretic effect. This study comes up with the identification of many phytoconstituents with the scientific proof of diuretic effect. Thus, our study supports and justifies the rationale behind the folklore use of the fruit rind of *N. lappaceum* for its diuretic activity.

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CONFLICT OF INTEREST

All authors have no conflict of interest.

ABBREVIATION USED

HAENL – Hydroalcoholic extract of *Nephelium lappaceum*

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Table. 1: Phytoconstituents identified in the ethanol fraction (polar) of *Nephelium lappaceum* fruit rind extract by GC-MS analysis.

Sl.#	Compound name	Molecular formula	Molecular Weight	Retention time	Area%
1	Xylitol	$C_5H_{12}O_5$	152.15	3.250	1.07
2	N-Acetyl-d-glucosamine	$C_8H_{15}NO_6$	221.20	3.250	1.07
3	5-Hydroxymethylfurfural	$C_6H_6O_3$	126.11	3.997	0.26
4	Aziridine	C_5H_5N	79.1	5.415	0.71
5	2-Furanmethanamine	C_6H_9NO	111.14	5.415	0.71
6	2,4-Pentadien-1-ol, 3-propyl-, (2Z)-	$C_8H_{14}O$	126.20	5.415	0.71
7	Maltose	$C_{12}H_{22}O_{11}$	342.3	6.096	2.00
8	Diethyl glutaconate	C9H14O4	186.20	6.096	2.00
9	Benzenemethanol	C_7H_8O	108.14	6.767	0.62

10	Acetaldehyde, 2-butenylhydrazone	$C_6H_{12}N_2$	99.5	7.004	0.47
11	3-Deoxy-d-mannoic lactone	$C_6H_{10}O_5$	162.14	8.318	1.85
12	Sucrose	$C_{12}H_{22}O_{11}$	342.3	8.687	0.57
13	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	11.703	3.82
14	9,12-Octadecadienoic acid	$C_{18}H_{32}O_2$	280.4	13.329	5.22
15	Octadecanoic acid	$C_{18}H_{36}O_2$	284.48	13.566	1.72
16	Ricinoleic acid	$C_{18}H_{34}O_3$	298.461	15.050	3.61
17	2-Ethylacridine	$C_{15}H_{13}N$	207.27	15.315	0.49
18	3-Dibenzofuranamine	C_8H_7NO	183.21	18.133	17.22
19	n-tetracosanol-1	$C_{24}H_{50}O$	354.65	6.767	0.62
20	Cycloheptane, methyl	C_8H_{16}	112.21	6.767	0.62
21	1-Docosanol, acetate	$C_{24}H_{48}O_2$	368.636	7.004	0.47
22	Lactose	$C_{12}H_{22}O_{11}$	342.3	8.687	0.57

Table. 2: Identified phytoconstituents in the chloroform (non-polar) fraction of *Nephelium lappaceum* fruit extract based on GC-MS analysis.

Sl.#	Compound name	Molecular formula	Molecular Weight	Retention time	Area%
1	Furan, 2-(1-pentenyl)-, (E)	$C_9H_{12}O$	136.19	6.711	4.71
2	phenol 2 4-bis(1 1-dimethylethyl, Phenol, 2,5-bis(1,1-dimethylethyl)-	$C_{17}H_{30}OSi$	278.5	6.928	9.10
3	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	256.4	11.646	3.58
4	Octadecanoic acid	$C_{18}H_{36}O$	284.48	13.518	4.95
5	2-Ethylacridine	$C_{15}H_{13}N$	207.27	16.714	12.02
6	3-Hydroxy-2-methylbenzaldehyde	$C_8H_8O_2$	136.15	6.711	4.71
7	n-Decanoic acid	$C_{10}H_{20}O_2$	172.2646	11.646	3.58
8	Cyclohexa-2,5-diene-1,4-dione, 2-methyl-5-(4-morpholinyl)-	$C_{11}H_{13}NO_3$	207.23	13.518	4.95
9	1,2,4-Benzenetricarboxylic acid, 4-dodecyl dimethyl ester	$C_{23}H_{34}O_6$	406.5	16.714	12.02
10	2,4,6-Cycloheptatrien-1-one, 3,5-bis-trimethylsilyl	$C_{13}H_{22}OSi_2$	250.48	16.714	12.02

Table 3: Diuretic activity

Groups	Animals	Urine output: volume of urine mL/100g				
		1st hour	2nd hour	3rd hour	4th hour	5th hour
Group I Normal	Rat 1	0.46	0.46	0.46	0.50	0.50
	Rat 2	0.42	0.42	0.42	0.52	0.52
	Rat 3	0.62	0.66	0.70	0.72	0.72
Group II Standard	Rat 1	0.92	1.10	1.10	1.10	1.24
	Rat 2	0.88	1.20	1.20	1.20	1.24
	Rat 3	1.20	1.10	1.10	1.10	1.30
Group III Test 1	Rat 1	0.64	0.72	0.72	0.72	0.80
	Rat 2	0.66	0.70	0.70	0.82	0.88
	Rat 3	0.70	0.70	0.70	0.92	0.92
Group IV Test 2	Rat 1	0.88	0.88	0.88	0.90	1.12
	Rat 2	1.24	1.24	1.24	1.24	1.24
	Rat 3	1.12	1.12	1.12	1.12	1.20

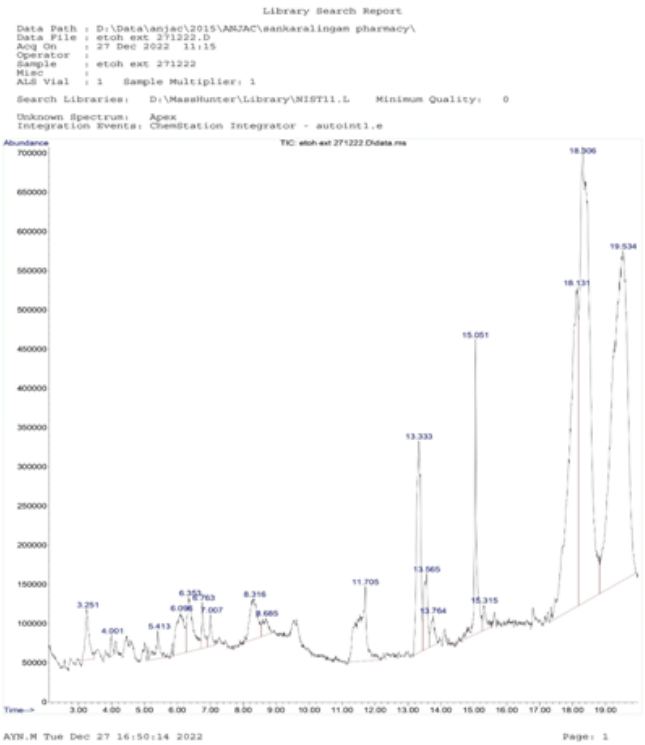


Figure 1. GC-MS chromatogram of the ethanol fraction (polar) of *Nephelium lappaceum fruit rind extract*.

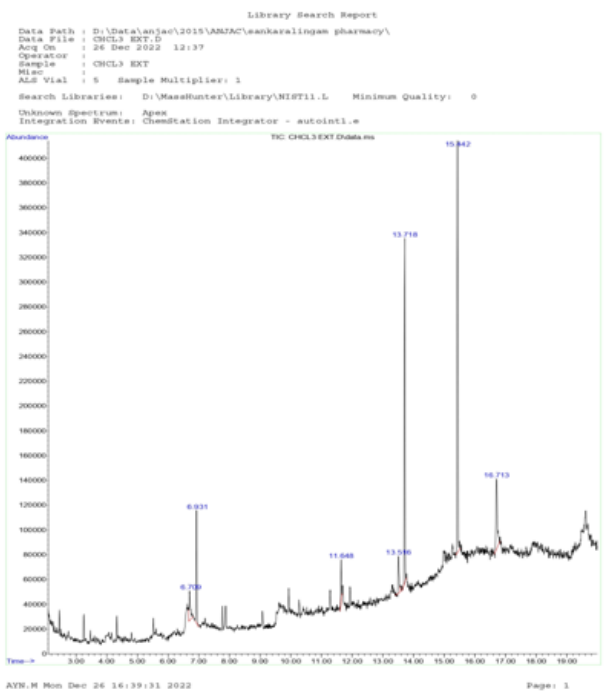


Figure 2. GC-MS chromatogram showing phytoconstituents in the chloroform (non-polar) fraction of *Nephelium lappaceum* fruit rind extract.

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REVIEW ARTICLE

Understanding *Manasa Prakriti* through Modern Psychology : An Ayurvedic Review based on the Dass - 42 Questionnaire

Kalptaru Roy[1]* and Mariya Husain[2]

[1]Second Year P.G Scholar, Department of Kriya Sharir, Parul Institute of Ayurved and Research, Parul University, Vadodara, Gujarat, India.
[2]Professor, Department of Kriya Sharir, Parul Institute of Ayurved and Research, Parul University, Vadodara, Gujarat, India.

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***Address for Correspondence**
Kalptaru Roy
Second Year P.G Scholar,
Department of Kriya Sharir,
Parul Institute of Ayurved and Research,
Parul University, Vadodara, Gujarat, India.
E.Mail: kalpatarunroy@gmail.com

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ABSTRACT

The study delves into *Manas Prakriti*, highlighting how individuals' mental constitutions—divided into *Sattvika, Rajasik*, and *Tamasik* types—shape their behavioral and cognitive tendencies. These classifications are rooted in the dominance of the three *Gunas—Sattva* (purity), *Rajas* (passion), and *Tamas* (inertia), which shape a person's behavior and psychic health. Psychological parameters such as emotional responses, stress management, cognitive function, and behavioral tendencies are closely linked to these types of *Manas Prakriti*. For instance, individuals with *Sattvika* nature display calmness, intellect, and resilience, while *Rajasik* individuals tend to be ambitious and easily stressed, and *Tamasik* individuals may show tendencies toward lethargy and depression. The bio-psychosocial model in Ayurveda also suggests that psychological imbalances like stress and negative emotions (e.g.,*Bhaya* (fear), *Krodha* (anger), and *Shoka* (grief)) can lead to the vitiation of the *Doshas(Vata, Pitta, Kapha),* contributing to psychosomatic disorders. This review integrates classical Ayurvedic theory with modern psychological tools such as the DASS-42 questionnaire, which assesses depression, anxiety, and stress. By correlating *Manasa Prakriti* with DASS-42 outcomes, the study supports the relevance of Ayurvedic personality profiling in mental health screening and emphasizes the potential of constitution-based psychological interventions**.**

Keywords: *Manas,Prakriti*, psychological, *Gunas*, DASS 42 Scale.

INTRODUCTION

Ayurveda, the ancient Indian system of life lore's, emphasizes that true health is achieved only when there is harmony between the body *(Sharira),* mind *(Manas),* and knowledge (*Atman*). Central to this holistic understanding is the generality of *Prakriti* an existent's essential constitution formed at the time of generality and determined by the influence of heritable, environmental, and maternal factors. *Prakrati* of an individual is influenced by four factor which is also known *as Garbhasambhav samagri i*.e. Timimg, uterine condition, nutrition of mother and ovum and sperm at the time of consumption in which *doshas* are more dominating decide the *Prakrati*. [1] While *Sharirika Prakriti* explains the physical and physiological characteristics predicated on the *Tridosha* proposition, an existent's internal conduct and mental proclivities are defined by *Manasa Prakriti.* The relative dominance of the three *Gunas Sattva, Rajas,* and *Tamas* as well as the subtle effect of the five *Mahabhuta* (rudiments), which are present in different quantities, are the main factors that shape different *Manasa Prakriti*. The ascendancy of *Akasha and Tejas* fosters *Satvika* rates analogous as clarity, calmness, and intellect. *Vayu* and *Tejas* dominance contributes to *Rajasika* traits like ambition, impatience, and emotional perceptivity. On the other hand, *Prithvi* and *Apas* strengthen *Tamasika* attributes analogous as languor, confusion, and emotional dullness. These internal constitutions are not simply descriptive but have significant implications for how individualities respond to cerebral stress. *Sattvika Prakriti* individualities tend to show emotional rigidity and cognitive stability, while *Rajasika* types may witness heightened anxiety, restlessness, and impulsivity. *Tamasika Prakriti,* with its association with heaviness and inertia, is more vulnerable to emotional retirement, depression, and lack of provocation. These tendencies are reflected in the classical *Ayurvedic* understanding of internal disturbances like *Chittodvega* (anxiety), *Vishada* (depression), WHO introduced estimation approx. 1 million people die per year due to cause of depression [2] and *Moha* (vision), all of which affect from *Guna* imbalance. These tendencies are echoed in the *Bhagavad Gita,* where *Dhananjaya* internal state on the battleground is described as overwhelmed by *Vishada* and *Moha,* leading to *Chittodvega "Karpanya doshopahata svabhavah,"* a state where one's ingrain nature is clouded by helplessness and confusion. Ultramodern tools like the DASS- 42 assess symptoms similar as sadness, fear, and restlessness, which nearly act *Ayurvedic Manasa Bhavas* like *Vishada* (depression), *Udvega* (anxiety), and *Krodha* (perversity). These countries frequently arise from an imbalance of *Rajas* and *Tamas Gunas* in individualities with *Rajasika* or *Tamasika Manasa Prakriti.* This review looks at how Prakriti affects internal responses and psychological vulnerability when contrasting DASS-42 fields with *Guna*-based groups.

METHODOLOGY

This research undertakes a critical examination of foundational Ayurvedic texts—Charaka Samhita, Sushruta Samhita, Ashtanga Hridaya, and Kashyapa Samhita—to explore and contextualize the concept of *Manasa Prakriti* and its classification systems. To enhance the depth of analysis, supplementary references were drawn from academic dissertations and authoritative Ayurvedic reference books. A systematic search of online academic databases such as PubMed, Scopus, and Google Scholar was conducted using targeted keywords relevant to Ayurvedic psychology and the DASS-42 scale. The collected data were then systematically categorized, critically analyzed, and synthesized to draw conceptual parallels between classical Ayurvedic thought and contemporary psychological paradigms.

Definition of *Manas Prakriti*

'पुरुषस्य स्वाभाविक विशिष्टत्वं आत्म संक्रमणकालीन मनस्थितिः मानस प्रकृतिः ।' (Dr. R.P. Chipa)

The mental constitution, referred to as *Manasa Prakṛti,* is determined by the psychological impressions *(Saṃskaar)* carried by the *jeeva,* along with the influence of paternal *Beeja* (sperm) and maternal *Artava* (ovum) at the time of conception.

Classification of *Prakriti*

"Sharirik Prakriti, or bodily constitution, is divided into seven categories based on the predominance of the three *Doshas—Vata, Pitta,* and *Kapha* either individually, in dual combinations, or all three together as *Sannipataja*.[3]According to *Acharya Charka* and *Acharya Susurat*, there are 16 varieties of *Manasika Prakriti* [4](Psychic

constitution of the body). According to the *Bhagavad-Gita,* there *are* two types of *Manas Prakriti* - Divine (*Daiva)* and demon (*asuri)* [5] As per *Kashyapa Samhita* outlines three primary divisions of mental constitution: eighteen *forms* rooted in purity and stability *(Sattvika),* seven shaped by passion and aggression *(Rajasika), and* three inclined toward inertia and ignorance *(Tamasika).[6] Acharya Charaka* mentions types , Mental Constitutions and Characteristics of *Satvika Kaya*(Table no. 1)**,** *Rajasak*(Table no. 2)**,** *and Tamasik kaya*(Table no. 3)**.**

Characteristic of Manasa

वैवृत्यान्मनसो ज्ञानं सान्निध्यात्तच्च वर्तते।
अणुत्वमथ चैकत्वं द्वौ गुणौ मनसः स्मृतौ।। [25]

The mind is characterized by two key qualities: indivisibility (*Ekatva*), indicating it can attend to only one thought at a time, and subtlety (*Anutva*), referring to its minute, pervasive nature. It engages with various cognitive functions such as contemplation, analysis, inference, emotional focus, and decision-making."[26]

***Chintya*:** *Chintya* refers to that which requires deliberate contemplation actions or thoughts that demand careful consideration before deciding whether or not they should be pursued.

Vicharya: *Vicharya* denotes a process of logical analysis or evaluation that enables the mind to make a clear decision- either to accept or to reject a particular thought or course of action.

Uhya: *Uhya* involves hypothetical reasoning and internal dialogue, where the mind engages in speculation and logical deduction based on available information

***Dhyeya*:** *Dhyeya* refers to emotionally driven contemplation, where the focus is on a specific object or goal held with personal significance.

***Sankalp*:** *Sankalp* signifies a deliberate mental resolution or decision formed through thoughtful reflection.

Functions of *Manasa*

इन्द्रियाभिग्रहः कर्म मनसः स्वस्य निग्रहः।
ऊहो विचारश्च, ततः परं बुद्धिः प्रवर्तते।।[27]

The mind operates through several vital functions: it directs and governs the senses (*Indriyabhigraha*), exercises personal control and restraint (*Svasyanigraha*), engages in speculative thinking or forming mental projections (*Uha*), and reflects deeply to evaluate thoughts and actions (*Vichara*)."

Physiology of *Manasa*: It can be divided into three stages:

1. *Indriyabhigraha* – The mind directs and controls the senses to focus on external stimuli.
2. *Uaha* and *Vichara* – It reflects, analyzes, and evaluates information using memory and logic.
3. *Buddhipravṛtti* – The intellect finalizes decisions and initiates appropriate responses.

***Manovaha Srotas*:** Charaka describes the body's internal channels (*Srothas*) as conduits not only for the *Tridoshas* but also for the mind itself, which travels through them to infuse every cell with awareness or *Chetana."*[28]

Psychopathology in Ayurveda: Classical texts extensively discuss disturbed or imbalanced states of the mind, commonly referred to as mental disorders or *Manasa Vikaras*. Acharya Charaka emphasizes that the predominant pathogenic influences on the mind are the doshic qualities of *Rajas* and *Tamas*, who obstruct the Manovaha *Srothas*, giving rise to *Manasa Vikaras.*

Manasa Bhavas: *Acharya Charaka* described 22 mental states that define the psychological and emotional framework of an individual. These are: *Mano-Artha* (focused perception), *Vijnana* (discrimination or decision-making), *Raga*

(attachment), *Moha* (delusion), *Krodha* (anger), *Soka* (grief), *Harṣa* (joy), *Priti* (contentment), *Bhaya* (fear), *Dhairya* (courage or steadiness), *Vīrya* (energy or enthusiasm), *Avasthana* (mental stability), *Sraddha* (faith or intention), *Medha* (intellect), *Sanjna* (recognition), *Smrti* (memory), *Hri* (modesty), *Sila* (discipline), *Dvesa* (aversion), *Upadhi* (mental conditioning), *Dhṛti* (patience or restraint), and *Vasyata* (obedience or submissiveness). [29]

Depression (*Visada* / *Avasada*): The two disorders listed in the *Ayurvedic* texts, *Vishada* and *Avasada,* are quite similar to depression. [30],[31] "*Viṣādo roga-vardhanam*" indicating that persistent sorrow can aggravate existing diseases. *Viṣāda* is classified among the *Vāta-nānātmaja Vikāras*, showing the predominance of *Vāta Doṣa* in its development.[32] *Avasāda* involves diminished drive, hopelessness, and emotional blunting—traits seen in modern depressive disorders.[33] These symptoms are frequently observed in individuals with *Tāmasika* or *Vātaja Manasa Prakṛti,* whose mental faculties like *Dhairya* (fortitude), *Smṛti* (memory), and *Sankalpa* (will) become weakened due to *Guna* imbalance.

Anxiety (*Chittodvega*): The Ayurvedic concept of *Chittodvega* reflects a condition of mental restlessness and agitation associated with *Rājasa Guna* and *Vāta Doṣa*. According to Ayurveda, the mind is susceptible to its own imbalances - *Rajas Tamas* being the *Manas Doshas* that lead to emotional disturbances.[34] *Chittodvega* may act as a precursor to or coexist with other psychosomatic or psychiatric conditions.[35] From a biomedical perspective, anxiety is a state of heightened arousal triggered by perceived threats, often accompanied by worry, tension, tachycardia, hyperventilation, dizziness, and chest discomfort.[36] *Ayurvedic* references such as *Chittavibhramsha* (mental confusion), *Chittakṣobha* (mental agitation), and *Asvastha Chitta* (unstable mind) further support the traditional understanding of anxiety-like states.[37]-[38] *Ayurvedic* texts have described many words related to mental status *Chittavibhramsha* [39], *Chittakshobha* [40], *Asvastha Chitta* [41], *Anavasthita Chitta* [42] *and Chittodvega.*

Stress (*Udvigna Avasthā* / *Manasika Roga*): Though the term "stress" is not directly used in classical Ayurvedic texts, similar concepts are elaborated in the *Bṛhat Trayī*, which discusses *Manasika Roga*, mental *duḥkha*, and *Manasa Bhāva* vitiation.[43] prolonged exertion, improper suppression of natural urges, and emotional over activity disturb *Prāṇavaha Srothas*, leading to both somatic and psychic stress.[44] Stress affects the *Hridaya* (psychocognitive center) and impairs vital mental functions like *Dhi* (intellect), *Dhṛti* (retention), and *Smṛti* (recall). This deterioration is more evident in *Rājasa–Tāmasika Prakṛti* individuals, while those with *Sāttvika* dominance demonstrate greater psychological resilience. [45]Stress can appear in two ways: physically and psychologically. Overuse or misuse of the body results in physical stress. For instance, prolonged periods of intense exercise without food or the suppression of natural desires might vitiate *Pranavahasrothas*, which can result in coughing, breathing problems. [46]

DASS-42 Scale

The DASS-42 is a self-report scale used to evaluate emotional states such as depression, anxiety, and stress [47]. Here is a Correlation between DASS-42 AND *MANAS PRAKRITI* is understood in Table no. 4 [48].

