RESEARCH ARTICLE

Isolation and Identification of *Aspergillus niger* from Naturally Infected Jasmine Plants

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ABSTRACT

In this study, we have noticed the symptoms like brown and chlorotic leaf spots , leaf chlorosis and wilting on naturally infected jasmine plants (*Jasminum sambac*) from field crops at Moyilla kallava village of Kadapa district, Andhra Pradesh. Since, it is suspecting as fungal infection, the infected leaf bits were used for preparation of fungal pure culture by PDA plating method. Morphological features of the pure culture were observed under Scanning electron microscope (SEM) and amplified internal transcribed spacer (ITS) region by polymerase chain reaction (PCR). The resulted amplified PCR product of ~ 600 bp was subsequently sequenced. Both the SEM observations and ITS sequence analysis of the culture have confirmed that *Aspergillus niger* is the causal agent for the present study disease in jasmine.

Keywords: Jasmine, *Aspergillus niger(A. niger)*, Internal transcribed spacer (ITS), Polymerase chain reaction (PCR), Scanning electron microscope (SEM).

INTRODUCTION

Jasmine is a genus of shrubs and vines in the olive family Oleaceae. Although it has around 200 species, only three species namely *Jasminum sambac, Grandiflorum* and *Auriculatum* are used for commercial forming. Its flowers used for garlands, especially for weddings and other auspicious occasions and in perfume industries. In India, most of the states are cultivating Jasmine as a major flower crop, however, predominantly in Tamil Nadu. In Andhra Pradesh, the major jasmine producing areas are Kurnool, Kadapa, Anantapur, Chittoor, Guntur and Prakasam districts. However, the crop is vulnerable to various fungal, bacterial and viral infections and

account for significant crop yield loss. The most common bacterial diseases of jasmine are blight and gall disease caused by *Botrytis cinerea* and *Pseudomonas savastanoi* on *Jasminum officinalis* and winter jasmine, respectively (Aiello, Parlavecchio *et al.* 2008 and Taghavi and Hasani 2012). The severe fungal diseases are rust caused by *Uredo manilensis* in crepe jasmine (Martinez-de la Parte, Cantillo- Pere *et al.* 2011), sclerotial wilt disease by *Sclerotium rolfsii* in *Jasminum sambac* (Maheswari, Muthuswamy *et al.* 2002), cercosporoid leaf spots by *Colletotrichum* (Braun and Sivapalan 1999; Wikee, Cia *et al.* 2011) and *Colletotrichum gloeosporioides* infection in *Jasminum grandiflorum* (Sharma, Singh *et al.* 2012). The viruses like *Tobacco streak virus* (Goud, Vemana *et al.* 2013), *Tomato mosaic virus* (Kamenova, Adkins *et al.* 2006), *Groundnut bud necrosis virus* (Sujitha *et al.* 2013) have been reported from jasmine. In the present study, we have isolated a fungal pathogen from a infected jasmine plant and identified it as *A. niger* by SEM and ITS sequence analysis. *A. niger* (genus *Aspergillus*) is commonly found as a saprophyte grows on dead leaves, stored grains, compost pits and other decaying vegetation (Sharma 2012). Further, it causes black mold disease on fruits, vegetables, preserved food, crown rot diseases in *Arachis hypogea* (Schuster, Dunn-Coleman *et al.* 2002; Jackson 1962). In plants it is also a causative agent for many rot diseases (Sharma 2012). It has a total genome size ranges from 35.5 to 38.5 Mb and composed of 13000 genes (Debets, Holub *et al.* 1990). Most common symptoms of *A. niger* includes brown spots on the infected plant leaves, stunted growth with leaf chlorosis and wilting. As the disease is progressed, the dark brown and black powdery spores are observed on the surface of infected areas caused crown rot disease in *Arachis hypogea* (Schuster, Dunn-Coleman *et al.* 2002). Small rectangular yellow spots have been perceived on leaves of diseased *Zingiber officinale* and Grapes with *A. niger* (Pawar, Patil *et al.* 2008; Vitale, Castello *et al.* 2008). Some reports revealed that *A. niger* induced black rot of onions (El-Nagerabi and Ahmed 2001), spoilage of tomatoes (Sinha and Saxena 1987), grapes (Sharma and Vir 1986). On the other hand, this black fungus (*A.niger*) had great industrial importance in production of citric acid, gluconic acid, and enzymes such as amylases, pectinases and proteases (Godfrey and West, 1996). Allergic issues associated with humans have been noticed when exposed to spores and its vegetative forms present in air and consumable foods (Goutam *et al.* 2011). In the present study, we have reported that *A. niger* as a plant pathogen of jasmine and it might be the first report from India.