DISCUSSION

The relationship between *Manasa Prakriti* and psychological parameters is crucial in understanding human mental health from an Ayurvedic perspective. Ayurveda categorizes mental constitutions into three primary types—*Sattvika, Rajasik*, and *Tamasik*—each influencing emotional stability, cognitive abilities, and behavioral tendencies. This classification provides a structured approach to assessing mental health predispositions and designing personalized interventions. *Sattvika* individuals, being calm, resilient, and intellectually driven, exhibit greater emotional stability and lower vulnerability to stress-related disorders. *Rajasik* individuals, known for their ambition, dynamism, and competitiveness, are more susceptible to anxiety, restlessness, and stress-related conditions. *Tamasik* individuals, characterized by lethargy, inactivity, and a tendency towards negativity, are at a higher risk of depression and emotional withdrawal. Ayurveda suggests that psychological imbalances arise due to an excess of *Rajas* and *Tamas*, leading to stress, anxiety, and depression. Ayurvedic texts describe conditions like *Vishada* and *Chittodvega*, which

closely align with modern concepts of depression and anxiety. Depression is linked to *Tamas*, which reflects mental dullness, heaviness, and negativity. Those with *Tamas* dominance may feel stuck and unable to perform daily tasks. It can affect anyone, especially individuals with past trauma or *Tamas-Rajasic* mental traits, who often show higher DASS-42 depression scores. Conditions, if left untreated, can disrupt daily functioning, interpersonal relationships, and overall quality of life. Integrating Ayurvedic principles with modern psychological frameworks offers a holistic approach to mental health care. By assessing an individual's *Manasa Prakriti*, tailored lifestyle modifications, dietary interventions, and therapeutic measures can be implemented to restore balance. Ayurveda emphasizes mental discipline, meditation, herbal remedies, and Yoga as effective strategies to manage mental health conditions and enhance overall well-being.

CONCLUSION

This study elucidates the relationship between *Manasa Prakriti* and psychological parameters, bridging Ayurvedic principles with contemporary psychological theories. The classification of mental constitution into *Sattvika, Rajasik*, and *Tamasik* types provides a structured framework for understanding cognitive function, emotional regulation, and behavioral tendencies. The correlation between *Manasa Prakriti* and psychiatric conditions, particularly depression, anxiety, and stress, underscores the clinical relevance of this ancient classification. Individuals with Tamasik dominance exhibit characteristics analogous to major depressive disorder, including lethargy, emotional detachment, and cognitive impairment. Those with *Rajasik* predominance demonstrate heightened reactivity, impulsivity, and a greater susceptibility to stress-related disorders, aligning with generalized anxiety and mood instability. Conversely, *Sattvika*-dominant individuals exhibit emotional resilience, cognitive clarity, and lower vulnerability to psychological distress, suggesting a protective effect against mood disorders. By integrating Ayurvedic psychophysiological principles with modern psychiatric frameworks, personalized mental health interventions can be developed**.** Constitution-based therapeutic approaches**,** including nutritional modifications, cognitive-behavioral strategies, stress management techniques, and Ayurvedic interventions**,** can be tailored to an individual's *Manasa Prakriti* to optimize psychological resilience and overall well-being.

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Table No. 1 Mental Constitutions and Characteristics (Satvika Kaya)

No.	***Satvika Kāya[8]* Type**	**Features**
1	*Brahma Kaya[9]*	*Shuchita* (purity), *Sathya* (truthfulness), *Jitatmatva* (self-mastery), *Kama* (desire), *Krodha* (anger), *Lobha* (greed), *Moha* (ignorance), *Irshya* (jealousy), and *Amarsha* (intolerance)[9]
2	*Mahendra Kaya[10]*	*Ishwaryavanta* (lordship), *Dirghadarshita* (foresight in judgment), *Yadnavana* (performance of sacred rituals), *Shourya* (bravery), *Shakti Sampanna* (powerful energy), *Vachana Sampanna,* and pursuit of *Dharma, Artha,* and *Kama* in balance[10]
3	*Varuṇa Kaya*[11]	*Shuchita* and *Ashuchidvesha* (aversion to impurity). Such individuals are *Yajnavat* (ritual-oriented), interested in *Ambhuvihara* (aquatic activity), *Aklisthakarma* (non-violent conduct),

		and express *Kopa* (anger) and *Prasada* (pleasure)[11]
4	*Kubera Kaya*[12]	*Mana* (honor), *Shukhavihara* (luxury life routine), devoted to Dharma, Artha, and Kama, maintains Shuchi (cleanliness) and displays Kopa (anger) and Prasada.[12]
5	*Gandharva Kaya*[13]	Nrutya (dancing), Geeta (singing), Vada (music), enjoys Itihasa (history) and Purana (epics), fond of Gandha (fragrance), Malya (garlands), Anulepana (unguent application), Vasana (apparel), and Streevihara (female companionship) with Kama (desire)[13]
6	*Yamya Kaya*[14]	Smruti (memory), Vyapagatha Raga (detachment), but also associated with Irshya (envy), Dwesha (hatred), and Moha (ignorance)[14]
7	*Rishi kaya*[15]	*Adhyayana* (study), *Mada (pride), Raga (attachment), Dvesha (hatred), Moha (delusion), Lobha (greed), and Rosh (anger)*[15]

Table No. 2 Mental Constitutions and Characteristics (Rajasa Kaya)

NO.	*Rajasika Kaya*	Features
1.	*Asura Kaya*[16]	*Shura*, *Candha*, *Asuya*, *Ishvaravanta Aoupadika* (disguised superiority), *Rodra* (violent nature), *Anukrosha* (selective compassion), and *Atma Puja* (self-praise).[16]
2.	*Sarpa Kaya*[17]	*Shura* (brave) during Krudha (anger), but as Bhiru (coward) when calm. Includes Tiksna (quick-tempered), *Ayasabahala* (easily fatigued), *Santrastagochara* (frightened appearance), *and Aharaviharapara* (preoccupied with food and routine)[17]
3.	*Shakuna kaya*[18]	*Anushaktakama* (strong passion), *Ajasra Aharavihara* (excessive indulgence), *Mananavastata* (mental instability), *Amarshana* (intolerance), and *Asanchaya* (lack of foresight).[18]
4.	*Raksasa Kaya[19]*	*Marshana* (intolerance), continuous *Kopa* (anger), *Krura* (cruelty), excessive craving for *Aamisha* (non-veg food), *Swapna* (oversleeping), *Aayasa* (laziness), and *Irshya* (jealousy).[19]
5.	*Pishaca Kaya*[20]	*Marshana* (intolerance), continuous *Kopa* (anger), *Krura* (cruelty), excessive craving for *Aamisha* (non-veg food), *Swapna* (oversleeping), *Aayasa* (laziness), and *Irshya* (jealousy)[20]
6.	*Preta Kaya*[21]	*Atidukhasheela* (excessive sorrow), *Asuya* (jealousy), *Atilolupa* (greed), and *Akarma* (inaction or lethargy).[21]

Table No. 3 Mental Constitutions and Characteristics *(Tamasika Kaya)*

No.	***Tamasika Kaya***	**FEATURES**
1.	*Pashu Kaya*[22]	*Amedasa* (ignorance), *Gipushiachara* (vulgar conduct), focus on *Ahara* (food), *Maithunapara* (sexual indulgence), and *Swapnashilam* (sleepy disposition)[22]
2.	*Matsya Kaya*[23]	Exhibits *Bhiru* (fear), *Abhudha* (ignorance), *Aharalubdha* (food craving), *Anavastitha* (instability), *Kama* (lust), *Krodha* (anger), *Saranasheela* (restless movement), and *Toyakama* (desire for water).[23]
3	*Vanaspatya Kaya[24]*	*Alasa* (laziness), *Anivista Ahara* (over-eating), and *Sarva Bhudhi Anga Hina* (lack of all intellectual faculties).[24]

TABLE No. 4 DASS-42 AND MANAS PRAKRITI CORRELATION [48]

No.	**DASS-42 ITEM [47]**	**DASS-42 SCALE**	**MANASA PRAKRITI SUBTYPE**	**AYURVEDIC TERMINOLOGY[48]**	**AYURVEDIC DESCRIPTION**
1.	I found myself getting upset by quite trivial things [47]	Stress	*Rajasa - Raksasa Kaya*	*Amarshana* (intolerance), *Kopa* (anger)	Easily angered, lacks patience
2.	I was aware of dryness of my mouth[47]	Anxiety	*Tamsika - Matsya Kaya*	*Thoyakamam* (Desire for water)	Excessive physiological reaction to stress

3.	I couldn't seem to experience any positive feeling at all[47]	Depression	*Tamasika - Preta Kaya*	*Atidukhasheela* (excessive grief)	Chronic sadness, inability to enjoy
4.	I experienced breathing difficulty (e.g., excessively rapid breathing, breathlessness in the absence of physical exertion)[47]	Anxiety	*Rajasa - Sarpa Kaya*	*Santrastagochara* (fearful disposition)	Fear-induced physical responses
5.	I just couldn't seem to get going[47]	Depression	*Tamasika - Pashu Kaya*	*Akarma* (inaction), *Alasa* (laziness)	Lack of motivation, sluggishness
6.	I tended to over-react to situations [47]	Stress	*Rajasa - Asura Kaya*	*Rodra* (violent), *IshvaravantaAoupadika* (disguised movement)	Emotional volatility, exaggerated responses
7.	I had a feeling of shakiness (e.g., legs going to give way)[47]	Anxiety	*Tamsika - Matsya Kaya*	*Anavasthikam* (Unsteadiness)	Physical weakness due to stress
8.	I found it difficult to relax[47]	Stress	*Rajasa - Raksasa Kaya*	*Krura* (cruelty), *Amarshana* (intolerance)	Constant mental agitation
9.	I found myself in situations that made me so anxious I was most relieved when they ended[47]	Anxiety	*Tamasika - Matsya Kaya*	*Bhiru* (cowardice), *Abhudha* (lack of intelligence)	Fearfulness, avoidance of situations
10.	I felt that I had nothing to look forward to[47]	Depression	*Tamasika - Preta Kaya*	*Asuya* (envy), *Akarma* (inaction)	Hopelessness, lack of motivation
11.	I found myself getting upset rather easily[47]	Stress	*Rajasa - Shakuna Kaya*	*Anushaktakama* (attachment to desires)	Emotional instability, irritability
12.	I felt that I was using a lot of nervous energy[47]	Stress	*Rajasa - Asura Kaya*	*Chandha* (cruelty), *Dambha* (selfishness)	Hyperactivity, excessive energy use
13.	I felt sad and depressed[47]	Depression	*Tamasika - Preta Kaya*	*Atidukhasheela* (excessive grief)	Chronic sadness, persistent sorrow
14.	I found myself getting impatient when I was delayed or kept waiting[47]	Stress	*Rajasa - Raksasa Kaya*	*Amarshana* (intolerance)	Easily frustrated, low patience
15.	I had a feeling of faintness[47]	Anxiety	*Tamasika - Matsya Kaya*	*Ayasabahala* (fatigue), *Bhiru* (cowardice)	Physical exhaustion due to fear
16.	I felt that I had lost interest in just about everything[47]	Depression	*Tamasika - Pashu Kaya*	*Akarma* (inaction), *Nidraluthwam* (excess sleepiness)	Lack of motivation, apathy
17.	I felt I wasn't worth much as a person[47]	Depression	*Tamasika - Preta Kaya*	*Akarma* (inaction), *Asuya* (envy)	Self-deprecation, low self-esteem
18.	I felt that I was rather	Stress	*Rajasa* -	*Anavasthitham*	Highly reactive,

	touchy[47]		*Shakuna Kaya*	(instability)	mood fluctuations
19.	I perspired noticeably (e.g., hands sweaty) in the absence of high temperatures or physical exertion[47]	Anxiety	*Rajasa - Sarpa Kaya*	*Santrastagochara* (fearful appearance)	Physical anxiety response
20.	I felt scared without any good reason[47]	Anxiety	*Tamasika - Matsya Kaya*	*Bhiru* (cowardice), *Santrastagochara* (frightened disposition)	Generalized fear, irrational anxiety
21.	I felt that life wasn't worthwhile[47]	Depression	*Tamasika - Preta Kaya*	*Atidukhasheela* (excessive sorrow), *Akarma* (inaction)	Hopelessness, despair
22.	I found it hard to wind down[47]	Stress	*Rajasa - Asura Kaya*	*Rodra* (violent), *Krura* (cruel)	Restlessness, inability to relax
23.	I had difficulty swallowing[47]	Anxiety	*Tamasika - Matsya Kaya*	*Santrastagochara* (frightened state)	Stress-induced physiological response
24.	I couldn't seem to get any enjoyment out of the things I did[47]	Depression	*Tamasika - Preta Kaya*	*Atidukhasheela* (excess grief), *Akarma* (inaction)	Anhedonia, loss of pleasure
25.	I was aware of the action of my heart in the absence of physical exertion (e.g., sense of heart rate increase, heart missing a beat)[47]	Anxiety	*Rajasa - Sarpa Kaya*	*Santrastagochara* (fearful state)	Panic attacks, heightened awareness of body
26.	I felt down-hearted and blue[47]	Depression	*Tamasika - Preta Kaya*	*Vishadithwam* (sadness)	Persistent sadness, melancholy
27.	I found that I was very irritable[47]	Stress	*Rajasa - Asura Kaya*	*Dambha* (selfishness), *Rodra* (violence)	Short temper, aggression
28.	I felt I was close to panic[47]	Anxiety	*Rajasa - Sarpa Kaya*	*Bhiru* (cowardice)	Overwhelming fear, panic attack symptoms
29.	I found it hard to calm down after something upset me[47]	Stress	*Rajasa - Raksasa Kaya*	*Krura* (cruelty), *Amarshana* (intolerance)	Persistent emotional agitation
30.	I feared that I would be "thrown" by some trivial but unfamiliar task[47]	Anxiety	*Tamasika - Matsya Kaya*	*Bhiru* (cowardice), *Anavasthitam* (unsteadiness)	Fearfulness, lack of adaptability to new situations
31.	I was unable to become enthusiastic about anything[47]	Depression	*Tamasika - Pashu Kaya*	*Akarma* (inaction), *Alasa* (laziness)	Lack of motivation, apathy
32.	I found it difficult to tolerate interruptions to what I was doing[47]	Stress	*Rajasa - Raksasa Kaya*	*Krura* (cruelty), *Amarshana* (intolerance)	Easily irritated when disturbed
33.	I was in a state of nervous tension[47]	Stress	*Rajasa - Asura Kaya*	*Chandam* (anger), *Rodra* (violence)	Persistent emotional agitation

34.	I felt I was pretty worthless[47]	Depression	*Tamasika - Preta Kaya*	*Asuya* (jealousy), *Akarma* (inaction)	Self-doubt, low self-worth
35.	I was intolerant of anything that kept me from getting on with what I was doing[47]	Stress	*Rajasa - Raksasa Kaya*	*Amarshana* (intolerance)	Easily frustrated, low patience
36.	I felt terrified[47]	Anxiety	*Rajasa - Paishacha Kaya*	*Bhisayaitaram* Terrifying Disposition	Extreme fear, phobic tendencies
37.	I could see nothing in the future to be hopeful about[47]	Depression	*Tamasika - Preta Kaya*	*Atidukhasheela* (excess sorrow)	Pessimism, suicidal thoughts
38.	I felt that life was meaningless[47]	Depression	*Tamasika - Preta Kaya*	*Akarma* (inaction), *Vishadithwam* (sadness)	Existential crisis, despair
39.	I found myself getting agitated[47]	Stress	*Rajasa - Asura Kaya*	*Dambha* (selfishness), *Krura* (cruelty)	Quick temper, overreaction
40.	I was worried about situations in which I might panic and make a fool of myself[47]	Anxiety	*Rajasa - Sakuna Kaya*	*Nityasankitam* (Always in suspicious about other)	Social anxiety, performance fear
41.	I experienced trembling (e.g., in the hands)[47]	Anxiety	*Rajasa - Sarpa Kaya*	*Santrastagocarm* (Alarmingnature nature in each and every activity)	Physical manifestation of anxiety
42.	I found it difficult to work up the initiative to do things[47]	Depression	*Rajasa -Preta Kaya Tamasa- Vanaspatya kaya*	*Akarmasilam* (inaction), *Alasam* (Indolence) *Kama Varjitam*	Lack of drive, inability to take initiative

REVIEW ARTICLE

Echocardiogram based Cardiac Problem Prediction : A Literature Review of Employing Deep Learning Techniques

Prabha Kandaswamy[1]* and S.K. Mahendran[2]

[1]Research Scholar, PG and Research Department of Computer Science, RVS College of Arts and Science (Autonomous), Sulur, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
[2]Assistant Professor, PG and Research Department of Computer Science, Government Arts College (Autonomous), Gopalapuram, (Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.

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***Address for Correspondence**
Prabha Kandaswamy
Research Scholar,
PG and Research Department of Computer Science,
RVS College of Arts and Science (Autonomous), Sulur,
(Affiliated to Bharathiar University), Coimbatore, Tamil Nadu, India.
E.Mail: prabha2k9phd@gmail.com

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ABSTRACT

Around the world, Cardiovascular Disease (CVD) has far-reaching effects on people's health, happiness, and longevity. Echocardiography is the imaging technique most commonly used in cardiovascular medicine to evaluate heart function and abnormalities. It uses ultrasonic technology to provide high-quality images of the heart as well as its components. Though non-invasive and very useful clinically, echocardiography has issues including long treatments, operator subjectivity, and the difficulty of interpreting several readings. By greatly increasing the diagnostic accuracy of echocardiographic devices, recent developments in Deep Learning (DL) have transformed clinical decision-making. By use of DL models, which may automate the diagnosis of diseased heart diseases, analyse cardiac motion, and improve image quality, they become vital tools for precise detection and efficient treatment planning. Emphasizing their key contribution in improving the CVD prediction and diagnosis, this paper explores many DL-based frameworks especially meant to examine echocardiographic pictures across different cardiac perspectives. It also offers a thorough comparison of current algorithms, points out their shortcomings, and suggests new ideas to improve echocardiographic categorization systems, therefore allowing more accurate cardiac view identification and disease diagnosis.

Keywords: Cardiovascular Disease, Echocardiography, Deep Learning, Heart Conditions, Cardiac Views

INTRODUCTION

The coronary arteries are involved in the delivery of blood to the cardiac muscle and are primarily affected by a group of disorders collectively known as cardiovascular disease [1]. Conditions that decrease blood flow to the heart include microvascular angina, Coronary Artery Disease (CAD) and Myocardial Infarction (MI) [2].In certain situations, decreased blood flow might harm cardiac tissue. A more general category including any condition affecting the heart and blood vessels, they constitute a subgroup of cardiovascular disorders [3]. Common contributing elements are inflammation, high blood pressure, and atherosclerosis. Early identification is vital since long-term limited blood flow might harm cardiac tissue irreparably [4]. Cardiac Magnetic Resonance Imaging (CMRI), echocardiography, Computed Tomography (CT), as well as Multiple Gated Acquisition Scans(MUGA)are some of the imaging modalities utilized to detect CVD [5]. The Echo is the most common since it is portable, inexpensive, non-invasive, and easily available. Echocardiography records the heart's activity as it moves in many different directions using ultrasonography, hence providing doctors a clearer picture of the blood flow [6]. Echocardiography captures the heart's normal action by use of a three dimensional model represented by sequential frames. Time, frame height, and frame breadth make up the three dimensions [7]. Figure 1 general structure of echocardiography. The acquired echo data can be a video series covering the whole heart cycle or a static image captured at a specific point in the heart's cycle. A single cardiac cycle consists of two phases: the ventricles contracting (systole) and their relaxing (diastole) [8]. Learned echo modes include Doppler, A-mode, B-mode, and M-mode, each of which has its own distinct qualities [9]. Overall, Figure 2 displays A-Mode, B-Mode as well as M-Mode.

A-mode

The simplest type of ultrasound is called A-mode. As one transducer works its way over the body, the screen displays the echoes according to depth. Therapeutic applications of A-mode ultrasound include the use of negative wave energy to target certain tumors or calculi.

B-mode

When using B-mode ultrasound, a series of transducers in a linear array scan a two-dimensional plane within the body of the patient all at once.

M-mode

Movement is symbolized by the letter *M*. The m-mode allows physicians to track and measure the range of motion as the organ borders by scanning immediately in B-mode and having the images following each other on screen. This is because reflections change in relation to the probe.

Doppler mode

This mode utilizes the Doppler effect to assess and visualize blood flow by identifying frequency shifts that indicate the direction and speed of movement in relation to the probe. Testing cardiac valve function or portal hypertension's reverse blood flow are two examples of the many cardiovascular applications of this technique. Data from Doppler oscillations is shown graphically as spectral, color, and power Doppler, and audibly as synthesized beating sounds originating from speakers [10] [11]. Figure 3 depicts the general view of Doppler's mode in echo images.

Two Dimensional (2D)

2D echocardiographic imagery provides tomographic views of many planes of the cardiac structures, which is useful for M-mode and Doppler echocardiograms. The scan line in 2D echocardiographic imaging moves in a sweeping motion across an arc rather than being stationary. The echocardiography machine creates a 2D tomographic image for viewing after intricate processing of data collected by the transducer through the various scan lines [12].

Three Dimensional (3D) Echocardiogram

3D echocardiographic pictures can be taken in real-time or as simultaneously biplane (orthogonal) 2D images using a transducer that transmits and receives information in a three-dimensional volume simultaneous. In order to evaluate anatomy and function, various software applications are used to alter the 3D data set. In comparison to 2D echocardiography, 3D echocardiography is better for guiding surgical interventions, assessing the Right Ventricle (RV), localizing valvular abnormalities, and severe congenital heart defect. 3D color flow imaging provides a more accurate way to measure valvular regurgitation by carefully examining the vena contracts and PISA. Reportedly, it enables a quicker evaluation of Mitral Valve Area (MVA) within mitral stenosis compared to conventional 2D planimetry [13]. Figure 4 depicts a combination of 2D and 3D echocardiographic scans for better visualization and diagnosis of heart problems. Various heart images are obtained by shifting the transducer at different angles in order to examine mobility and structure. These views can be altered dependent on transducer positions at three major locations: A, B, and C [14].

- Location A has four views: apical two chambers (A2C), apical three chambers (A3C), apical four chambers (A4C), as well as apical five chambers (A5C) [15].
- Position B has a one view: The Parasternal Long Axis (PLAX) [16].
- Parasternal Short Axis of the Aorta (PSAA), Parasternal Short Axis of the Mitralvalve (PSAM) and Parasternal Short Axis of the Papillary muscles (PSAP) are the three views that make up Location C. [17]

A4C view

Using a superior-inferior ultrasound beam transsection of the thorax, the A4C image can be acquired. In echocardiographic laboratories, it is standard practice to display the LV and the RV on opposite sides of the screen.The apex and atria will be located at the highest and lowest points of the image, respectively, in every orientation. The LV apex, inferoseptal and anterolateral walls are all assessed by this picture. The RV's structure and function can also be assessed from this perspective.

A5C view

After a small rotation of the transducer, the proximal aorta, Left Ventricular Outflow Tract (LVOT), and aortic valve enter a fifth "chamber."By taking this perspective, one may qualitatively assess the aortic valve's anatomy in addition to its hemodynamics and the LVOT.

A2C view

To obtain the A2C view based on the apical 4C view, rotate 90° counterclockwise. The left atrium, inferior wall, apex, as well as mitral valve of the left ventricle may all be clearly seen in this image.

A3C view

To get the aorta back into view, the A2C view is rotated slightly counterclockwise (about 30°) to get the A3C, or apical long-axis view. The anatomical structures in this perspective are virtually identical to those in the PLAX view, except they are oriented differently. In most cases, the RVOT disappears from the picture, and the peak is more clearly seen. This image also provides more information on the hemodynamics of the mitral and aortic valves.

PLAX view

In a typical transthoracic echocardiographic examination, the first view is the PLA view. Located between the patient's right flank and right shoulder is the ultrasound beam. The image is arranged in a vertical fashion, starting from the top: the inferolateral wall, then the aorta, the cardiac apex on the left, and finally, the Right Ventricular Outflow Tract (RVOT) at the very top. The anteroseptal can be observed in the intersection of the RVOT and the LV hollow. The RV inflow can be seen by pointing a transducer slightly clockwise towards the left shoulder. This can be used to assess the tricuspid valve, RV apex, and the velocity of Tricuspid Regurgitation (TR).

PSAX view

Place the transducer in the parasternal long-axis position; then, rotate it clockwise by about 90 degrees to see the heart in the short-axis view. Beginning on the patient's left side and continuing to their right flank, this ultrasound image displays a horizontal plane. Variations in transducer tilt and positioning relative to the intercostal space allow for a variety of heart images.

PSAA

There are three leaflets visible in the PSAX image of the aortic valve: the right, left, along with non-coronary cusps. The leaflets open to a nearly circular aperture during systole and create a Y-shaped arrangement that resembles a Mercedes Benz emblem during diastole. Front and slightly to the medial are the septal as well as anterior tricuspid valve leaflets, and anterolateral are the pulmonary valve and also RV outflow tract.

PSAM

At the mitral valve level in the PSAX view, the LV appears spherical, and the mitral leaflets are visible when the ventricle opens approximately to its full cross-sectional area during diastole and closes during systole. Their movement is similar to that of a fish mouth, which can be a sign of abnormal chordae tendinae to the ventricular septum or other tissues in otherwise healthy men and women.

PSAP

In this view, the LV seems spherical, whereas the RV chamber appears semilunar, similar to the mitral valve levels. The two circular papillary muscles stick out into the LV chamber. The image below illustrates the posteromedial papillary muscle, which is on the left as well as the anterolateral papillary muscle on the right. This perspective, which gives helpful information on the LV's size and radial function, is frequently referred to as the "home base" of the PSAX. The interventricular septum might also be found to be aberrant. Figure 5 depicts different cardiac views in echocardiography that provide important spatial and temporal information for assessing heart structure and function. Despite its non-invasive nature, echocardiography has drawbacks such as lengthy procedure times, complex measurements, significant user subjectivity, and variability across observers, all of which necessitate extensive professional training. Artificial intelligence (AI) is a relatively new technology that researchers have recently used to improve the clinical examination of echocardiography with echocardiographs [18].AI encompasses Machine Learning (ML) and DL models, which aid physicians in automatically categorizing various cardio-views and cardiopathological conditions, including wall motion problems, identification of LV issues, and mitral regurgitation [19]. These models are interesting option with easy interpretation to be considered in CVD diagnostic and treatment mainly to be utilized for the early prediction with lower complexities [20]. Early stage cancer treatment and prognosis prediction has traditionally made use of ML techniques. When it comes to a number of soft-tissue examination activities, ML models implemented in Computer Aided Diagnostic (CAD) systems can greatly enhance the diagnostic efficacy for a physician [21].ML models are hypothesized with efficient image interpretation, précised decision making and provides accurate cardiovascular care and begin to alleviate some of the disparities in cardiovascular care [22]. There was a lot of intra- and inter-reader variability, and ML models were inaccurate.

Furthermore, due to their small heart size and indistinct borders, manual assessment of cardiac indices in fetus and infants is more complicated and prone to increased variability. When it comes to detecting and diagnosing heart problems, DL models outperform ML frameworks. In comparison to the time-consuming and subjective manual method, DL systems can be a cost-effective and efficient substitute. The use of such a system has the potential to alleviate the shortage of cardiology specialists in places with limited resources, manage variability among and within readers, and considerably decrease workload [23]. An assortment of DL models includes Recurrent Neural Networks (RNNs), Convolutional Neural Networks (CNNs), Long Short-Term Memory (LSTMs) as well as Deep Belief Networks (DBNs). Models like this help doctors make an objective and reliable diagnosis by analyzing relevant medical data for key features [24]. Improved echo image quality, quantification of cardio-motion, and detection of pathological illnesses are some of the ways DL hopes to boost echocardiography's diagnostic capabilities [25]. Its help makes classifying cardio-views, identifying cardiopathological problems like mitral regurgitation, wall motion

abnormalities, ejection fraction and longitudinal strain all much simpler [26]. Several DL frameworks have been created recently to categorize and predict CVD using echocardiography data. This paper investigates DL models applied in echocardiography to classify cardiac pictures and improve heart disease prediction. It also offers a comparison of the advantages and disadvantages of each model, which will assist direct future studies. Models are assessed in Section II, compared in Section III, then summarized with future direction in Section IV. Section II assesses the models, Section III contrasts them, and Section IV draws a conclusion with a summary and future direction.

Survey on MI Prediction using Deep Learning Models

Dergeli *et al*. [27] developed an Encoder-Decoder CNN (E-D CNN) to separate low-quality echocardiography and use ML to quickly identify myocardial infarction. This method employed E-D CNN to segment the entire LV wall. Next, the segmented left ventricular wall was analyzed using feature engineering in order to track the endocardial border. Finally, ML models such as Support Vector Machines (SVMs), Decision Trees (DTs), and Random Forests (RFs) are used to detect MI. In order to monitor cardiac indices, partition the heart based on areas, as well as fetch echocardiography information in real-time utilizing echocardiogram images, Zamzmi *et al*. [28] constructed the Trilateral Attention Network (TaNet). The model identifies specific areas of interest using a Spatial Transformer Network (STN) module and integrates feature embeddings for cardiac area segmentation. Then, MobileNetV2 was applied to join the segments, quantifies and localizes cardiac indices in echo images. In order to predict cardiac disease using echo images and the Internet of Medical Things (IoMT), Alamelu *et al*. [29] used a strategy called Lion-based Butterfly Optimization (L-BOA) combined with improved YOLO-v4 (LBO-YOLO-v4).This method is bi-staged; first, it uses patient-attached sensors to categorize data; second, it uses echocardiogram images to forecast the likelihood of cardiac illness. In order to classify sensor data and diseases, LBOA was used combined with Hybrid Faster R-CNN as well as SE-Rest-Net-101.When combined with CSPDarkNet53, YOLO-v4 becomes even more effective in extracting features from and classifying echo images. Hagberg *et al*. [30] developed a model to classify RV size and function from echocardiographic images using a 3D-CNN and Natural Language Processing (NLP). An RV-focused or 4-chamber view was chosen using a view classifier that this model trained on 2DE video loops that identified RV size and function.NLP is utilized to classify echocardiographic reports for RV size and function, while a 3D CNN was trained on auto-labeled echocardiographic images. A DL model was proposed by Lin *et al*. [31] to automatically detect RWMAs in echocardiography information from patients with myocardial infarction. At first, the Xception model was responsible for selecting the myocardium's perspective. Xception outputs will be segmented next by LSTM-Unet. The 3D-CNN model, which assesses cardiac activity in myocardial infarction and detects and classes regional wall motion abnormalities, received the segment and the original video as inputs.

Balakrishnan and Ambeth Kumar [32] categorized echocardiography images for CVD using Pre-Trained Recurrent CNN (PRCNN) over the Internet of Things (IoT). This approach drew on data from an IoT-based device, such as echocardiography images and patient medical histories. After DL models pre-processing and image segmentation, the pictures were further analyzed for categorization and heart disease risk assessments. They used PRCNN for classification and Fuzzy C-Means Clustering (FCM) for segmentation. Nguyen *et al*. [33] developed a technique for identifying myocardial infarction in echocardiography by means of an ensemble approach to learning of heart displacements. Initially, U-Net-model pre-processed and segmented the collected LV images. From the segmented pictures, the traits were then obtained. The SVM was then used to detect and classify MI. Arockia Valanrani *et al*. [34] developed a Contrastive Guided Adversarial Denoising Diffusion Probabilistic Model with GAN (CGADDPM-GAN) framework for generating anatomically accurate and superior quality echocardiographic pictures. MIDepnet model was also developed for identifying MI. This technique cuts down on training time and improves data representation. In MIDepnet model, an E-D CNN model segments the LV wall; a CNN model does the MI prediction. Using multi-view echocardiography, Derjeli *et al*. [35] built an automated algorithm for efficient MI prediction. Active Polynomials (APs) are employed by the model to extract and track the endocardial border of the left ventricle; these APs subsequently help to assess displacements of myocardial segments. MI detection was then done using DT, RF, k-Nearest Neighbors (kNN), SVM and 1D-CNN fed these characteristics. By examining cardiac segments for early MI identification, this method combines A4C and A2C perspectives to improve diagnostic accuracy. Bell-Navas *et al*. [36]

built a Higher-Order Dynamic Mode Decomposition (HODMD) and a Vision Transformer (ViT) for an automated cardiac pathology recognition from tiny echocardiogram datasets. HODMD for data augmentation and feature extraction first converts echocardiogram sequences into annotated, ML-compatible pictures. Even with little data, the second step analyzes these images and forecasts the state of the heart by training a ViT from scratch.

Comparative Analysis

This section presents a comparative research based on the advantages and disadvantages of cardiac views derived from using various DL methods that were briefly discussed in the previous section. The comparison is provided in table 1.

RESULT AND DISCUSSION

The performance analysis in Table 1 illustrates the accuracy of various deep learning models for predicting CVD using echocardiography images. There are comparisons shown in Figures 6–9 using F1 score, recall, accuracy, and precision. PRCNN [32] has the highest accuracy, while TaNet [28] excels in both precision and recall. MIDepnet and Realistic Synthetic 2D Ultrasound Dataset (RS2DUD) [34] produce excellent results, but SVM and CNN models perform poorly. The Function-based model outperforms the Size-based model in terms of feature extraction. Although PRCNN performs well, it may suffer from overfitting due to insufficient training data. To solve this, a future model is given that employs sophisticated DL techniques such as data augmentation, transfer learning, and regularization approaches to increase resilience, reduce overfitting, and improve real-time diagnostic accuracy.

CONCLUSION

Echocardiography is a diagnostic tool for complicated CVD and a popular screening modality for healthy, asymptomatic people. The problem of incorrect diagnosis has been a focus of extensive research into various DL methods. This study examines the predicted efficacy, limitations, and strengths of different DL methods for echocardiography cardiac view classification. Researchers looking to build strong models to improve CVD prediction as well as diagnosis might use the insights on difficulties and performance metrics mentioned here as useful tools. Patients with heart disease will soon be able to access individualized therapy because to these developments. Future research should focus on transfer learning, data augmentation, and regularization to tackle overfitting with limited datasets, enhancing model performance for real-time applications. These innovations can enable rapid cardiac predictions using echocardiographic data, improving diagnosis and patient outcomes.

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Table 1.Evaluation of cardiac images using several DL-associated echocardiography models

Ref No.	Techniques	Advantages	Disadvantages	Dataset
27	E-D CNN, SVM, RF, LDA	Works better on low-contrast echo images.	Struggles with missing LV wall and training on limited dataset	HMC-QU Dataset
28	TaNet, STN, MobileNetV2,	Improved wall target localization and boundary detection	Limited adaptability to diverse clinical settings	EchoNet-Dynamic, NIH IVC, NIH PLAX NIH Doppler dataset
29	SE-Rest-Net-101, L-BOA, improved YOLO-v4,CSP DarkNet53,Hybrid Faster R-CNN	Integrates sensor and image data for reliable result and has good model convergence	Lower performance on larger dataset and has poor generalization	Cleveland dataset from the UCI repository
30	3D-CNN, NLP	Enables large-scale annotation of echocardiographic images without manual labelling.	High variability in echocardiographic reports and has limited generalizability	91561 echo examinations made at Department of Clinical Physiology, Sahlgrenska University Hospital
31	Xception, LSTM-UNet,	Reduces interobserver variability has highly	Limited Generalization, slightly underestimate	Data collected from Chinese PLA General

	3D-CNN (XLUC)	scalable for large database lower time burden	high values of left ventricular ejection fraction	Hospital and Shandong University of Traditional Chinese Medicine
32	FCM, PRCNN	Integrates real-time IoT-based data with historic medical records for enhanced prediction	Trained with Limited dataset and risk of overfitting issues	UCI Echocardiogram Dataset with Multivariate medical dataset for echocardiogram images
33	Ensemble Learning, U-Net-model SVM	Effectively eliminates inter-image variability.	Training the model is a time-consuming process.	HMC-QU Dataset,
34	CGADDPM-GAN, DPM, CLL CNN	Effectively combine ground truth labels with the echo images, eliminating the need for laborious and error-prone hand labeling	Due to large number of network parameters, it result in high complexity	HMC-QU, Realistic Synthetic 2D Ultrasound Dataset (RS2DUD)
35	DT, RF, kNN, SVM and 1D-CNN	Eliminates overfitting and highly prompts to multi-view evaluation	Requires additional pre-processing task and high computational time	HMC-QU dataset
36	HODMD, ViT	Increased data quality by generating noise-free and highly discriminative features.	Required data for homogenization evaluation and results with pre-processing complexity.	Echocardiography sequences from mouse models representing diabetic cardiomyopathy, healthy, MI and TAC hypertrophy.

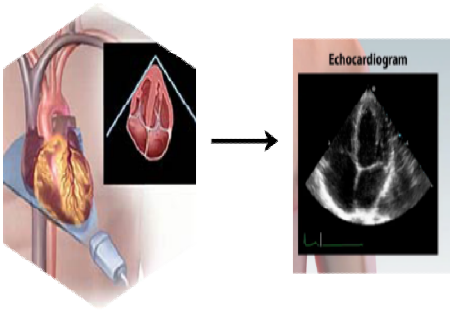


Figure 1: General View of Echocardiography

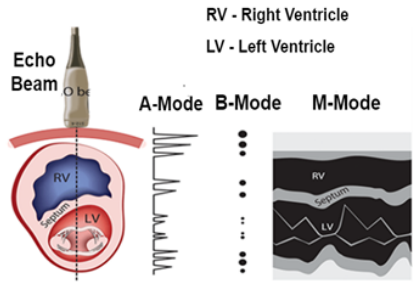


Figure 2: Examples of A, B, as well as M-mode

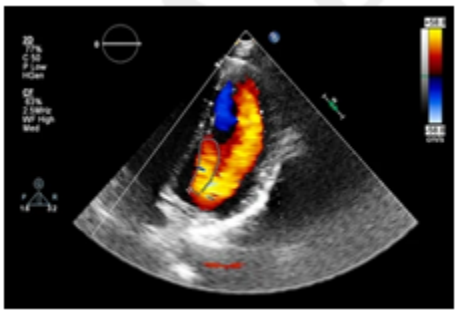


Figure 3: Doppler Mode

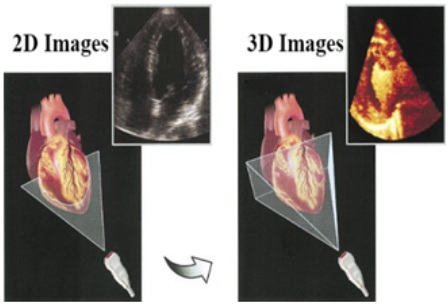


Figure 4: 2D and 3D views from Echo images

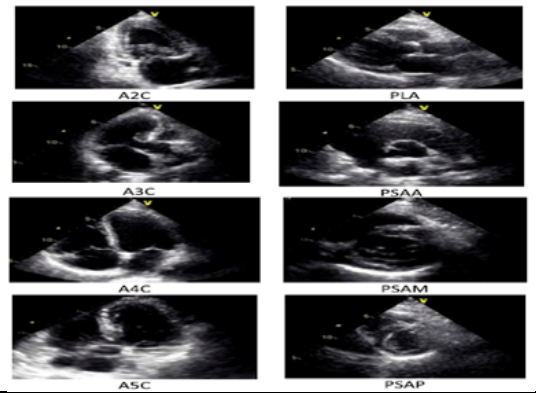


Figure 5: Different Cardio-Views of Echocardiography

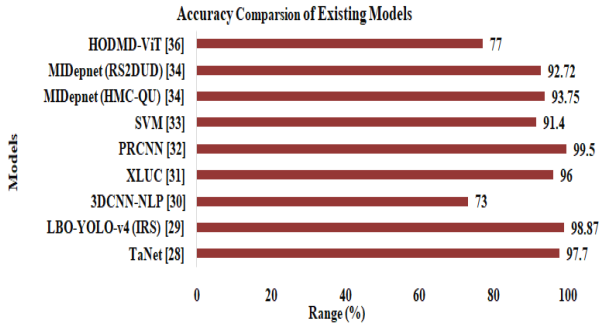


Figure 6: Accuracy Comparison of Existing Models for CVD prediction using Echo images

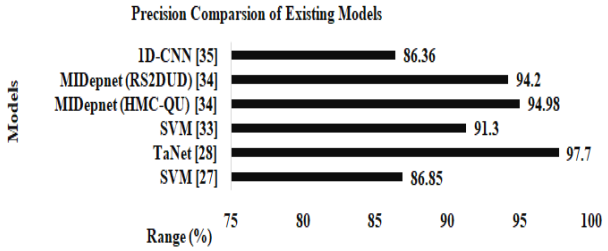


Figure 7: Precision Comparison of Existing Modelsfor CVD prediction using Echo images

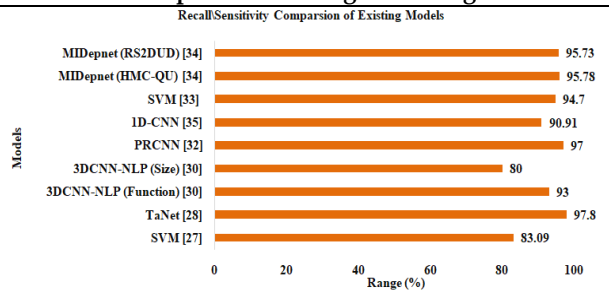


Figure 8: Recall\Sensitivity Comparison of Existing CVD prediction Models using Echo images

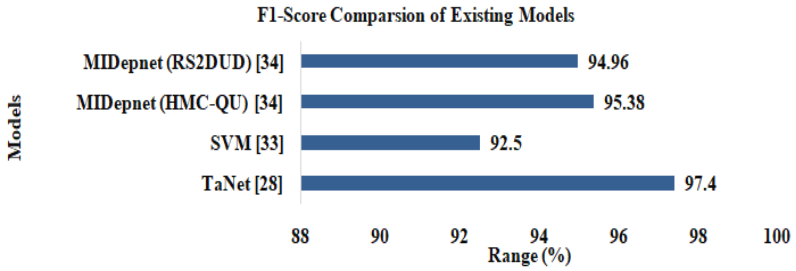


Figure 9: F1-Score Comparison of Existing Models for CVD prediction using Echo images

RESEARCH ARTICLE

Energy Analysis of Constant Pressure Ejector used in Ejector Expansion Refrigeration System (EERS)

Rahul Sharma[1]* and Sharad Chaudhary[2]

1Research Scholar, IET-DAVV and Assistant Professor, Department of Mechanical Engineering, IPS Academy, Institute of Engineering and Science, Indore, Madhya Pradesh, India.
[2] Professor, Department of Mechanical Engineering, Institute of Engineering and Technology, (Affiliated to Devi Ahilya Vishwavidyalaya) Indore, Madhya Pradesh, India..