MATERIALS AND METHODS

Sample Collection and Preparation of Pure Fungal Culture
During the field inspection at Moyilla kallava village of Kadapa district, AP, we have noticed that jasmine crop was severely infected. The infected plants have shown small, irregular to rectangular brown leaf spots, leaf chlorosis and wilting. As it is suspecting fungal infection, the infected plant leaves were collected to the laboratory and used for pure culture preparation. Leaves were initially washed with tap water and later with sterile distilled water. The symptomatic leaves were cut into pieces (1x1 cm in size) with a sterile lancet and soaked in 70% ethanol for 2 min. Later these pieces were washed again with sterile distilled water and dried by blotting with sterile tissue paper. The leaf bits were placed on PDA plates and incubated at 28⁰C for 3 days. The characteristic fungal colonies were further sub-cultured until the development of its pure culture and subjected to microscopic observation.

Microscopic Observation
A fungal colony from the pure culture was mounted on a slide containing a drop of lacto phenol cotton blue staining agent and covered with the cover slip for light microscopic observation. For SEM observations, the fungal mycelium from PDA was mounted on aluminium stubs with double-sided carbon adhesive tape and observed under SEM.

Isolation of Genomic DNA

To isolate genomic DNA, Prabha et al., procedure was followed with few modifications (Prabha *et al.* 2013). A loop full of fungal culture was inoculated into 100 ml of Potato dextrose broth (PDB) and incubated at 28^0C for 3 days in a shaking incubator. The mycelium grown on 3rd day was allowed to drain and air dried for 10 mins on a sterile filter paper. To the mycelium, 500 µl of extraction buffer (200 mM Tris HCl, 25 mM EDTA, 250 mM NaCl and 0.5% SDS, pH 7.5) was added and ground with sterile mortar and pestle. Later, the sample was vortexed for 5 sec and incubated for 30 min at room temperature. After incubation, the sample was centrifuged at 13000 rpm for 1 min. Supernatant was initially extracted with equal volume of cold phenol: chloroform (1:1, v/v) and later with chloroform by centrifugation at 13,000 rpm for 2 min. To the supernatant, 300 µl of cold iso-propanol was added and incubated for 30 min at -20^0C. Later it was centrifuged at 13,000 rpm for 5 min and the obtained pellet was washed with 70% cold ethanol. Finally, the air dried DNA was resuspended in 50 µl of sterile distilled water and stored at $-20\ ^0C$ for further use. 10 µl of isolated DNA was resolved in 1 % agarose gel and the bands were observed under Gel documentation system.

ITS Amplification and Sequence Analysis

PCR amplification was made by using universal ITS primers for identification of fungus. Reaction was set upto 25 µl containing 1 µg of DNA, 10 X Taq buffer, 10 mM dNTPs, 2 mM $MgCl_2$, 1.25U of Taq polymerase (EP0402, Thermo Scientific) and 50 nmol of ITS primers (ITS1 5′TCCGTAGGTGAACCTGCGG 3′, ITS4 5′ TCCTCCGCTTATTGATATGC 3′) for 35 cycles at 94 ^0C for 30 s, 55 ^0C for 30 s, 72 ^0C for 30 s, followed by a final extension at 72 ^0C for 10 mins. Further, the amplified PCR product was resolved on 1% agarose gel and analysed by Gel documentation system. The obtained PCR product was commercially sequenced (Eurofins, Bangalore) and the sequence was respectively analysed and submitted in NCBI-BLAST and GenBank (MH341159). Multiple sequence analysis and phylogenetic tree construction were made by MEGA 5.

RESULTS

The infected jasmine plants have shown symptoms like small, irregular to *rectangular* brown and chlorotic lesions on the leaves, leaf chlorosis and severe wilting (Fig1). The leaf bits of the infected jasmine plants have produced black colonies on PDA plates (*Fig2).* Pure culture of the fungus was prepared by subsequent subculture and their morphological features have been identified by Scanning electron microscope (SEM). It revealed that the fungus has a long stalk filamentous conidiophore with a globose head having conidial spores (Fig3). These characteristics are very similar to *Aspergillus niger.* For further confirmation, we have made an attempt to amplify and sequence ITS (*ITS1-5.8S-ITS2*) region of the fungus. Genomic DNA of the pure culture was isolated and PCR amplified with ITS universal primers (*ITS1 and ITS4*). The size of the amplified product of approximately 600 bp was subsequently sequenced. The resulted sequence of the amplified product was analysed by NCBI - BLAST tool. In the analysis, we found that the present study ITS sequence (MH341159) has more than 99 % sequence similarity with *A.niger* isolates of Delhi (KX011017, KX928746), Italia (KU686682), Tamil Nadu (KJ881376, KU865178, KJ865316) and Haryana (KY030729). A phylogenetic tree was constructed using MEGA 5 to the above isolates of *A.niger* with an out group of A. flavus, *Origanum syriacum*, Egypt. Two clear cut clades were formed in the phylogram where the present study A. niger isolate was closely grouped with Delhi and Haryana isolates of *A. niger* (Fig4). Both the SEM observations and ITS sequence analysis have confirmed that the present investigating pathogen of jasmine is as *A.niger.*