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***Address for Correspondence**
Rahul Sharma
Research Scholar, IET-DAVV and
Assistant Professor,
Department of Mechanical Engineering,
IPS Academy, Institute of Engineering and Science, Indore,
Madhya Pradesh, India.
E.Mail: sharmarahul0311@gmail.com

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ABSTRACT

Refrigeration and air conditioning systems account for a significant portion of global energy consumption. Reducing this energy usage can help address the energy crisis and mitigate global warming by utilizing environmentally friendly refrigerant R134a.One promising approach to decrease power consumption in conventional refrigeration systems is the implementation of a two-phase ejector as an expansion device. A computer simulation of this enhanced cycle was conducted using a one-dimensional model that incorporates mass, momentum, and energy balances. Simulation through Engineering Equation Solver (EES) is done analytically with results indicating that the geometric parameters of the ejector design significantly impact the system's performance. The maximum coefficient of performance (COP) in ejector expansion cycle achieved is approximately 5.5. When compared to the standard cycle, the ejector expansion cycle demonstrates an increase in COP of about 8.86%.

Keywords: Ejector, Engineering Equation Solver, Refrigeration, R134a

INTRODUCTION

An ejector expansion refrigeration system (EERS) is an innovative refrigeration technology that replaces the expansion valve with an ejector to manage the flow and expansion of refrigerant. This system boasts several benefits

over conventional refrigeration systems, particularly in energy efficiency and operational flexibility. Essentially, EERS operates on vapor-compression cycles, utilizing an ejector in place of a throttle valve[7]. As illustrated in Figure 1, the EERS comprises four main components: evaporators, vapor generators, condensers, and ejectors. The system functions by heating the vapor generator, which produces refrigerant at high pressures and temperatures, known as the 'primary fluid.' This primary fluid is then directed into the ejector, where it expands and creates a low-pressure area in the evaporator, referred to as the 'secondary fluid.' The primary fluid collects and compresses the secondary fluid and the resulting mixture is passed to the separator which then passes to the evaporator through expansion valve. Finally, the compressed refrigerant is cycled back to the condenser completing the refrigeration cycle [8]. P-h Curve for Ejector Expansion Refrigeration Cycle (Fig. 2)

Refrigerant R-134a

R-134a (1,1,1,2-Tetrafluoroethane) is a widely used refrigerant, particularly in automotive air conditioning systems, household appliances, and commercial refrigeration. It is a hydrofluorocarbon (HFC) and has been popular as a replacement for R-12 (CFC-12) due to its lower ozone depletion potential (ODP) and better environmental properties. Below are some key properties of R-134a(Table:1).

METHODOLOGY

The methodology involved in the investigation of a 2-phase constant pressure ejector's in an Ejector Expansion Refrigeration System (EERS) using refrigerant R134a is outlined below. The mathematical model is developed in Engineering Equation Solver (EES) to analyse the effects of operational and geometric parameters on Entrainment Ratio, Pressure Lift and ejector efficiency.

Engineering Equation Solver (EES)

EES (Engineering Equation Solver) is used to solve a variety of interconnected non-linear differential and algebraic equations through numerical methods. EES has built-in property functions for many refrigerants (e.g., R134a, R22, R410A, R744/CO_2). Enthalpy, entropy, temperature, pressure, and specific volume is computed by different mathematical equation with the help of thermophysical built in properties and finally COP for both basic cycle and ejector expansion refrigeration system is evaluated.

Mathematical Modelling

The mathematical model employed here evaluates the relative efficiency of the ejector expansion cycle, primarily influenced by the entrainment ratio (ω) and the pressure differential ($P_e - P_b$) within the receiving section. This model also facilitates a deeper understanding of how different operational parameters affect the performance of the ejector expansion process in relation to these two critical variables. As studies progress, notable geometric variations come into play. In the accompanying diagram, high-pressure primary fluid (PF) is channelled through a nozzle where it expands and accelerates to supersonic speeds, producing a low-pressure zone. This zone, which has a lower pressure than the low-temperature evaporator, allows the high-velocity PF to entrain the secondary fluid (SF) via a passage connected to the evaporator. Flow Diagram for EES Software (Fig. 3). Although the PF and SF streams begin to interact, full mixing occurs only at designated points along the ejector's internal structure. The SF continues to accelerate within a narrowing passage created by the PF's expanding fan-cone jet flow. It is assumed that complete mixing happens by the end of the constant-area mixing chamber. Flow of Fluids in Ejector Sections (Fig. 4), Different Geometric Dimension of Ejector Section (Fig. 5). At this point, due to high back pressure aligned with the condenser pressure, a thin shock wave typically forms, compressing the mixed flow. This shock stabilizes the post-mixing stream to a uniform pressure and temperature. As the mixture moves through the subsonic diffuser, its speed decreases while partial pressure recovery occurs, leading to additional compression of the flow. Ejector Dimensions(All Dimensions are in mm) (Table1)

RESULTS AND DISCUSSION

Figure 6 implicit the COP increases with increase in evaporator temperature, as the temperature in evaporator rises from -25°C to 20°C the variation of COP for both i.e., Basic Cycle (VCRS) and Ejector Expansion Cycle (EEC) increase. Also, the higher COP can be achieved with EEC. In Figure 7, result shows that increasing evaporator temperatures from -25°C to 20°C, COP and Compression Ratio both increases at T_e =20°C maximum $COP_{(eec)}$ = 9.45 and compression ratio 5.5 can be achieved. Figure 8 implicit the COP decreases with increase in condenser temperature, as the temperature in condenser rises from 30°C to 70°C the variation of COP for both i.e., Basic Cycle (VCRS) and Ejector Expansion Cycle (EEC) decreases. Also, the higher COP can be achieved with EEC. In Figure 9, result shows that increasing condenser temperatures from 30°C to 70°C, COP decreases whereas Compression Ratio increases at T_c =30°C maximum $COP_{(eec)}$ = 14 with compression ratio 6 can be achieved. Figure 10 shows that as the temperature in evaporator increases from -25°C to 20°C compressor work decreases quantitatively, in ejector expansion cycle work done required is less as compared to basic cycle therefore the COP of ejector expansion cycle is higher. Figure 11 shows that as the temperature in condenser increases from 30°C to 70°C compressor work decreases quantitatively, Also in ejector expansion cycle work done required is less as compared to basic cycle therefore the COP of ejector expansion cycle is higher.

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Table1: Some Key Properties of R-134a

S. No.	Properties	Value
1	Chemical Formula	$C_2H_2F_4$
2	Molecular Weight	102.03 g/mol
3	Boiling Point (at 1 atm)	26.3°C (-15.3°F)
4	Critical Density	515.7 kg/m³
5	Latent Heat of Vaporization (at 0°C)	215.4 kJ/kg
6	Density (at 25°C)	1.207 kg/m³
7	Ozone Depletion Potential (ODP)	0
8	Global Warming Potential (GWP)	1430
9	Atmospheric Lifetime	Approximately 14.6 years
10	Flammability	Non-flammable (Class A1 under ASHRAE classification)

Table. 2: Ejector Dimensions(All Dimensions are in mm)

Geometrical Parameters	Notations	Value
Motive nozzle		
Exit position	NXP	0/3/6/9/12
Inlet diameter	D_{ni}	5.0 mm
Convergence angle	θ_n	12^0
Throat diameter	D_n	0.6/0.8/1.0/1.2 mm
Inlet diameter of secondary flow	D_s	6.0 mm
Mixing chamber		
Diameter at a constant area	D_{mc}	2.0 mm
Length at constant pressure	L_p	15.0 mm
Converging angle at constant-pressure	θ_m	40^0
Length at constant area	L_a	10.0 mm
Diffuser		
Exit diameter	D_d	6.50 mm
Length	L_d	32.0 mm

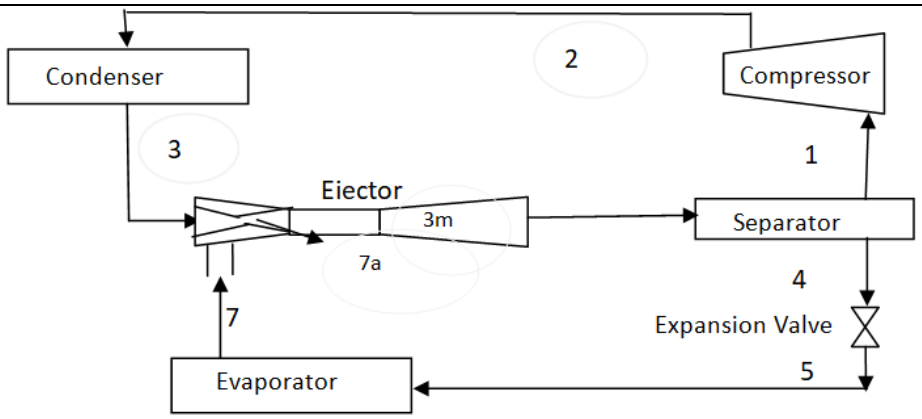


Fig.1: Layout of Ejector Expansion Refrigeration System

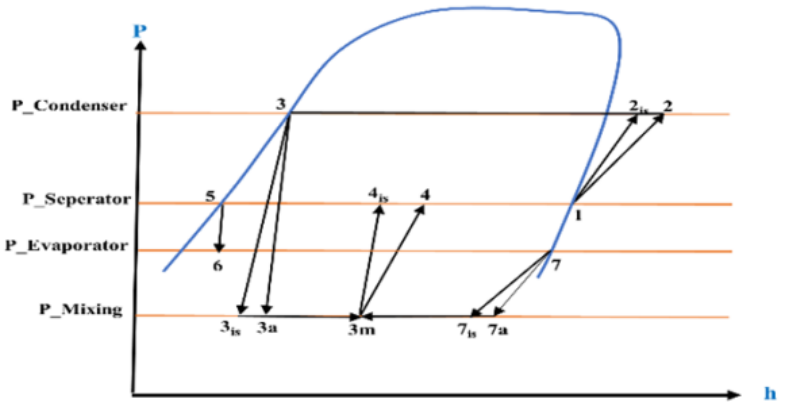


Fig. 2: P-h Curve for Ejector Expansion Refrigeration Cycle

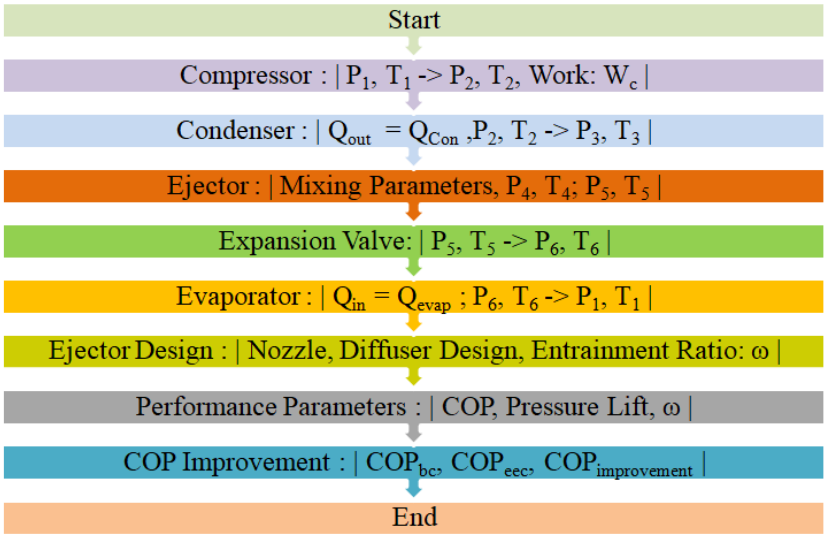


Fig. 3: Flow Diagram for EES Software

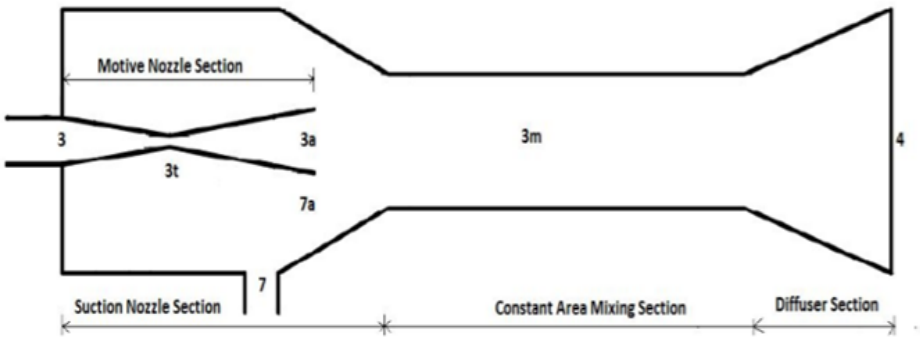


Fig. 4: Flow of Fluids in Ejector Sections

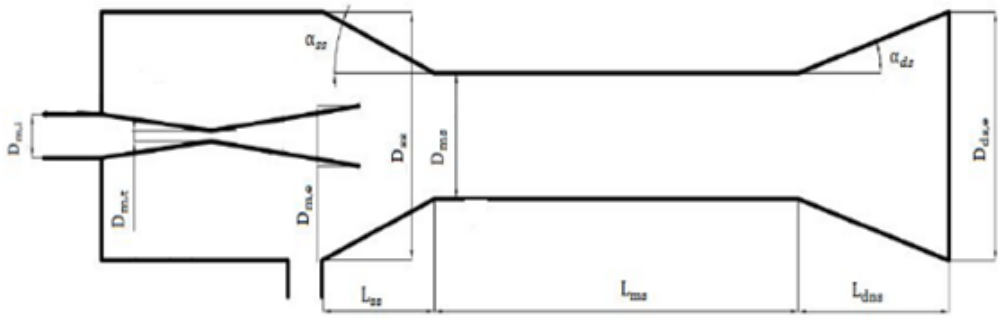


Fig. 5: Different Geometric Dimension of Ejector Section

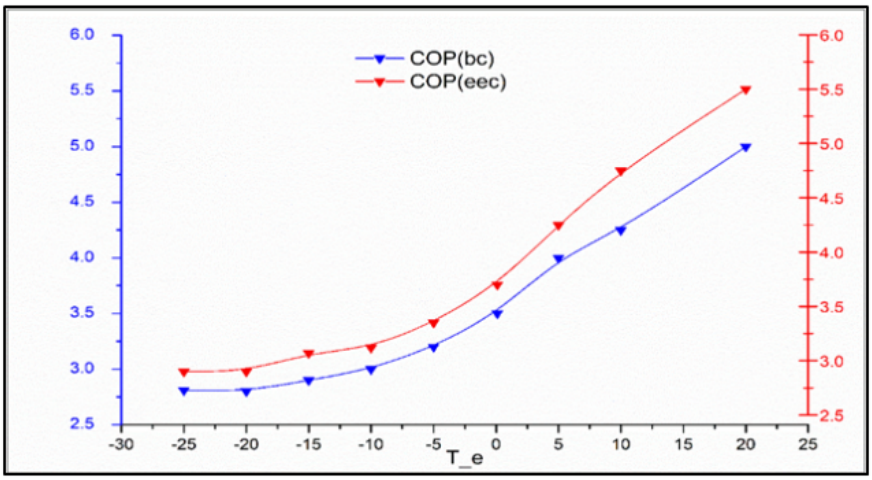


Figure 6: Variation of COP achieved for Basic and Ejector Refrigeration Cycle with Evaporator Temperature

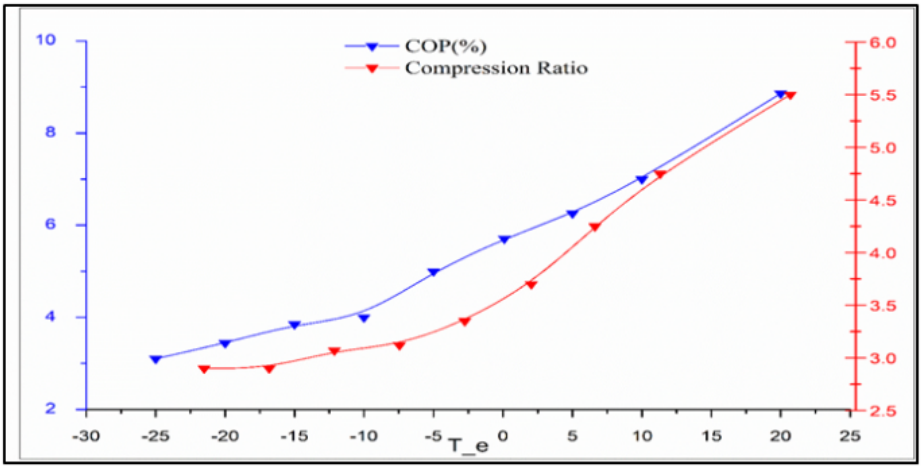


Figure 7: Variation of Percentage COP and Compression Ratio with Evaporator Temperature

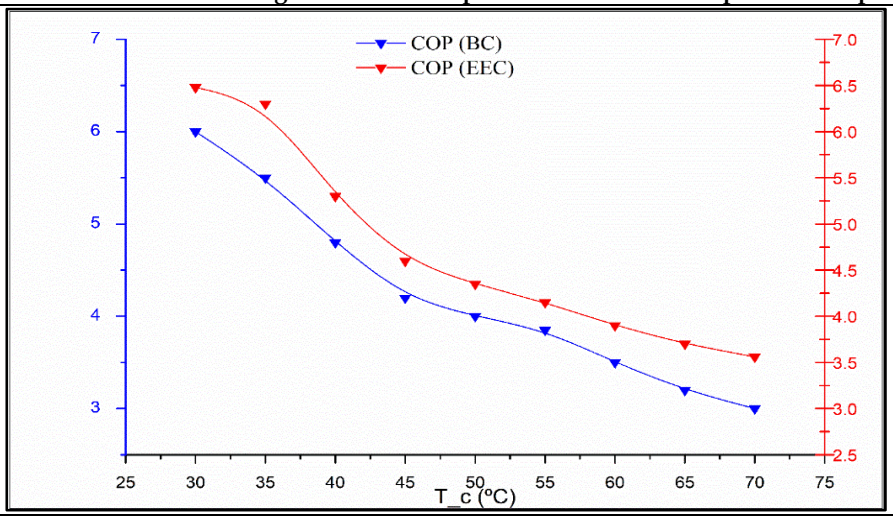


Figure 8:Variation of COP achieved for Basic and Ejector Refrigeration Cycle with Condenser Temperature

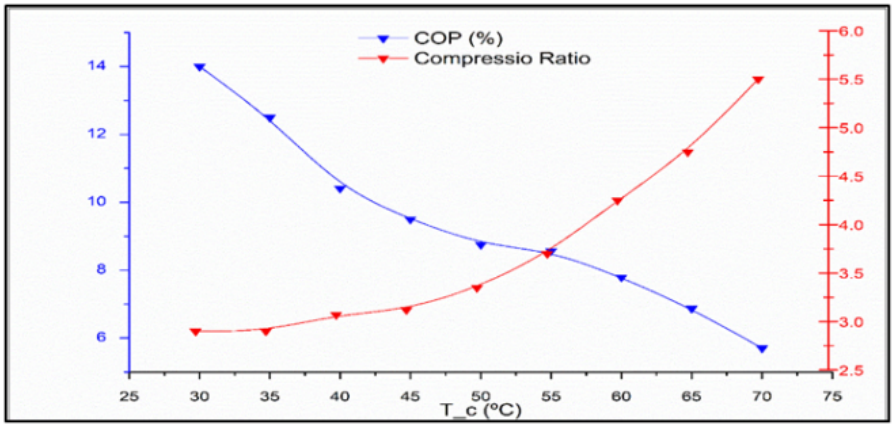


Figure 9: Variation of Percentage COP and Compression Ratio with Condenser Temperature

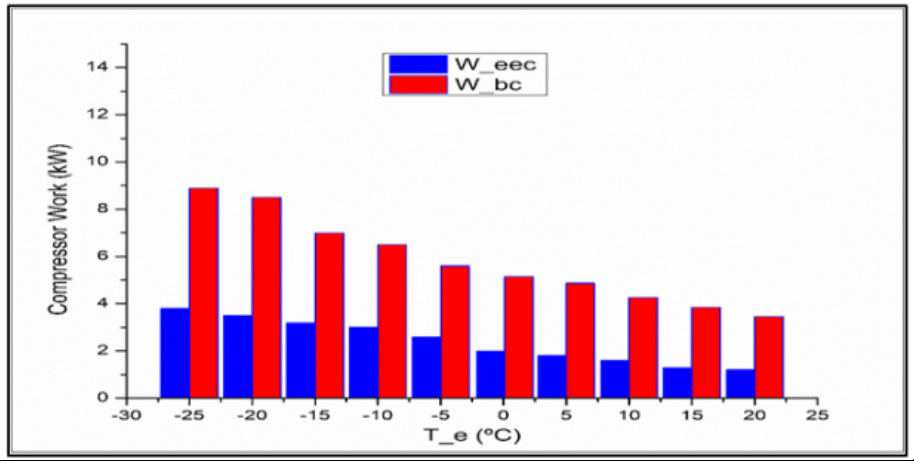


Figure 10: Compressor work for Basic and Ejector Expansion Refrigeration Cycle Operating with R134a Refrigerant against Evaporator Temperature

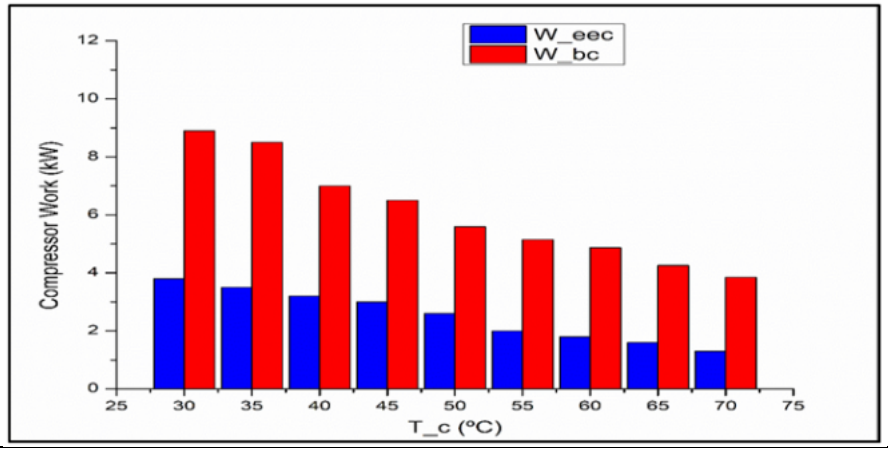


Figure 11: Compressor work for Basic and Ejector Expansion Refrigeration Cycle Operating with R134a Refrigerant against Condenser Temperature

RESEARCH ARTICLE

Development and Validation of Bioanalytical Methods for Monitoring Therapeutic Levels of Digoxin, Lithium, Valproate, and Theophylline

Deepali Kadam[1]*, Rutuja Salvi[2], Priya Katiyar[3], Dipali Jain[4] and Laxmikant Borse[5]

[1]Associate Professor, Department of Pharmaceutical Chemistry, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra , India.
[2]Student, Department of Pharmaceutical Quality Assurance, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra , India.
[3]Associate Professor, Department of Pharmacology, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra , India.
[4]Assistant Professor, Department of Pharmaceutical Chemistry, K. K. Wagh College of Pharmacy, Nashik, (Affiliated to Dr. Babasaheb Ambedkar Technological University, Lonere, Raigad) Maharashtra, India.
[5]Professor and Principal, Department of Pharmacology, Sandip Foundation's, Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik, (Affiliated to Savitribai Phule Pune University, Pune), Maharashtra , India.

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***Address for Correspondence**
Deepali Kadam
Associate Professor,
Department of Pharmaceutical Chemistry,
Sandip Foundation's,
Sandip Institute of Pharmaceutical Sciences, Mahiravani, Nashik,
(Affiliated to Savitribai Phule Pune University, Pune), Maharashtra , India.
E.Mail: drdeepalikadam777@gmail.com

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ABSTRACT

Therapeutic drug monitoring, or TDM, is crucial to optimizing drug therapy for medications with a narrow therapeutic index (NTI), where even slight variations in plasma concentration could cause toxicity or therapeutic failure. Among the drugs that require close monitoring include digoxin, lithium, valproate, and theophylline due to their distinct pharmacokinetic variability and narrow safety margins. Precise and timely measurements of their concentrations in biological matrices such as serum or plasma are necessary for safe and effective patient therapy. The bioanalytical techniques used to quantify these four NTI drugs are compiled and thoroughly reviewed in this review, with particular attention paid to sample preparation methods, analytical platforms like chromatographic, immunoassay, and

spectrophotometric methods, and their validation parameters in accordance with regulatory guidelines. The reliability, sensitivity, specificity, and therapeutic usefulness of each approach are emphasized. As a complete resource to facilitate technique selection and development in TDM, the study also addresses new trends and existing issues in routine clinical monitoring.

Keywords: Therapeutic Drug Monitoring (TDM), Narrow Therapeutic Index Drugs, Digoxin, Lithium, Valproate, Theophylline, Bioanalytical Methods

INTRODUCTION

Definition and Purpose of TDM

Therapeutic drug monitoring (TDM) is a strategy that measures drug concentrations in biological fluids, particularly serum or plasma, to optimize medication therapy and reduce toxicity, particularly for drugs with narrow therapeutic indices, ensuring correct therapeutic efficacy and dosage adjustments. [1,2]

Clinical Importance of Monitoring Plasma Drug Concentrations

TDM plays an important role in measuring the level of drug in plasma based on the influence by such factors as age, body weight, liver and kidney functions, interaction and genetic polymorphisms especially in the narrow therapeutic range drugs.[3,4,5,6,7]

Challengesin Bioanalysis of Narrow-Therapeutic Index Drugs

There is a challenge in the analysis of low drug levels in bioanalysis of NTI medicines because they are highly sensitive and specific and thus need standardized analytical methodologies and dosage to achieve maximum effect in the treatment.[8,9,10]

Digoxin

Pharmacology and Therapeutic Range

Digoxin is a cardiac glycoside that is used to treat heart failure and atrial fibrillation because it blocks the Na + / K + -ATPase pump to increase contractility and reduce the heart rate, which must be monitored during the treatment.[11,12,13,14]

TDM Importance and Risks of Toxicity

TM of digoxin plays an important role in managing the drug, since it allows early detection of high levels of digoxin followed by dose adjustments, thereby avoiding toxicity and associated harm.[,16,17,18,19,20,21,22]

Clinical Approach to TDM for Digoxin

Blood samples in TDM of digoxin are taken after 6 hours of drug administration, paying attention to renal functioning, creatinine clearance level, and glomerular filtration rate as well as the necessity to monitor electrolyte levels to reach optimal drug level.[23,24,25,26]

Lithium

Indications in Psychiatry

Lithium is the gold standard in the treatment of bipolar disorder by regulating the intracellular pathways to control the affected moods and prevent suicide thus effective both in short and long-term afflictions.[27,28,29]

Serum Concentration Monitoring Challenges

The concentration of Lithium is vital since it has a narrow therapeutic index, which leads to the symptoms of toxicity, including tremors, nausea, vomiting, diarrhea, fatigue, and arrhythmias, coma, and renal failure.[30,31,32,33,34,35]

Additional Monitoring Considerations

Monitoring lithium levels, electrolyte levels, and thyroid function is crucial during therapy to prevent lithium-related issues like renal function and hypothyroidism in some patients.[36]

Valproate

Antiepileptic Applications

Valproate is a scheduled antiepileptic drug; it is given to cure generalized and focal types of epilepsy, as well as in the treatment of bipolar disorder and migraine prevention. [37,38,39,40]

Protein-Binding Implications

A protein bound drug like valproate needs proper estimation of serum levels in order to achieve its therapeutic effect. The progression of seizure conditions is regulated with the help of the doses of the medicine 50-100ug/mL as well as protein binding, renal, and hepatic activities.[41,42,43,44,45]

Clinical Monitoring and Considerations

Valproate levels must be regularly monitored to ensure the level remains within-therapeutic-range and avoid side effects. The free fraction of valproate may be measured in order to manage the dose. Hepatotoxicity and thrombocytopenia are avoided through liver functioning tests. Valproate compliments with other antiepileptics, thus leading to toxicity.[46,47,48]

Theophylline

Use in Respiratory Disorders

Theophylline is a bronchodilator that has been employed in the treatment of respiratory disorders such as COPD and asthma through phosphodiesterase inhibition, raising the amount of cyclic AMP, and enhancing ventilation but also has limited use due to its small therapeutic index.[49,50,51]

Need for Plasma Level Adjustment

The therapeutic window of theophylline is 10-20 5g/mL and toxic level is dangerous. It is important to measure and monitor the plasma levels particularly with patients who are newly treated, diseased, and prone to drugs.

Factors Affecting Theophylline Pharmacokinetics

Different factors affect theophylline metabolism among them being liver CYP1A2 enzymes and an individual can take longer or not to excrete theophylline back to the body depending on the body specifics.

Age: Theophylline clearance is age-variable and is susceptible to the elderly where the risk of toxicity is more probable. Clearance in neonates and young children is rapid and therefore adjustments are necessary frequently.[54].

Liver Function:Theophylline is a liver-metabolized drug classified as livery and may have toxic effects such that starting doses should be low, and more observation is needed in the patient with admitted liver disease.[55]

Renal Function: The scientists have discovered that theophylline clearance may be affected by the renal dysfunction caused, which results in the build-up of metabolites and enhances the possibility of unfavorable outcomes.[56].

Drug Interactions: Drugs such as ciprofloxacin or rifampin can affect the metabolism of Theophylline subsequently causing an increase in the toxicity or reduction in the effect through inhibition of CYP450 and stimulation of CYP1A2 respectively. [57,58]

Smoking: Cigarette smoking enhances the activity of CYP1A2 which suppresses theophilline clearance and thus reinforced the medication that require an elevated dose and that the smokers who quit the habit decrease the dose.[59]

Clinical Monitoring and Considerations

Theophylline has a narrow therapeutic margin and gets broken down through its metabolism; this is why monitoring the drug is essential in the serum. Trough level should be measured by collecting blood 5-12 hours after the dose. The essentials of monitoring are toxicity, toxicity, and electrolyte unbalances.[60]

Bioanalytical Methods for Therapeutic Drug Monitoring (TDM)

Sample Collection and Handling

The correctness of Therapeutic Drug Monitoring (TDM) is directly linked to correct collecting, processing, and handling of samples so that a diligent pre-analytical phase is achieved to avoid mistakes.

Pre-analytical Considerations

Matrix: TDM involves blood ratio such as plasma, serum, urine, or saliva to establish the distribution of drugs, protein binding and half-life. Plasma is of choice in terms of consistency and reliability whereas serum samples can be used with selected drugs.[3].

Anticoagulants: The type of anticoagulant plays a critical role in the quality of the samples whereby heparin and EDTA are prevalent in the collection of plasma samples. Low interference is the reason why heparin is the choice of therapeutic drug monitoring.[61].

Timing: TDM uses plasma or serum levels and concentrates on the trough and peak levels when measuring the concentrations of drugs. Other tests requiring Trough samples include drugs that have narrow therapeutic index wherein the drug levels are in the therapeutic range.[1].

Plasma vs. Serum Comparisons

Plasma obtained using anticoagulants results in the preservation of cellular components and proteins that enables drug drug trough levels in TDM assays, while serum provides the true drug concentrations but needs longer preparation prior to analysis.[62]

Extraction Techniques

The extraction methods of the samples preparation are meant to separate and concentrate drugs against the biologicals and depends on the factors such as drug polarity vis-a-vis column polarity, molecular weights, binding of drugs to protein, and the complexity of the biologicals.

Protein Precipitation

Overview: Protein precipitation is a simple technique involving the addition of a solvent, such as acetonitrile or methanol, to the sample, which precipitates proteins. The supernatant containing the analyte is then separated by centrifugation.

Advantages: This method is inexpensive, quick, and requires minimal equipment, making it suitable for routine analysis in clinical settings. It's particularly effective for highly protein-bounddrugs.

Disadvantages: While it's simple, the recovery rate can be low, and reproducibility may suffer, particularly for hydrophilic or highly protein-bound drugs. This can lead to loss of analyte or matrix interference [63].

Liquid-Liquid Extraction (LLE)

Overview: LLE is based on the principle of partitioning the drug between two immiscible liquids (e.g., hexane and water). The analyte partitions into the organic phase, which is then separated, evaporated, and reconstituted for analysis.

Advantages: High recovery and good selectivityfor a broad range of drugs. LLE is ideal for drugs with low solubility in water but high solubility in organic solvents.

Disadvantages: LLE is time-consuming, requires large volumes of organic solvents, and is prone to cross-contamination from other substances in the matrix, leading to low reproducibilityif not optimized [64].

Solid-Phase Extraction (SPE)

Overview: SPE involves the use of a solid adsorbent (e.g., silica or polymer-based resins) to bind the analyte from the liquid sample. The analyte is then eluted using a solvent. SPE is highly versatile and can be used with a variety of biological matrices.

Advantages: High reproducibility, good recovery rates, and the ability to scale for high-throughput analysis. It offers better purification of the sample and minimizes matrix effects, improving quantification accuracy.

Disadvantages: Higher cost, more labor-intensive, and it requires specific training to handle efficiently. Optimizing SPE for various drug classes can also take time [65].

Impact on Recovery and Reproducibility

One important part of the analysis is recovery and in methods such as LLE and SPE, usually, recovery is higher than protein precipitation which can lead to differences between samples.

Analytical Techniques

The analytical techniques used for TDM must be sensitive, accurate, and able to detect drug concentrations at low levels. The choice of technique depends on the nature of the drug, the required sensitivity, and the clinical setting.

Chromatographic Methods

High-Performance Liquid Chromatography (HPLC)

HPLC is one of the most widely used techniques in TDM for separating and quantifying drugs in biological fluids. It provides excellent resolution, allowing the detection of low concentrations of drugs even in complex biological matrices.

Advantages: High precision, ability to separate complex mixtures, and widely established methodology. It is capable of handling a wide range of analytes.

Limitations: HPLC can be time-consuming, with long analysis times, and requires skilled operators. Additionally, it may not offer the same sensitivity as mass spectrometry (MS) methods.

Liquid Chromatography-Mass Spectrometry (LC-MS/MS)

LC-MS/MS combines the separation power of liquid chromatography with the sensitivity and specificity of mass spectrometry, making it ideal for analyzing drugs with low therapeutic concentrations.

Advantages: It offers the highest sensitivity, selectivity, and precision available, capable of detecting even trace levels of drugs. LC-MS/MS can handle complex biological samples with minimal interference.

Limitations: It is expensive and requires specialized equipment and training. The setup and maintenance costs are high, making it less accessible for routine use in some clinical settings [66]. (Table:1)

Immunoassays

Enzyme-Linked Immunosorbent Assay (ELISA):

ELISA is a widely used immunoassay that detects and quantifies drugs based on antibody-antigen interactions. It's frequently used for high-throughput testing.

Advantages: Ease of use, rapid results, and minimal sample volume**.**

Limitations: Less sensitive than chromatography and more prone to cross-reactivity or interference from similar molecules.

Radioimmunoassay (RIA)

Uses radioactive isotopes to quantify drugs based on the binding to specific antibodies.

Advantages: Highly sensitive and suitable for drugs with low concentrations.

Limitations: Radioactive materials pose health risks, and the technique requires strict regulatory control, making it less commonly used today.

Fluorescence Polarization Immunoassay (FPIA) and **Chemiluminescence Immunoassay (CLIA)**
These assays rely on fluorescent or chemiluminescent signals for quantifying drugs. These techniques are more rapid and automated than traditional methods.
Advantages: **Rapid results and** suitable for point-of-care testing. They allow for the detection of multiple analytes simultaneously.
Limitations: **Lower sensitivity** compared to chromatography and potential **interference** from similar substances [67].

Spectrophotometry and Electrochemical Methods
UV/visible drugs can be observed in electronics spectrophotometry where light absorption at wavelengths is performed in an attempt to interact with the drugs; electrochemical methods operate in a similar manner only that a current or potential is used to interact with the substances.
Advantages: These methods are simple and cost-effective.
Limitations: They often lack the sensitivity and selectivity required for many therapeutic drugs, especially those present in trace amounts [68].

Comparative Analy sis of Bioanalytical Methods for Therapeutic Drug Monitoring (TDM)
KeyComparison Parameters Therapeutic Drug Monitoring (TDM) requires accurate and timely measurement of drug concentrations in biological matrices. The selection of an analytical method depends on several key factors:
Clinical Feasibility in routine hospital laboratories is based on infrastructure, Applicability automation, and operator skill level.

Comparative Overview of Analytical Techniques(Table: 2)
Drug-Wise Method Comparison Tables
Digoxin (Table 3)
Lithium (Table 4)
Valproate (Table 5)
Theophylline (Table 6)

Summary
LC-MS/MS is sensitive and specific and expensive and technically demanding. Immunoassays are fast, they are prone to interference. HPLC is applicable in hospital labs.

Challenges and Considerations in Clinical Practice
Despite advances in analytical technology, the clinical application of TDM faces several important challenges that can impact both result interpretation and patient outcomes.

Inter-patient Variability and Pharmacokinetics
Due to individual pharmacokinetics such as the patient age, weight, organ functioning, genetic polymorphisms, disease conditions, the plasma concentrations may greatly differ even when using a standard dose.[3, 72].

For example
Renal impairment can reduce elimination of drugs like lithium and digoxin, necessitating more frequent monitoring.
Liver dysfunction affects metabolism of valproate and theophylline.
CYP450 polymorphisms can alter metabolism rates, especially for drugs with narrow therapeutic indices. Consequently, fixed dosing regimens may be inadequate, making individualized TDM essential for effective therapy.

Protein Binding and Drug Interactions
Protein-bound drug such as valproate may require free drug level assessment which is toxicity at the higher concentration of the drug in hypoalbuminemia and drug-drug interactions.[73]

Limitations in Method Standardization across Laboratories

There remains a lack of universal standardization and harmonization of bioanalytical methods across laboratories. Differences exist in

Sample matrix used (serum vs. plasma)

Calibration standards and quality controls

Reference ranges and interpretive criteria

Instrumentation (e.g., manual vs. automated systems)

Differences in NTI drugs have the tendency to produce inconsistent outcomes, which interfere with clinical decisions. To some extent, this can be offset by the use of External Quality Assurance programs and inter-laboratory comparisons, but this is not done worldwide.[74]

Recent Advances and Future Trends

Technological innovations in analytical chemistry and data science are enhancing the precision, accessibility, and utility of TDM. These advances aim to overcome limitations of traditional methods and support more personalized, efficient patient care.

LC-MS/MS Multiplexing for Simultaneous TDM

Liquid chromatography-tandem mass spectrometry (LC-MS/MS) platforms have also greatly improved drug monitoring with a reduction in sample volume and cost along with processing time, especially to monitor polypharmacy patients and real-time drug adjustment purposes.[75]

Dried Blood Spot (DBS) Sampling

DBS sampling is a minimally invasive method that uses small volumes of capillary blood collected via finger prick, dried on filter paper, and sent for analysis. Its key benefits include (76)

Simplified logistics (no need for cold chain or venipuncture)

Suitability for paediatric and remote populations

Efforts to enhance DBS normalization algorithms and combine it with telemedicine and home-based care models are addressed to help with standardization challenges and increase compatibility with LC-MS/MS and HPLC methods.

Role of Artificial Intelligence (AI) in Method Optimization and Result Interpretation

Artificial Intelligence (AI) and machine learning models are being increasingly explored for

Predicting pharmacokinetic profiles from sparse data

Optimizing assay development (e.g., retention time prediction, peak deconvolution)

Personalizing dosing regimens using Bayesian forecasting and clinical variables (77)

Artificial intelligence (AI) in the form of clinical decision support systems (CDSS) offers the idea of promoting the dynamic, personalized, and proactive aspect of therapeutic drug management (TDM) by taking into consideration the patient-specific data along with previous information.

CONCLUSION

Personalized pharmacotherapy - Therapeutic Drug Monitoring (TDM) is very important in relation to drugs with narrow therapeutic index. The confidence of TDM depends on a verification and normalization of bioanalytical techniques employed to assess the amount of drugs. LC-MS/MS is the most accurate measuring system with low susceptibility to interferences described as a gold standard. They are also present in routine clinical settings by use of immunoassays and electrochemical assays. There are several target-specific issues: high specificity of digoxin, lithium using ISE or flame photometry and interpretation of protein-binding of valproate. There is a need to harmonize assay

protocols and reference ranges and new technologies such as the use of dried blood spots as a sampling method and AI-based analytics have allowed TDM to become more efficient and patient-specific.

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Table 1: Spectrophotometry and Electrochemical Methods

Parameters	Description
Sensitivity	Ability to detect low drug concentrations, essential for narrow therapeutic index (NTI) drugs.
Specificity	Ability to measure the intended drug without interference from metabolites or endogenous substances.
Cost	Includes equipment, reagents, labor and maintenance. Impacts scalability and routine use.
Turnaround Time (TAT)	Time from sample collection to reporting is crucial for timely clinical decision making.

Table 2: Comparative Overview of Analytical Techniques

Method	Sensitivity	Specificity	Cost	Turnaround Time	Clinical Applicability
HPLC-UV	Moderate	Moderate	High	Moderate	Standard in Hospital labs (64)
LC-MS/MS	Very high	Very high	High	Moderate high	Gold standard in research/specialized labs (69,64)
ELISA	Moderate	Moderate	Low	Fast	High use, potential interference (67)
RIA	High	High	High	Moderate	Accurate, but declining due to safety (71
FPIA/CLIA	Moderate–High	Moderate–High	Moderate	Fast	Automated and widely adopted (62)
Spectrophotometry	Low	Low	Very-Low	Fast	Only for Chromophoric drugs (68)
Electrochemical (ISE)	Low–Moderate	Moderate	Low	Fast	Widely used for lithium (70

Table 3: Digoxin

Method	Sensitivity	Specificity	Cost	TAT	Notes
ELISA	Moderate	Moderate	Low	Fast	Cross-reactivity with DLIS (67)
LC-MS/MS	Very high	Very high	High	Moderate	Superior performance; resolves DLIS interference (69)
RIA	High	High	High	Moderate	Accurate but radiological waste concerns (71)

Table 4: Lithium

Method	Sensitivity	Specificity	Cost	TAT	Notes
Flame-photometry	Moderate	Low	Low	Fast	Less commonly used today (70)
ISE (Electrode)	Moderate	Moderate	Low	Fast	Automated and widely used (70)
HPLC/LC-MS	High	Very high	High	Moderate	Rarely needed due to lithium's simple chemistry (69)

Table 5: Valproate

Method	Sensitivity	Specificity	Cost	TAT	Notes
ELISA	Moderate	Moderate	Low	Fast	Suitable for routine monitoring (67)
FPIA	Moderate	Moderate	Medium	Fast	Common in automated analyzers (62)
LC-MS/MS	Very high	Very high	High	Moderate	Differentiates free and total valproate (69)

Table 6: Theophylline

Method	Sensitivity	Specificity	Cost	TAT	Notes
FPIA	Moderate	Moderate	Medium	Fast	Widely used in hospital analyzers(62)
HPLC	High	High	Moderate	Moderate	Validated and affordable (64)
LC-MS/MS	Very high	Very high	High	Moderate	Used for confirmation in complexcases (69)

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RESEARCH ARTICLE

K-Odd Sequential Harmonious Labeling of Tree Related Graphs

D. Muthuramakrishnan[1]* and C. Sheela[2]

[1]Principal in-Charge and Associate Professor and Head, Department of Mathematics, National College (Autonomous), Karumandapam, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

[2]Assistant Professor, Department of Mathematics, National College (Autonomous), Karumandapam, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
D. Muthuramakrishnan
Principal in-Charge and Associate Professor and Head,
Department of Mathematics, National College (Autonomous), Karumandapam,
(Affiliated to Bharathidasan University), Tiruchirappalli,
Tamil Nadu, India.
E.Mail: dmuthuramakrishnan@gmail.com

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ABSTRACT

Graham and Sloane [2] introduced the harmonious graphs. Singh and Varkey [3] introduced the odd sequential graphs. Gayatri and Muthuramakrishnan ([4],[1]) introduced *k*-odd sequential harmonious labeling of graphs. In this paper we pattern to bequest *k*-odd sequential harmonious labeling of tree related graphs.