DISCUSSION

Jasmine is a well-known ornamental plant cultivated in many southern states of India for commercial importance of flowers. In AP, major harvesting regions were Kadapa, Kurnool, Anantapur, Chittoor, Prakasam, and Guntur districts. We have visited the jasmine crops in the surrounding areas of Moyilla kallava village of Kadapa district and noticed many plants of the crop were severely infected and expressed symptoms like small, irregular brown and chlorotic lesions on the leaves. Based on the symptomatology of the plants we have suspected that it was fungal infection and, hence to confirm the exact pathogen, the symptomatic leaves were further processed and used for preparation of fungal pure culture by a PDA plating method (Zhang, Xi *et al.* 2016). The surface sterilized leaf bits on PDA plates over 3 days of incubation have produced black coloured colonies. These colonies are initially white in colour and later turned into black colour colonies. The culture was carefully observed under light and scanning electron microscope and found very clear morphological features of pathogen include a long stalk filamentous conidiophores with a globose head having conidial spores. According to the previous reports these observed features are the characteristic features of *A. niger*. The common characteristic features of *A. niger,* septate hyphae attached with flexous filamentous particles with long stalk asexual conidiophores with a globose head at the tip ejecting its own conidial spores (Clark 1981 and Sharma 2012). *A. niger* isolated from *Allium cepa* was also showed black colour colonies on PDA as well as aforementioned morphological characteristic under microscope (Dilendra, Shweta *et al.* 2014; Raper and Fennell 1965). Based on these morphological observations of the present study fungus was identified as *A.niger*. It is commonly regarded as a saprophyte growing on dead leaves, stored grain, compost piles and other decaying vegetation. However, for further confirmation, we have employed PCR amplification and subsequent sequencing of ITS region of the fungus. The PCR amplified product of 600 bp was further sequenced and confirmed as *A.niger*. The amplification of the ITS1-5.8S-ITS2 region of ribosomal DNA (rDNA) for the *Aspergillus* isolates, using the universal primers ITS1 and ITS4 originated a fragment of ~ 600 bp (White *et al.* 1990). In a study, a total of 108 isolates of *Aspergillus* spp. was identified from contaminated coffee beans at the species level by sequencing the internal transcribed spacer (ITS1-5.8S-ITS2) of rDNA (Magnani, *et al.* 2005). ITS region exhibits the highest variation within the species and used as a universal barcode region (Aslam *et al.* 2017). Similarly, sequencing of ITS region combined with *m*orphological identification confirmed that *A. niger is* the causal pathogen of flower black mold disease of big onion (Fernando *et al.* 2018), leaf spot disease in bindweed (Zhang, Xi *et al.* 2016), Crown rot disease of peanut (*Arachis hypogaea* L.) (Magnoli *et al.* 2006). From the above studies, suspects that *A.niger* becomes an emerging plant pathogen account for great loss in agriculture. In this study, based on SEM and ITS sequence study we have confirmed that jasmine is infected by *A. niger* .

CONCLUSION

The naturally infected jasmine plants have showed rectangular brown colour leaf spots, leaf chlorosis and severe wilting. Based on the symptomatology, we have suspected it as fungal infection. For further confirmation, the pure fungal culture was prepared and subjected for SEM, PCR amplification (ITS region) and sequencing. Based on these studies, it was confirmed that the pathogen of jasmine is *Aspergillus niger* .