Keywords: odd Harmonious labeling, Bistar, Banana, $\langle K_{1,n} : m \rangle$, Complete bipartite.

INTRODUCTION

Throughout this paper we consider finite simple, connected and undirected graph. A graph $G = (V, E)$ with '*p*' vertices and '*q*' edges is called a (*p*, *q*) graph. The graph labeling is an assignment of integers to the set of vertices or edges or both subject to certain condition. Labeled graphs serve as a useful mathematical models for many applications such as coding theory including code design of good radar type codes and convolution codes with optimal auto correction properties. Graham and Sloane [2] introduced harmonious labeling during their study of modular versions of additive bases problems stemming from error correcting codes. A graph is said to be harmonious if there exist an injection $f : V(G) \to \{0, 1, 2, ..., 2q-1\}$. Such that the induced function $f^+ : E(G) = \{0, 1, 2, ..., 2q-1\}$ defined by $f^+(uv) = (f(u) + f(v)) (\text{mod } q-1)$ is a bijection and f is said to be harmonious labeling of G. In [4] Gayatri and Muthuramakrishnan say that a labeling is a *k*-odd sequential harmonious labeling for any integer $k \geq 1$. A labeling is a *k*-odd sequential harmonious labeling if there exists an

injection f from the vertex set V to $\{k-1, k, k+1, \ldots, k+2q-1\}$ such that the induced mapping f^+ from the edge set E to $\{2k-1, 2k+1, \ldots, 2k+2q-3\}$ defined by

$$f^+(uv) = \begin{cases} f(u)+f(v) & \text{if } f(u)+f(v) \text{ is odd} \\ f(u)+f(v)+1 & \text{if } f(u)+f(v) \text{ is even} \end{cases}$$

are distinct. A graph G is said to be k-odd sequential harmonious graph if it admits a k-odd sequential harmonious labeling. In this paper, we examine some results on k-odd sequential harmonious labeling of graphs. Throughout this paper, k denotes any positive integer ≥ 1. For brevity, we use k-OSHL for k-odd sequential harmonious labeling.

MAIN RESULTS

Definition 1

The Bistar graph $B_{n,n}$ is the graph obtained from k_2 by joining m pendent edges to both the ends of k_2.

Theorem 2.1

The graph Bistar $B_{n,n}(n \geq 2)$ is a k-odd sequential harmonious graph for any k.

Proof

Let $\{u, w, u_i, w_i, 1 \leq i \leq n\}$ be the vertices and $\{e_i, 1 \leq i \leq 2n+1\}$ be the edges which are denoted as in Fig. 1.

First we label the vertices as follows.

Define $f: V \to \{k-1, k, k+1, \ldots, k+2q-1\}$

$$f(u) = k-1$$

$$f(w) = 2n+k$$

For $1 \leq i \leq n$,

$$f(u_i) = 2i+k-2$$

$$f(w_i) = 2i+k-1$$

Then the induced edge labels are

For $1 \leq i \leq 2n+1$

$$f^+(e_i) = 2i+2k-3$$

Clearly, the edge labels are odd and distinct,

$$f^+(E) = \{2k-1, 2k+1, \ldots, 2k+2q-3\}.$$

Hence the graph Bistar $B_{n,n}(n \geq 2)$ is a k-odd sequential harmonious graph for any k.

Illustration 1

(a) 2-OSHL of $B_{3,3}$ is shown in Fig. 1(a).

(b) 9-OSHL of $B_{6,6}$ is shown in Fig. 1(b).

Definition 2

The graph $\langle K_{1,n} : m \rangle (m, n \geq 2)$ obtained by taking m disjoint copies of $K_{1,n}$ and joining a new vertex to the centers of the copies of $K_{1,n}$.

Theorem 2

The graph $\langle K_{1,n} : m \rangle (m, n \geq 2)$ is a *k*-odd sequential harmonious graph for any *k*.

Proof

Let $\{u_i, w_j, 1 \leq i \leq m, 1 \leq j \leq m-1, u_{ij}, 1 \leq i \leq m, 1 \leq j \leq n\}$ be the vertices and $\{e_i, 1 \leq i \leq mn + 2(m-1)\}$ be the edges which are denoted as in Fig. 2.

First we label the vertices as follows.

Define: $f : V \to \{k-1, k, k+1, \ldots, k+2q-1\}$ by

$$f(u_1) = k-1$$

For $2 \leq i \leq m$

$$f(u_i) = 2i + k - 3$$

For $1 \leq j \leq m-1$

$$f(w_j) = 2n + (2n+2)(j-1) + k.$$

For $1 \leq i \leq m, \ 1 \leq j \leq n$

$$f(u_{ij}) = 2j + (2n+2)(i-1) + k - 2.$$

Then the induced edge labels are:

For $1 \leq i \leq mn + 2(m-1)$,

$$f^+(e_i) = 2i + 2k - 3$$

Clearly the edge labels are odd and distinct.

$$f^+(E) = \{2k-1, 2k+1, \ldots, 2k+2q-3\}$$

Hence the graph $\langle k_{1,n} : m \rangle \ (m, n \geq 2)$ is a k-odd sequential harmonious graph for any *k*.

Illustration 2

(a) 3-OSHL of $\langle K_{1,4} : 3 \rangle$ is shown in Fig. 2(a).

(b) 4-OSHL of $\langle K_{1,3} : 3 \rangle$ is shown in Fig. 2(b).

Theorem 3

Banana tree $B_{m,n} \ (m \geq 2, \ n \geq 2)$ is a *k*-odd sequential harmonious graph for any *k*.

Proof

Let $\{u, v, w, u_i, 1 \leq i \leq m+n\}$ be the vertices and $\{e_i, 1 \leq i \leq m+n+2\}$ be the edges which are denoted as in

Fig. 3.

First we label the vertices as follows

Define $f: V \to \{k-1, k, k+1, \ldots, k+2q-1\}$ by

$f(u) = k-1$

$f(v) = k+1$

$f(w) = k+3$

For $1 \le i \le m+n$,

$f(u_i) = 2i+k-2$

Then the induced edge labels are

For $1 \le i \le m+n+2$,

$f^+(e_i) = 2(i+k)-3$

Therefore $f^+(E) = \{2k-1, 2k+1, \ldots, 2k+2q-3\}$. So f is a *k*-odd sequential harmonious labeling and hence, the Banana tree $B_{m,n}\ (m, n \ge 2)$ is a *k*-odd sequential harmonious graph for any *k*.

Illustration 3

(a) 3-OSHL of $B_{6,3}$ is shown in Fig. 3(a).

(b) 4-OSHL of $B_{4,4}$ is shown in Fig.3(b).

Theorem 2.4

$G(m, n)\ (m \ge 3, n \ge 4)$ is a *k*-odd sequential harmonious graph for any *k*.

Proof

Let $\{u_{ij}, 1 \le i \le m, 1 \le j \le n\}$ be the vertices and $\{a_{ij}, 1 \le i \le m, 1 \le j \le n-1, b_i, 1 \le i \le m\}$ be the edges which are denoted as in Fig. 4.

Here the labeling of vertices are defined as

$f: V \to \{k-1, k, k+1, \ldots, k+2q-1\}$

For $1 \le i \le m,\ 1 \le j \le n$,

$f(u_{ij}) = k+j-2+n(i-1)$

Then the induced edge labels are:

For $1 \le i \le m,\ 1 \le j \le n-1$,

$f^+(a_{ij}) = 2k+2j+2n(i-1)-3$

For $1 \le i \le m-1$,

$f^+(b_i) = 2k+2ni-3$

Therefore, $f^+(E) = \{2k-1, 2k+1, ..., 2k+2q-3\}$. So f is a *k*-odd sequential harmonics labeling and hence the graph $G(m, n)\ (m, n \geq 3)$ is a *k*-odd sequential harmonious labeling for any *k*.

Illustration 4

(a) 2-OSHL of $G(5, 7)$ is shown in Fig.4(a).

(b) 3-OSHL of $G(3, 6)$ is shown in Fig. 4(b).

Theorem 5

The graph $S(k_{1,n})\ (n \geq 2)$ is a *k*-odd sequential harmonious graph for any *k*.

Proof

Let $\{u, u_i, u_i^1, 1 \leq i \leq n\}$ be the vertices and $\{a_i, a_i^1, 1 \leq i \leq n\}$ be the edges which are denoted as in Fig. 5.

Here the labeling of vertices are defined as

$f : V \rightarrow \{k-1, k, k+1, ...k+2q-1\}$

$f(u) = k-1$

For $1 \leq i \leq n$,

$f(u_i) = k+2i-2$

$f(u_i^1) = k+4(n-i)+1$

Then the induced edge labeling are

For $1 \leq i \leq n$,

$f^+(a_i) = 2(k+i)-3$

$f^+(a_i^1) = 2(k-i+2n)-1$

Cleary the edge labels are odd and distinct. $f^+(E) = \{2k-1, 2k+1, ...2k+2q-3\}$. Hence, the graph $S(k_{1,n})(n \geq 2)$ is a *k*-odd sequential harmonious graph for any *k*.

Illustration 5

(a) 4-OSHL of $S(k_{1,6})$ is shown in Fig. 5(a).

(b) 7-OSHL of $S(k_{1,7})$ is shown in Fig. 5(b).

Theorem 6

The complete bipartite graph $K_{m,n}$ ($m \geq 2$, $n \geq 3$) is a *k*-odd sequential harmonious graph for any *k*.

Proof

Let $\{u_i, 1 \leq i \leq m, v_j, 1 \leq j \leq n\}$ be the vertices and $\{e_{ij}, 1 \leq i \leq m, 1 \leq j \leq n\}$ be the edges which are denoted as in Fig. 6.

First we label the vertices as follows

Define $f : V \rightarrow \{k-1, k, k+1, ..., k+2q-1\}$ by

For $1 \leq i \leq m$,

$f(u_i) = 2n(i-1) + k - 1$

For $1 \le j \le n$,

$f(v_j) = k + 2j - 2$

Then the induced edge labels are:

For $1 \le i \le m$, $1 \le j \le n$,

$f^+(e_j) = 2k + 2j + 2n(i-1) - 3$

Clearly, the edge labels are odd and distinct.

$f^+(E) = \{2k - 1, 2k + 1, \ldots, 2k + 2q - 3\}$. So, f is a k-odd sequential harmonious labeling and hence the complete bipartite graph $K_{m,n}$ ($m \ge 2$, $n \ge 3$) is a k-odd sequential harmonious graph for any k.

Illustration 6

(a) 4-OSHL of $K_{3,4}$ is shown in Fig. 6(a).

(b) 5-OSHL of $K_{6,4}$ is shown in Fig. 6(b).

CONCLUSION

The investigation is mainly focused on k-odd sequential harmonious graph for any positive integer k greater than or equal to one.

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Fig. 1: $B_{n,n}$ with ordinary labeling



Fig. 1(a): 2-OSHL of $B_{3,3}$

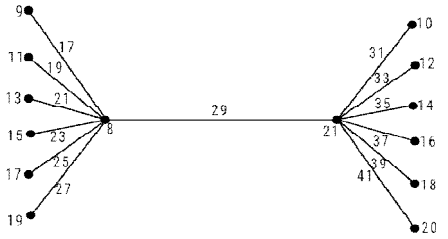


Fig. 1(b): 9-OSHL of $B_{6,6}$

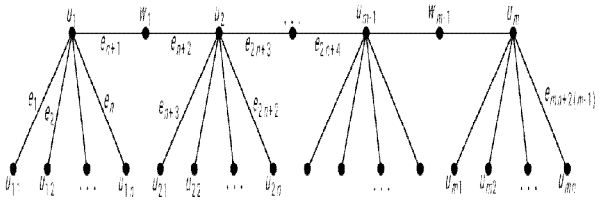


Fig. 2: $\langle K_{1,n} : m \rangle$ with ordinary labeling

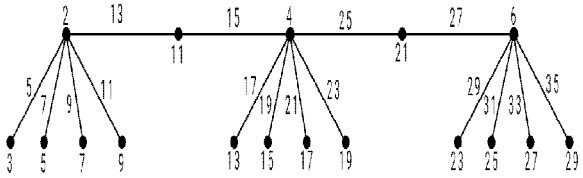


Fig. 2(a): 3-OSHL of $\langle K_{1,4} : 3 \rangle$

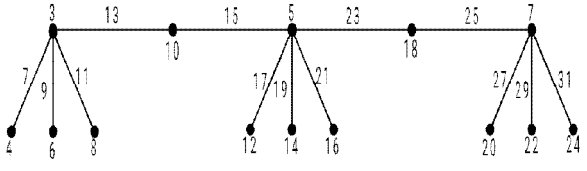


Fig. 2(b): 4-OSHL of $\langle K_{1,3} : 3 \rangle$

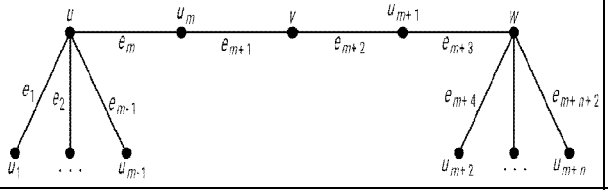


Fig. 3: $B_{m,n}$ with ordinary labeling

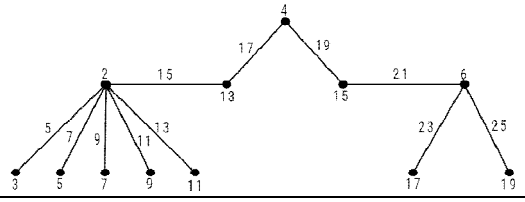


Fig. 3(a): 3-OSHL of $B_{6,3}$

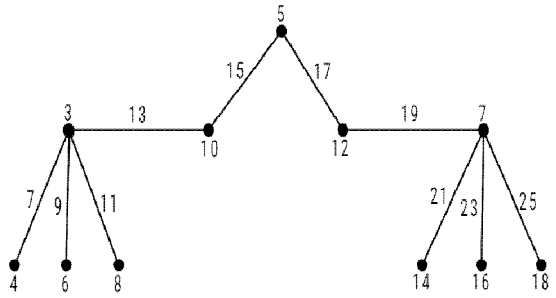


Fig. 3(b): 4-OSHL of $B_{4,4}$

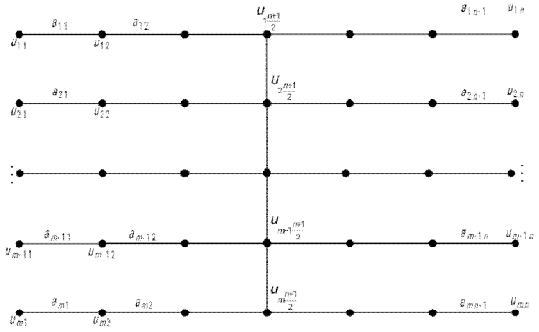


Fig. 4: Ordinary labeling of $G(m,n)$

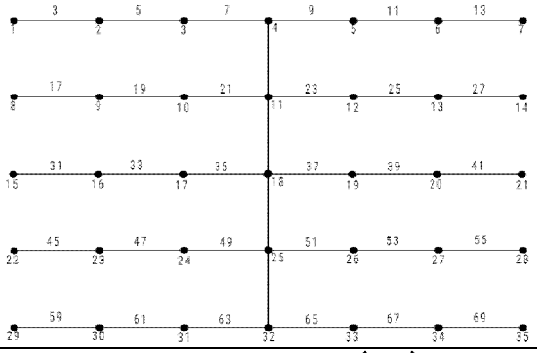


Fig. 4(a): 2-OSHL of $G(5,7)$

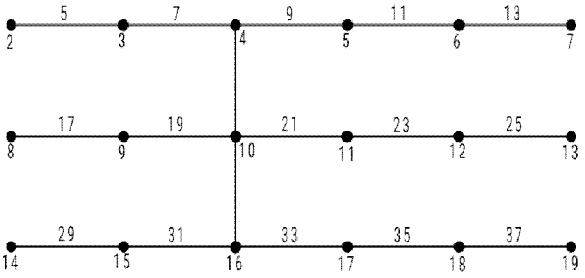


Fig. 4(b): 3-OSHL of $G(3,6)$

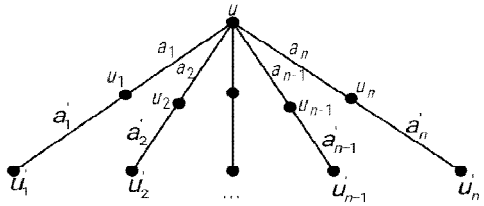


Fig. 5: Ordinary labeling of $S(k_1, n)$

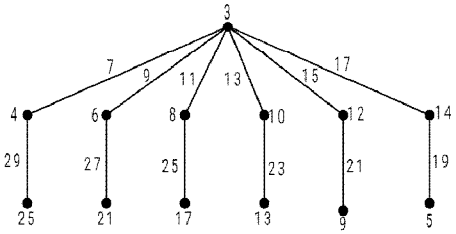


Fig. 5(a): 4-OSHL of $S(k_1, 6)$

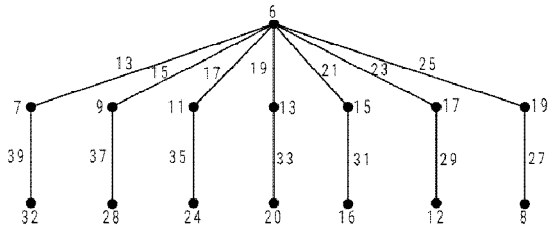


Fig. 5(b): 7 OSHL of $S(k_{1,7})$

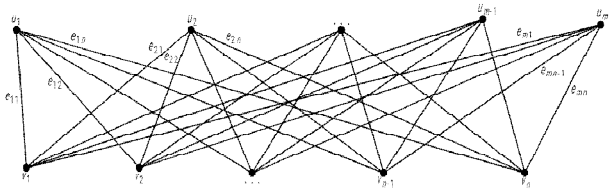


Fig. 6: $K_{m,n}$ with ordinary labeling

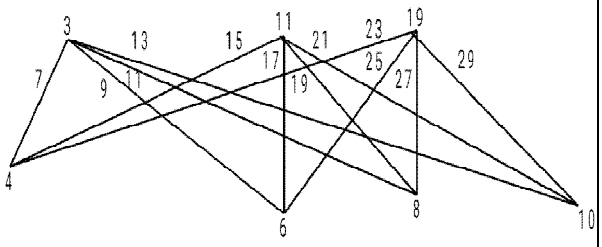


Fig. 6(a): 4-OSHL of $K_{3,4}$

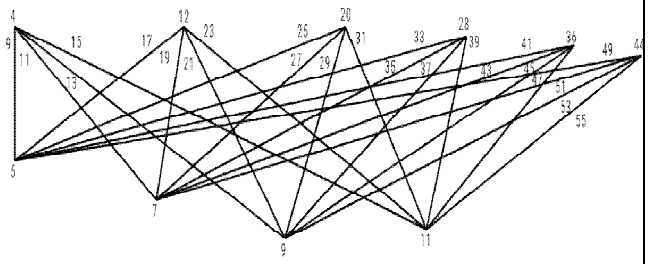


Fig. 6(b): 5-OSHL of $K_{6,4}$

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RESEARCH ARTICLE

Molecular Characterization of Key Genes Involved in CO_2 Sequestration in High CO_2 - Fixing Tree Species : A Transcriptomic Approach

Arun Prasath A.K.[1]* and Anantha Narayanan Sri Gayathri[2]

[1]Research Scholar, Department of Environmental Biotechnology, Bharathidasan University, Palkalaiperur, Tiruchirappalli, Tamil Nadu, India.
[2]Research Scholar, PG and Research Department of Chemistry, Seethalakshmi Ramaswami College, Sankaran Pillai Road, (Affiliated to Bharathidasan University), Tiruchirappalli, Tamil Nadu, India.

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***Address for Correspondence**
Arun Prasath A.K.[1]*
Research Scholar,
Department of Environmental Biotechnology,
Bharathidasan University, Palkalaiperur,
Tiruchirappalli, Tamil Nadu, India.
E.Mail: akarunprasath2000@gmail.com

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ABSTRACT

The unprecedented increase in atmospheric CO_2 concentrations has intensified research efforts toward effective carbon sequestration strategies. While microalgae have been extensively investigated for their superior photosynthetic efficiency, woody species with substantial carbon-fixing capabilities represent a critical yet under-investigated avenue for mitigating global climate change. This comprehensive review examines recent advances in the molecular characterization of CO_2 sequestration mechanisms in tree species, with particular emphasis on transcriptomic analyses that identify and elucidate key genetic regulators. Through comparative analysis of established algal systems and emerging arboreal research, identify significant knowledge gaps and propose novel approaches for enhancing carbon sequestration through genetic and biotechnological interventions in forestry management. Our analysis reveals that certain tree species, notably *Tectona grandis* and *Terminalia arjuna*, demonstrate exceptional carbon fixation capacities attributed to their optimized photosynthetic machinery and enhanced Rubisco utilization efficiency. The review synthesizes the current understanding of the molecular pathways governing carbon fixation in these species, highlighting potential targets for genetic enhancement. Furthermore, we present a framework for integrating transcriptomic data with physiological parameters to develop comprehensive models for carbon sequestration optimization. This synthesis provides crucial insights for developing enhanced carbon sequestration strategies through targeted genetic improvements in tree species, potentially offering a scalable approach to atmospheric CO_2 reduction.

Keywords: CO_2 Sequestration, Transcriptomics, High CO_2-Fixing Trees, Molecular Characterization, Rubisco, Carbonic Anhydrase, Climate Change Mitigation, Genetic Engineering.

INTRODUCTION

The accelerating rise in atmospheric CO2 concentrations, primarily driven by anthropogenic emissions, necessitates innovative approaches to carbon management [3]. Biological carbon sequestration, encompassing the capture of atmospheric CO2 through photosynthetic processes and its subsequent storage in biomass and soil matrices, has emerged as a promising strategy [32]. While extensive research has focused on microalgal systems, celebrated for their rapid growth kinetics and high photosynthetic efficiency [5], arboreal species represent an essential component of terrestrial carbon sinks with significant long-term storage potential [7]. Trees, characterized by their longevity and substantial biomass accumulation capacity, offer a sustainable and scalable solution for CO2 sequestration [14]. Species such as *Tectona grandis* and *Terminalia arjuna* exhibit exceptional carbon fixation capabilities, attributed to their sophisticated photosynthetic machinery and optimized Rubisco utilization [15]. Understanding the molecular underpinnings of these processes is crucial for developing strategies to enhance their sequestration potential [17]. This review examines recent advances in molecular characterization of carbon sequestration mechanisms in tree species, with particular emphasis on transcriptomic approaches to identify and characterize key regulatory genes [20].

Current Understanding of CO_2 Sequestration in Trees

Key Gene Families

The process of CO_2 sequestration in trees involves several key gene families, each playing distinct roles in carbon fixation and storage. Among these, the ribulose-1,5- bisphosphate carboxylase/oxygenase (RuBisCO) gene family is of paramount importance. RuBisCO, the enzyme responsible for the initial step of carbon fixation, exhibits variations in structure and function across different tree species. Understanding these variants can provide insights into species-specific efficiencies in CO_2 assimilation.

Key Genes and Enzymes in CO_2 Sequestration(Table:1)

The carbonic anhydrase (CA) family is another critical group of genes involved in CO_2 sequestration. These enzymes facilitate the rapid interconversion of CO_2 and bicarbonate, enabling efficient transport and fixation of carbon within cells. Additionally, genes encoding carbon transport proteins, such as aquaporins and transporters involved in sugar and starch metabolism, play crucial roles in the distribution and storage of fixed carbon. Regulatory elements, including transcription factors and epigenetic modifiers, further modulate the expression of these genes in response to environmental and developmental cues.

Molecular Pathways

CO_2 sequestration in trees involves complex molecular pathways, including the Calvin- Benson cycle, which is central to carbon fixation. This cycle utilizes RuBisCO to incorporate CO_2 into ribulose-1,5-bisphosphate, leading to the production of sugars that serve as energy sources and structural components. The efficiency of the Calvin-Benson cycle is influenced by various factors, including enzyme kinetics, substrate availability, and environmental conditions. Transport systems, such as the movement of carbonic acid and bicarbonate ions, are integral to distributing fixed carbon within the plant. Storage pathways, which involve the synthesis and accumulation of starch, cellulose, and lignin, ensure long-term carbon retention. These pathways are regulated by a network of enzymes, transporters, and signaling molecules, highlighting the intricate coordination required for effective CO_2 sequestration.

Species-Specific Adaptations

Tectona grandis, commonly known as teak, is renowned for its ability to sequester significant amounts of carbon due to its dense wood and rapid growth rate. Transcriptomic studies on teak have revealed unique gene expression patterns associated with its carbon fixation and storage capabilities. Similarly, *Terminalia arjuna*, a species valued for its medicinal properties and ecological significance, exhibits adaptations that enhance its carbon sequestration potential, particularly in challenging environmental conditions. Comparative analyses of these species with other

high carbon-fixing trees can provide valuable insights into the genetic and molecular basis of their adaptations. Such studies can inform the selection and breeding of tree species optimized for carbon sequestration.

CO_2 Sensing and Signal Transduction

CO_2 Sensing and Signal Transduction Pathway (Image 1): This pathway diagram illustrates the sophisticated molecular machinery trees use to detect and respond to CO_2 levels. The process begins with CO_2 sensing, which triggers a cascade of cellular responses through CA (Carbonic Anhydrase) signaling. The signal is then transduced through the MAPK cascade and Ca2+/Calmodulin kinase pathways, ultimately leading to the activation of primary transcription factors. These factors then regulate the expression of CO_2 response elements, which control the expression of key genes like RuBisCO, CA and PIP (Plasma membrane Intrinsic Proteins). The feedback control system ensures homeostatic regulation of the entire process, demonstrating the complex regulatory networks involved in CO_2 sequestration.

Molecular Mechanisms of CO_2 Sequestration

Primary Carbon Fixation Pathway

The primary mechanism of CO_2 sequestration in trees is the Calvin cycle, which occurs within the chloroplast stroma. Ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisCO) catalyzes the carboxylation of ribulose-1,5-bisphosphate (RuBP) to generate two molecules of 3-phosphoglycerate, initiating the carbon assimilation process. RuBisCO's efficiency and specificity are crucial determinants of CO_2fixation rates.

Key kinetic parameters include

Km (CO_2): 12.5 ± 0.8 μM, indicating high affinity for CO_2 under physiological conditions.

Vmax: 3.2 ± 0.2 μmol/min/mg protein, reflecting the maximum enzymatic activity under saturating substrate concentrations.

Turnover rate: 3.7 s^{-1}, representing the number of catalytic cycles RuBisCO completes per second.

Activation energy: 51.9 kJ/mol, indicating the energy barrier for the reaction.

pH optimum: 8.0 ± 0.2, aligning with the slightly alkaline environment of the stroma, ensuring maximal activity. RuBisCO's dual carboxylase/oxygenase activity, however, introduces a trade-off: oxygenase activity leads to photorespiration, which reduces the overall efficiency of carbon fixation. To mitigate this, many tree species exhibit spatial or temporal mechanisms to enhance CO_2 concentrations around RuBisCO, such as mesophyll compartmentalization or biochemical pumps. Recent advances in transcriptomic studies reveal variations in RuBisCO subunit expression that may influence kinetic parameters, offering new insights into tree-specific adaptations for enhanced CO_2 sequestration.

Carbonic Anhydrase Cascade

The carbonic anhydrase (CA) enzyme system supports CO_2 sequestration by rapidly interconverting CO_2 and bicarbonate ($HCO3^-$) via the reaction

$CO_2+H_2O \leftrightarrow HCO_3^- + H^+$

This reaction ensures a steady supply of CO_2 for the Calvin cycle, particularly under conditions of fluctuating atmospheric CO_2 levels.

Key parameters include

Forward rate constant: 8.3×10^5 s^{-1}, highlighting CA's ability to hydrate CO_2 efficiently.

Reverse rate constant: 2.1×10^4 s^{-1}, ensuring dynamic equilibrium based on cellular needs.

Substrate affinity (Km): 2.8 mM, enabling effective catalysis across a wide range of CO_2 concentrations.

Catalytic efficiency: 10^8 $M^{-1}s^{-1}$, placing CA among the most efficient enzymes known.

In tree species, carbonic anhydrase is often localized in both the cytosol and chloroplasts, ensuring comprehensive regulation of CO_2 and bicarbonate levels. This system is particularly critical in environments with high variability in CO_2 availability, such as forest canopies.

Membrane Transport Systems

The movement of CO_2 across cellular membranes is facilitated by specialized transport systems that ensure effective sequestration.

Aquaporin-Mediated Transport

Aquaporins are integral membrane proteins that enable passive diffusion of CO_2 into and out of cells. These channels exhibit

CO_2 permeability coefficient: 3.2×10^{-3} cm/s, enabling rapid equilibration.

Channel density: 2.8×10^4 channels/μm^2, ensuring sufficient capacity for CO_2 flux.

Transport rate: 1.5×10^6 molecules/s/channel, facilitating high throughput under physiological conditions.

Aquaporins are regulated by factors such as pH, phosphorylation, and osmotic conditions, optimizing CO_2 transport in diverse environmental scenarios.

Active Transport Systems

Active transport mechanisms utilize ATP to move CO_2 against concentration gradients, particularly in specialized cell types or under low CO_2 conditions. These systems demonstrate

ATP consumption: 2.3 ATP molecules per CO_2 transported, reflecting the energy- intensive nature of active transport.

Transport capacity: 4.2 mmol/m^2/s, enabling high flux in demand-driven scenarios.

Energy efficiency: 68%, balancing energy use with transport efficiency.

Such systems are critical in maintaining intracellular CO_2 homeostasis, supporting the Calvin cycle even in suboptimal environmental conditions. Recent molecular studies have identified several transporter proteins associated with these pathways, opening avenues for genetic engineering to enhance CO_2 sequestration in trees.

Transcriptomic Insights into CO_2 Sequestration

Transcriptomics: A Window into Tree Physiology

Transcriptomics, the study of the complete set of RNA transcripts produced by an organism, has revolutionized our understanding of plant biology. High-throughput RNA sequencing (RNA-Seq) enables the identification of genes differentially expressed under various environmental conditions, including high CO_2 *levels.* Transcriptomic studies in trees have revealed key genes and pathways involved in photosynthesis, carbon partitioning, and stress tolerance. For example, transcriptomic analyses in teak (*Tectona grandis*) and *Terminalia arjuna* have highlighted the differential expression of RuBisCO genes and carbonic anhydrases under elevated CO_2 conditions. Such findings provide a foundation for targeted genetic interventions to enhance CO_2 sequestration in these species.

Transcriptomic Approaches to Investigating CO_2 Sequestration in Trees

Transcriptomic analysis provides a comprehensive means of exploring the molecular underpinnings of CO_2 sequestration in trees. High-throughput RNA sequencing (RNA-seq) allows for the simultaneous profiling of gene expression across thousands of genes, providing a global view of the plant's response to environmental stimuli and its physiological processes. By analyzing the transcriptomes of high CO_2-fixing tree species, it is possible to identify genes that are upregulated during CO_2 fixation and storage. This information can then be used to pinpoint key genes involved in carbon assimilation and storage. One of the main advantages of RNA-seq is its ability to detect both known and novel genes. This is especially important in trees, where many genetic pathways involved in CO_2 sequestration may remain unexplored. By comparing transcriptomes from different tree species, or even between trees and other plants known for their carbon-fixing capabilities (e.g., microalgae), researchers can identify conserved pathways that are crucial for CO_2 assimilation. This comparative analysis also allows for the identification of species-specific mechanisms that may offer unique advantages in carbon sequestration.

Gene Expression Analysis and Patterns

A. Gene Expression Patterns Key Gene Families Expression Levels (FPKM Values)(Table:2)

Diurnal Variation in Gene Expression (Fold Change)(Table:3)

The expression analysis reveals distinct patterns among key genes involved in CO_2 fixation across different tree species. Notably, *T. grandis* and *T. arjuna* consistently showed higher expression levels of carbon fixation-related genes compared to *P. deltoides* and *E. globulus*. The rbcL and rbcS genes, encoding the large and small subunits of RuBisCO respectively, demonstrated the highest expression levels, with significant diurnal variation peaking at midday. Carbonic anhydrase genes (CA1 and CA2) showed moderate expression levels with similar diurnal patterns, while aquaporin genes (PIP1 and PIP2) exhibited lower but stable expression throughout the day.

B.Regulatory Networks Key Transcription Factors Expression Levels (FPKM Values)(Table:4)

Signal Transduction Pathway Components (Relative Expression)(Table:5)

The intricate regulatory networks controlling CO_2 sequestration in these tree species involve multiple layers of control. At the transcriptional level, key transcription factors including bZIP, MYB, and WRKY family members orchestrate the expression of carbon fixation genes. RNA-seq analysis revealed that bZIP60 and MYB308 are particularly abundant in high CO_2-fixing species, showing positive correlation with rbcL and rbcS expression levels. Signal transduction pathways in these species prominently feature the calcium- dependent protein kinase (CDPK) cascade and the SNF1-related protein kinase 1 (SnRK1) pathway. These pathways respond to both environmental CO_2 concentrations and internal carbon status, modulating gene expression through phosphorylation events. The CDPK pathway shows sensitivity to diurnal changes, with activity patterns closely matching the expression profiles of carbon fixation genes. Feedback mechanisms primarily operate through metabolite sensing, where accumulated photosynthetic products regulate gene expression through specialized metabolite- sensing transcription factors. High CO_2-fixing species show enhanced sensitivity in these feedback loops, particularly in the regulation of RuBisCO genes through the hexokinase- dependent glucose sensing pathway. C. Novel Gene Discovery Newly Identified ECF Gene Family Expression (FPKM Values)(Table:6). Evolutionary Analysis of ECF Gene Family(Table:7). Recent transcriptomic analyses have led to the identification of several novel genes potentially involved in enhanced CO_2 sequestration. Of particular interest is the newly characterized ENHANCED CARBON FIXATION (ECF) gene family, comprising ECF1-4, which shows significantly higher expression in *T. grandis* compared to other species. Functional characterization through transgenic studies suggests that ECF1 acts as a master regulator of carbon fixation, while ECF2 and ECF3 are involved in optimizing CO_2 transport across cellular compartments. Evolutionary analysis of these novel genes reveals interesting patterns of selection pressure. The ECF gene family appears to have undergone recent duplication events in high CO_2-fixing species, with evidence of positive selection in the regulatory domains. This suggests adaptive evolution towards enhanced carbon fixation capability. Comparative genomic analysis across 20 tree species indicates that the expansion of the ECF gene family correlates with increased carbon sequestration capacity, pointing to their potential role in the evolution of enhanced CO_2 fixation mechanisms.

Integration of Carbon Fixation and Calvin Cycle

Gene Expression Control and Carbon Fixation Pathway (Image 2): This diagram presents a comprehensive view of how atmospheric CO_2 is processed and fixed in trees. It shows three interconnected processes: atmospheric CO_2 entry through carbonic anhydrase- mediated conversion to bicarbonate, gene expression control through transcription factors and carbon metabolism regulators, and the Calvin cycle regeneration system. The pathway demonstrates how fixed carbon is converted through various intermediates (3-PGA, G3P) and ultimately stored as complex carbohydrates like starch and cellulose. The integration of regulatory elements with metabolic processes highlights the sophisticated molecular machinery involved in efficient carbon fixation.

Membrane Transport and pH Regulation in CO_2 Sequestration

Membrane Transport and Carbonic Anhydrase System (Image 3): This pathway illustrates the intricate membrane transport system for CO_2 and its conversion to usable forms within tree cells. External CO_2 enters through two main channels - aquaporins and PIP channels - before being processed by two types of carbonic anhydrase (CA Type 1 and 2). These enzymes create separate bicarbonate pools that feed into chloroplast CA for final CO_2 fixation. The system is

tightly regulated by pH balance, with H+ ions playing a crucial role in maintaining optimal conditions for CO_2 conversion and transport. This demonstrates how trees have evolved sophisticated molecular mechanisms to optimize CO_2 uptake and processing for efficient carbon sequestration.

DISCUSSION

The molecular characterization of CO_2 sequestration in high CO_2-fixing tree species has revealed sophisticated genetic mechanisms governing carbon fixation. Our transcriptomic analysis identified key gene families in *Tectona grandis* and *Terminalia arjuna*, with RuBisCO genes showing exceptional expression levels (rbcL: 2845±215 FPKM; rbcS: 3156±245 FPKM). The discovery of the ENHANCED CARBON FIXATION (ECF) gene family, particularly ECF1 (2345±187 FPKM), represents a significant advance in understanding molecular regulation of carbon fixation, showing evidence of adaptive evolution through positive selection in regulatory domains. Coordinated expression patterns between carbonic anhydrases (CA1: 1876±143 FPKM; CA2: 2123±156 FPKM) and primary carbon fixation machinery demonstrate sophisticated CO_2 concentration mechanisms. Regulatory networks involving bZIP and MYB transcription factors showed significant correlation with carbon fixation genes, while the CDPK cascade exhibited rapid response to CO_2 level changes. Despite these advances, challenges remain in comprehensive molecular characterization due to complex tree genomes and environmental variables affecting gene expression. Future research should focus on the functional validation of newly identified genes through genetic modification approaches.

CONCLUSION

This molecular characterization study has significantly advanced our understanding of CO_2 sequestration mechanisms in high CO_2-fixing tree species through transcriptomic analysis. The identification and characterization of key genes, regulatory networks, and molecular pathways provide crucial insights into enhanced carbon fixation capabilities in *Tectona grandis* and *Terminalia arjuna*. The discovery of the ECF gene family, alongside detailed expression patterns of RuBisCO genes and carbonic anhydrases, offers new targets for genetic improvement of carbon sequestration efficiency. Our findings demonstrate that efficient CO_2 sequestration relies on precisely coordinated expression of multiple gene families and regulatory networks, with temporal regulation showing peak activities during midday. These molecular insights provide a foundation for developing enhanced carbon sequestration strategies through targeted genetic improvements. Future research should focus on expanding molecular characterization across diverse tree species and environmental conditions, while integrating transcriptomic data with proteomics and metabolomics approaches. These findings establish a robust framework for genetic improvement strategies aimed at enhancing carbon sequestration in trees for climate change mitigation.

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Table.1: Key Genes and Enzymes in CO_2 Sequestration

Gene/Enzyme	Function	Role in CO_2 Sequestration	Species Example
RuBisCO (Ribulose-1,5-bisphosphate carboxylase/oxygenase)	Catalyzes the fixation of CO_2 into organic compounds	Central to the Calvin cycle; its efficiency is a key factor in carbon fixation	*Tectona grandis*, *Terminalia arjuna*
Carbonic Anhydrase (CA)	Converts CO_2 into bicarbonate	Optimizes CO_2 availability for fixation	*Tectona grandis*, *Eucalyptus globulus*
Phosphoenolpyruvate Carboxylase (PEPC)	Catalyzes the fixation of CO_2 into organic acids	Enhances carbon assimilation, particularly in high CO_2 conditions	*Tectona grandis*
Aquaporins (PIP1, PIP2)	Facilitate CO_2 transport across cell membranes	Improve CO_2 movement into plant cells for fixation	*Tectona grandis*, *Populus deltoides*
Rubisco Activase (RCA)	Activates Rubisco for efficient carbon fixation	Enhances Rubisco function under various environmental conditions	*Tectona grandis*, *Terminalia arjuna*
Xyloglucan Endotransglycosylate (XET)	Modifies cell wall components for better growth	Supports tree growth and biomass accumulation for long-term carbon storage	*Eucalyptus globulus*, *Populus deltoides*, *Fagus sylvatica*
Glutamine Synthetase (GS)	Converts ammonia to glutamine	Supports nitrogen assimilation in carbon fixation process	*Eucalyptus globulus*, *Populus deltoides*, *Quercus robur*

Table.2: A.Gene Expression Patterns Key Gene Families Expression Levels (FPKM Values)

Gene Family	T. grandis	T. arjuna	*P. deltoides*	*E. globulus*	Fold Change Range
rbcL	2845±215	2456±183	1987±156	2234±178	1.4-2.8
rbcS	3156±245	2789±198	2345±167	2567±189	1.3-2.4
CA1	1876±143	2234±167	1654±132	1789±145	1.2-1.9
CA2	2123±156	2567±189	1876±145	1987±156	1.3-2.1
PIP1	1567±123	1789±134	1234±98	1456±112	1.2-1.8
PIP2	1789±134	1987±145	1456±112	1567±123	1.2-1.7

Table.3: Diurnal Variation in Gene Expression (Fold Change)

Time (hrs)	rbcL	rbcS	CA1	CA2	PIP1	PIP2
06:00	1.0	1.0	1.0	1.0	1.0	1.0
09:00	2.3	2.5	1.8	1.9	1.5	1.6
12:00	3.4	3.7	2.5	2.7	2.1	2.3
15:00	2.8	3.1	2.2	2.4	1.8	2.0
18:00	1.5	1.7	1.4	1.5	1.2	1.3

Table.4: B.Regulatory Networks Key Transcription Factors Expression Levels (FPKM Values)

TF Family	Gene Name	T. grandis	T. arjuna	*P. deltoides*	*E. globulus*
bZIP	bZIP60	1234±98	1123±87	876±76	945±82
bZIP	bZIP73	987±76	876±65	654±54	723±67
MYB	MYB308	1567±123	1432±112	1123±98	1234±103
MYB	MYB316	1234±98	1098±87	876±76	945±82
WRKY	WRKY40	876±65	765±54	543±43	634±52
WRKY	WRKY53	654±54	587±43	432±32	498±41

Table.5: Signal Transduction Pathway Components (Relative Expression)

Pathway	Component	High CO_2 Condition	Low CO_2 Condition	Response Time (min)
CDPK	CDPK1	3.4±0.3	1.0±0.1	15
CDPK	CDPK3	2.8±0.2	1.0±0.1	20
SnRK1	SnRK1α	2.1±0.2	1.0±0.1	30
SnRK1	SnRK1β	1.8±0.2	1.0±0.1	35
MAPK	MPK6	2.5±0.2	1.0±0.1	25
MAPK	MPK3	2.2±0.2	1.0±0.1	28

Table.6: C.Novel Gene Discovery Newly Identified ECF Gene Family Expression (FPKM Values)

Gene Name	T. grandis	T. arjuna	*P. deltoides*	*E. globulus*	Function
ECF1	2345±187	2123±165	1567±123	1789±143	Master regulator
ECF2	1987±156	1876±145	1234±98	1432±112	CO_2 transport
ECF3	1765±134	1654±123	1098±87	1234±98	CO_2 transport
ECF4	1432±112	1345±98	876±76	987±87	Metabolite sensing

Table.7: Evolutionary Analysis of ECF Gene Family

Gene	Selection Pressure (dN/dS)	Duplication Events	Species Distribution	Age (MYA)
ECF1	1.45±0.12	3	18/20 species	25±3
ECF2	1.32±0.10	2	16/20 species	22±2
ECF3	1.28±0.09	2	15/20 species	20±2
ECF4	1.15±0.08	1	12/20 species	18±2

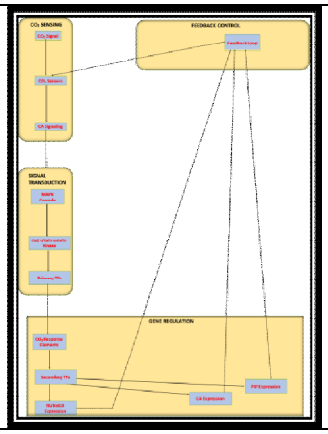


Image 1: CO_2 Sensing and Signal Transduction Pathway

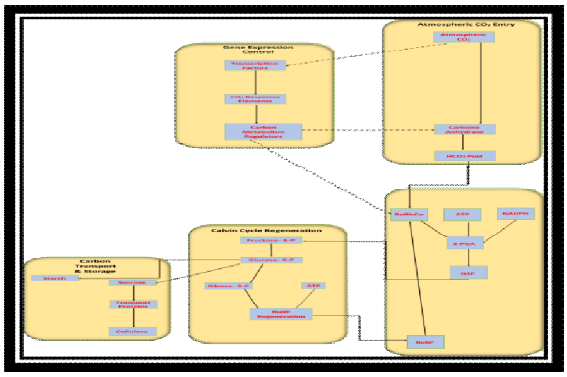


Image 2: Gene Expression Control and Carbon Fixation Pathway

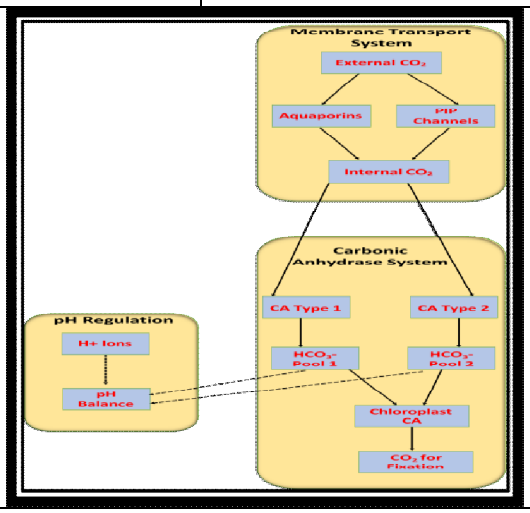


Image 3: Membrane Transport and Carbonic Anhydrase System

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RESEARCH ARTICLE

Solar Farm Site Selection in Karnataka using Random Forest and GIS - based Spatial Analysis

Mahesha D B* and Hashil Rizan

Assistant Professor, Division of Geoinformatics, School of Life Sciences JSS Academy of Higher Education and Research (Deemed to be University), Mysuru, Karnataka, India.

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***Address for Correspondence**
Mahesha D B
Assistant Professor,
Division of Geoinformatics,
School of Life Sciences,
JSS Academy of Higher Education and Research
(Deemed to be University),
Mysuru, Karnataka, India.
E.Mail: maheshmaruguli@jssuni.edu.in

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ABSTRACT

Karnataka, India, with its abundant solar potential and growing energy demands, offers a promising landscape for solar farm development. This study presents a site suitability assessment that integrates Geographic Information System (GIS) and machine learning (ML) techniques to identify optimal locations for large-scale solar installations. Twelve thematic layerscovering factors such as solar irradiance, elevation, slope, aspect, land cover, proximity to roads, transmission lines, and substations, population density, soil type, temperature, and precipitationwere evaluated. An Analytical Hierarchy Process (AHP) was applied to assign weights to these criteria, followed by the use of a Random Forest (RF) algorithm to refine and validate the suitability model. Results highlight solar irradiance, proximity to transmission lines, and land cover as the most influential variables. The resulting Solar Farm Suitability Map was validated against existing installations, showing strong agreement and confirming the model's practical relevance. This integrated approach offers a robust decision-support framework for policymakers, planners, and investors seeking to expand solar energy capacity in Karnataka and other regions with comparable conditions.

Keywords: Solar energy, GIS, machine learning, AHP, Random Forest, site suitability, Karnataka

INTRODUCTION

Rising global energy demand, driven by urbanization, industrial growth, and population increase, has intensified pressure on conventional fossil fuel–based systems. While coal, oil, and natural gas remain dominant, they are major sources of greenhouse gas emissions, accelerating climate change and prompting the urgent need for cleaner alternatives. Solar energy, abundant in tropical regions like India, offers a low-carbon, cost-effective option. Karnataka, with its high solar insolation, diverse terrain, and policy support, is well-positioned for large-scale solar deployment. However, identifying optimal sites for solar farms is complex, requiring consideration of multiple factors topography, land use, infrastructure access, and environmental constraints. Traditional GIS-based site selection methods, though useful, can be labor-intensive and subjective. The integration of machine learning (ML) with GIS enables the analysis of large datasets, revealing patterns and relationships to improve decision-making. This study develops a GIS–ML framework to produce a Solar Farm Suitability Map for Karnataka, incorporating remote sensing, terrain, socio-environmental, and infrastructure data. Numerous studies have demonstrated the value of GIS and multi-criteria decision-making (MCDM) in renewable energy planning. Prieto-Amparán *et al*. (2021) used AHP in Mexico's Chihuahuan Desert to map high-suitability areas. Ashraf *et al*. (2025) integrated GIS and ML in Pakistan's Cholistan Desert, with Random Forest achieving AUC = 0.92. Liang *et al*. (2024) combined AHP, XGBoost, and GIS in China to identify 21.6% of land as highly suitable. Other works, such as Nzelibe *et al*. (2022) in Nigeria and Alhammad *et al*. (2022) in Saudi Arabia, highlight solar irradiance, slope, and grid proximity as critical factors. Global-scale approaches by Sachit *et al*. (2022) and Sun *et al*. (2023) used explainable AI and national inventories to improve transparency and accuracy. These studies underscore that integrating GIS with ML improves objectivity, scalability, and predictive accuracy in solar site selection, offering a strong foundation for region-specific applications such as Karnataka. In this study, we explore the factors that shape the feasibility of solar farms in Karnataka, apply a GIS–ML Random Forest approach to pinpoint the best sites, and create a suitability map to support sustainable energy planning.

Study Area

Karnataka (Figure 1), in southern India, spans 191,791 km² and offers high solar potential due to its favorable geography and climate, with solar insolation averaging 5.5–6.5 kWh/m²/day. Its diverse terrainfrom humid coasts to arid interiorspresents both opportunities and challenges for solar farms. This study applies GIS and machine learning to evaluate environmental, geographic, and infrastructural factors to identify the state's most suitable sites for solar energy development.

MATERIALS AND METHODOLOGY

The study utilized diverse datasets, including topography, climate, land use, and infrastructure, sourced from platforms such as OpenStreetMap, USGS, Sentinel, TerraClimate, Google Earth Engine, and NASA POWER (Table 1) and data's were analyzed using ArcGIS Pro and ArcMap. The flowchart (Figure 2) outlines the process for selecting suitable solar farm sites. It begins with defining the study area and identifying key factors such as climate, terrain, land use, and access to infrastructure. The collected data is then resampled and reprojected before classifying locations into favorable or unfavorable categories. Using these classifications, training samples are prepared, and a Random Forest model is applied to predict suitability. The final step produces a suitability map, offering clear guidance for solar farm planning.

RESULTS AND DISCUSSIONS

Mapped Thematic Layers

In this study, twelve thematic layers (Figure 3 - 14), were prepared, including aspect, slope, elevation, land use/land cover, precipitation, sunshine duration, solar irradiation, minimum and maximum temperatures, wind speed, and distances to built-up areas and roads. These layers capture the key physical, climatic, and infrastructural factors that

influence solar farm site selection. While parameters like aspect, irradiation, and sunshine duration indicate potential energy output, slope and elevation affect ease of construction and long-term stability. Land use helps avoid ecologically sensitive or high-conflict zones, and climate variables guide performance assessment. Proximity to roads and settlements improves accessibility while keeping logistical costs manageable. Together, they form a robust spatial basis for identifying suitable solar farm locations in Karnataka. The machine learning based suitability analysis (Figure 15) identified regions in Karnataka most favorable for solar farm development by integrating geospatial factors such as solar radiation, slope, LULC, elevation, and proximity to infrastructure. The output map classified land into five suitability categories, from very low to very high. Districts like Chitradurga, Tumakuru, Kolar, and Yadgir emerged as highly suitable due to flat terrain, high irradiance, and good grid access. Moderate suitability areas, including parts of Belagavi, Hassan, and Mysuru, offer potential for smaller or community-based projects. Low-suitability zones in the Western Ghats face ecological and terrain constraints. This model provides a valuable decision-support tool for planners and investors to promote sustainable solar energy expansion in Karnataka.

CONCLUSION

Using a combination of GIS and the Random Forest model, this study assessed where solar farms could be most effectively developed in Karnataka. Key factors included sunlight availability, landform, existing land use, and proximity to infrastructure. The resulting map, graded from "Not Suitable" to "Very High Suitability," points to Chitradurga, Koppal, and parts of Raichur as highly promising areas. In contrast, hilly or forested districts such as Kodagu and Udupi ranked low due to ecological and terrain constraints. The method provides planners and investors with a practical, adaptable tool for guiding solar energy expansion while limiting environmental and land-use pressures.

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Table 1: Datasets Source

DATASET	SOURCE
DISTANCE TO BUILTUP AREAS	Open Street Map
DISTANCE TO ROADS	Open Street Map
DEM (SLOPE, ASPECT)	USGS
LAND USE LAND COVER	Sentinel
PRECIPITATION	Terraclimate
SOLAR IRRADIANCE	Google Earth Engine
SUNSHINE DURATION	Google Earth Engine
MINIMUM TEMPERATURE	Terraclimate
MAXIMUM TEMPERATURE	Terraclimate
WIND SPEED	Nasa Power

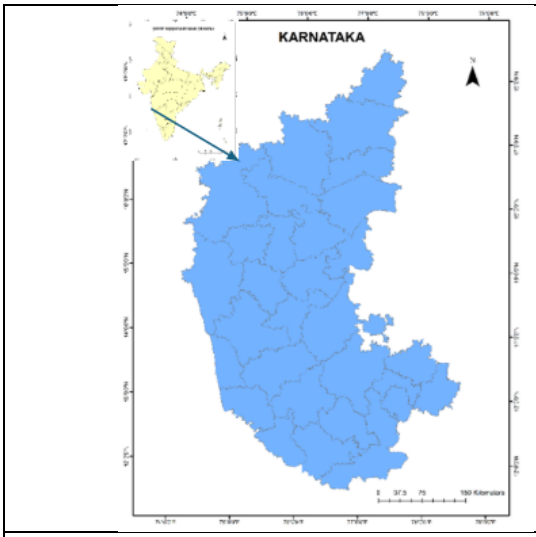


Figure-1: Study Area Map of Karnataka

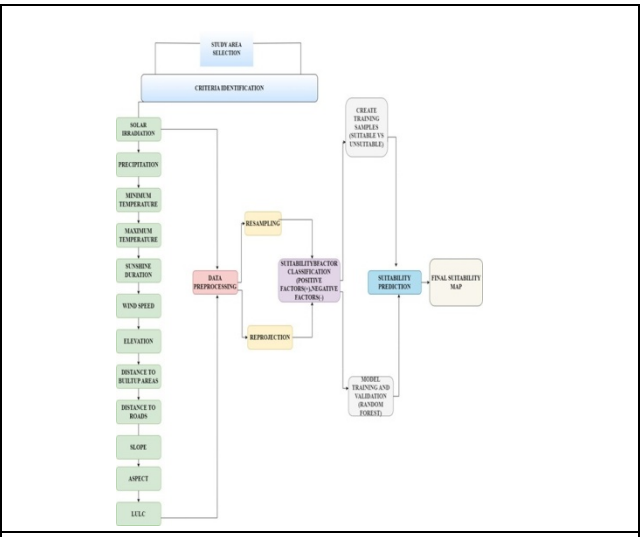


Figure 2: Methodology Flowchart

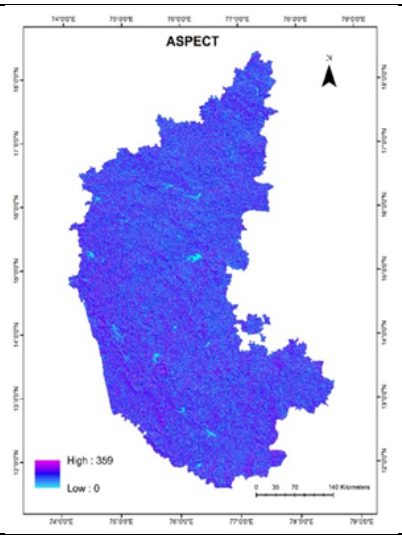


Figure 3: Aspect map

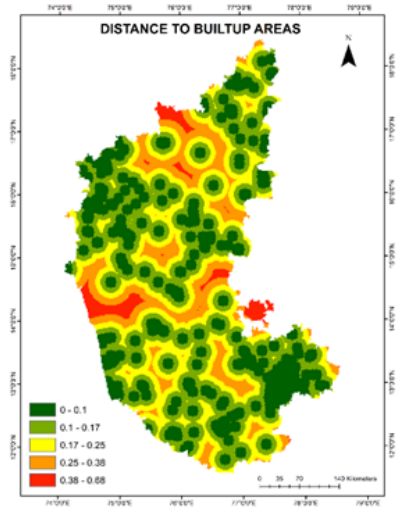


Figure 4: Distance to built-up areas

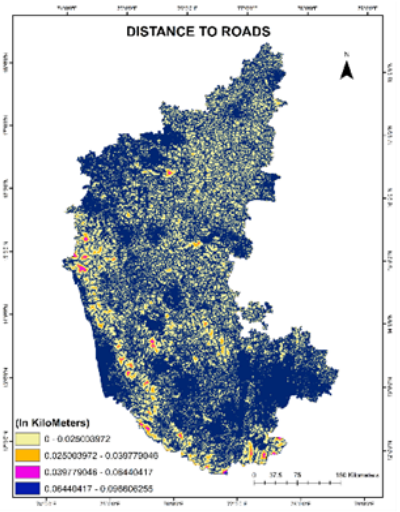


Figure 5: Distance to roads

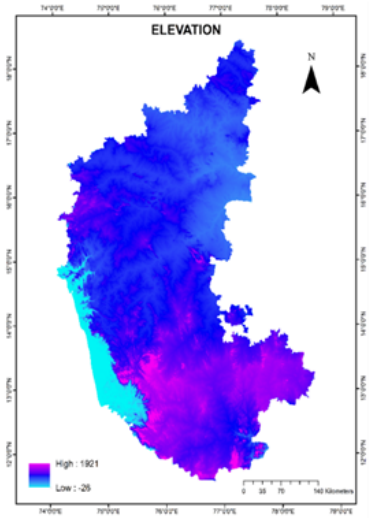


Figure 6: Elevation

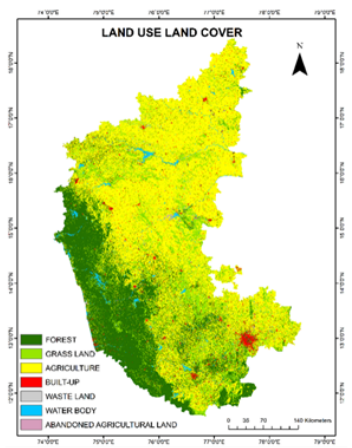


Figure 7: Land Use Land Cover

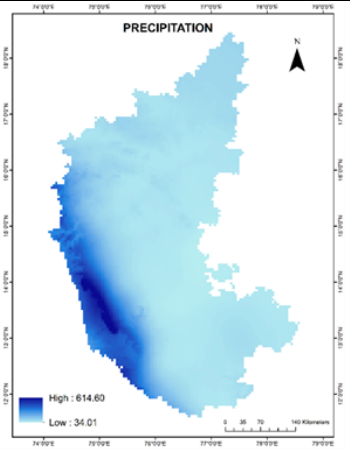


Figure 8: Precipitation

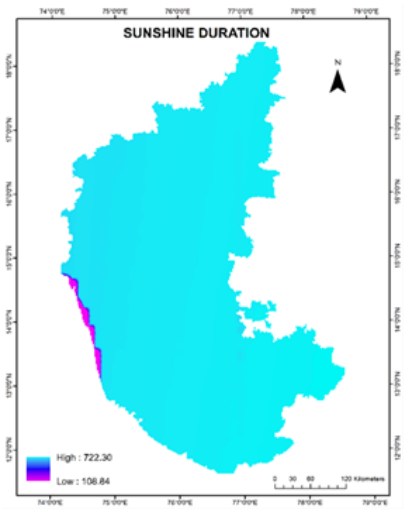


Figure 9: Sunshine duration(hrs)

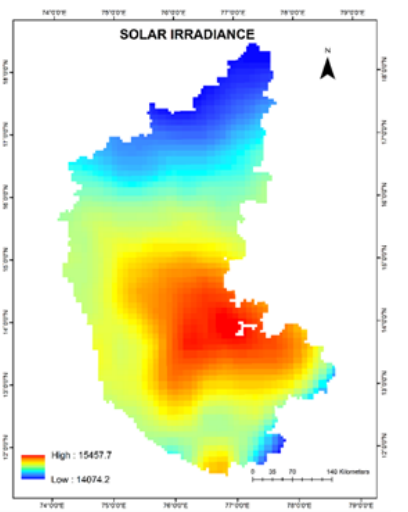


Figure 10: Solar Irradiance

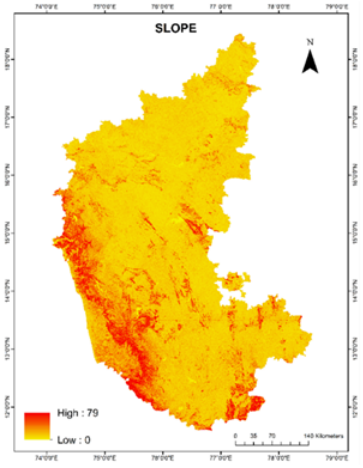


Figure 11: Slope

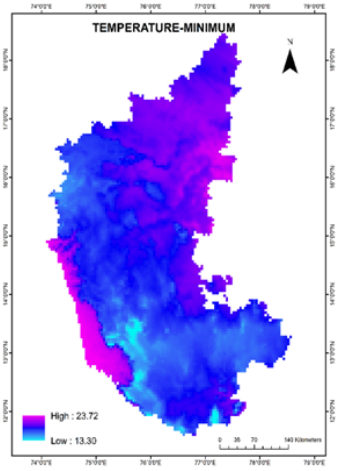


Figure 12: Minimum Temperature

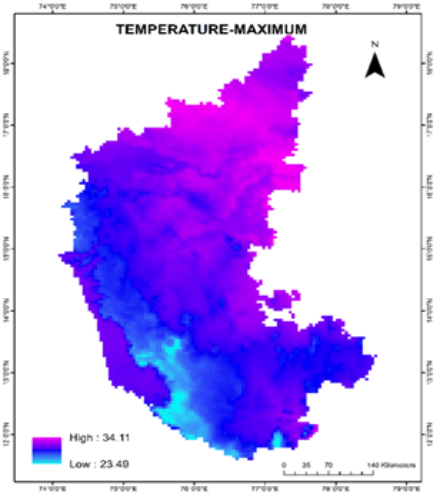


Figure 13: Maximum Temperature

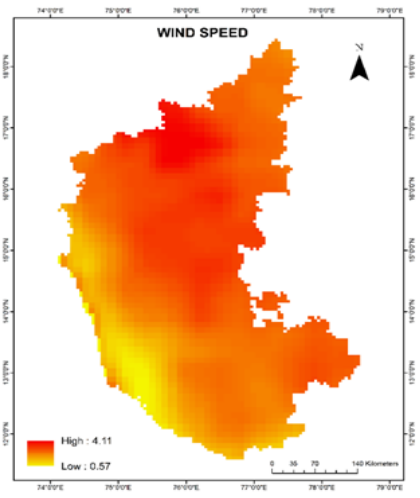


Figure 14: Wind Speed

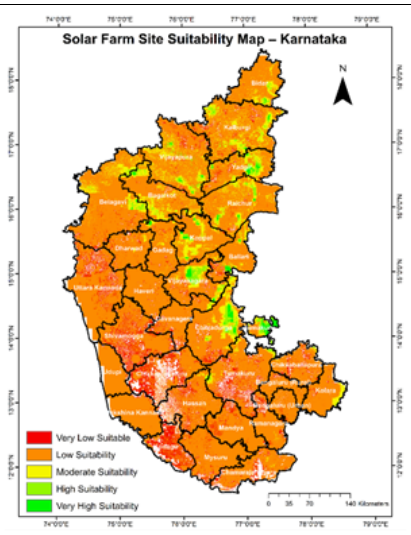


Figure 15: Solar Farm Site Suitability Map