ACKNOWLEDGEMENTS

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

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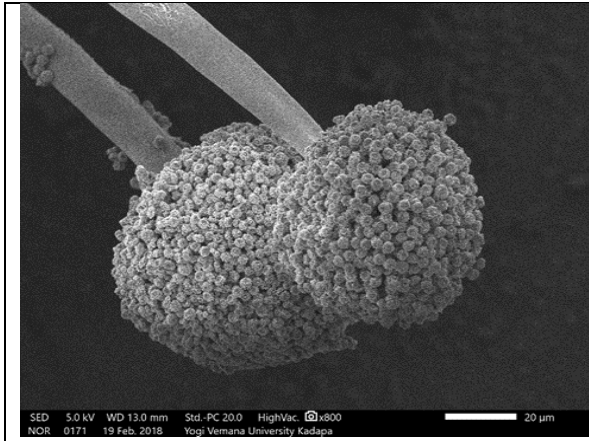
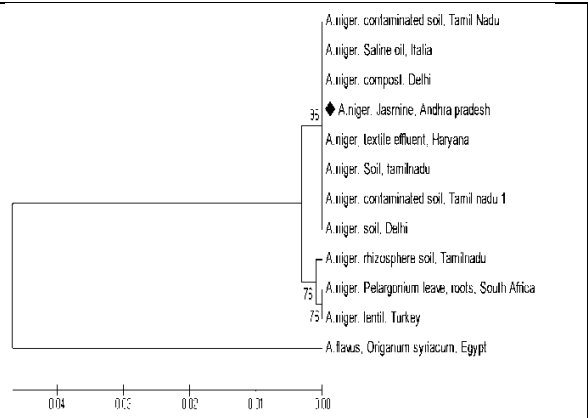**Fig1.Irregular brown and chlorotic lesions on the leaves of Jasmine**
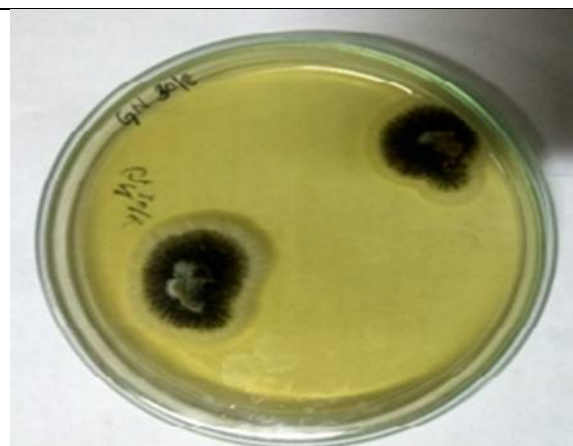
Fig 2. Characteristic black colonies for *A. niger*

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Fig.3.Scanning electron microscopy showing long *filamentous* stalk conidiophore with a globose head having conidial spores

Fig.4.The phylogram was established with the ITS sequence of *A. niger.*

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RESEARCH ARTICLE

Bill Deformity in Six Resident Birds of Jammu Region, J & K, India for the First Time

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ABSTRACT

This documentation reports bill deformity in six resident birds of Jammu region for the first time. All the birds studied were counted throughout the study area and five types of bill abnormalities were reported from different individuals of these species. Out of the five types of bill deformities, upper mandible elongation was found in maximum number of individuals. Furthermore, among different species under study, highest number of bill deformity was found in case of House Crow (*Corvus splendens*) followed by Common Myna (*Acridotheres tristis*) whereas intensity of deformed bill was maximum in case of House Crow. The individuals with deformed bill were found to have changed their foraging strategies and habits. Various reasons leading to deformed bill in these avian species has been discussed.

Keywords: Bill deformity, feeding, foraging strategies, breeding behavior, intensity and resident birds.

INTRODUCTION

Close correlation of the shape and size of a bird's bill with its feeding preferences has been demonstrated even within a species 1&2. Deformities have been found in a wide range of birds due to various reasons and mostly affect the beak and legs of birds. Bill deformities probably have not been reported previously in Indian birds 3. 4 defined it as any irregularity in the bird's bill which is sufficiently different from the normal to attract the observer's attention. Beak or bills of various shapes in birds play diverse ecological purposes and are a good indication of bird's feeding habits. The beak comprised of an upper and lower mandible, both of which have a bony base with a horny covering that continually grows, mainly at the tip, to make up for the wear and tear of use. A healthy beak should be smooth and symmetrical in appearance. Any change in the normal growth may lead to the formation of an abnormally shaped bill. The present piece of work documents some cases of bill deformity in six different resident bird species

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i.e. House Crow (*Corvus splendens*), Jungle Crow (*Corvus macrorhynchos*), Red-vented Bulbul (*Pycnonotus cafer*), Common Myna (*Acridotheres tristis*), Blue Rock Pigeon (*Columba livia*) and Indian Pond Heron (*Ardeola grayii*) for the first time from the study area.

MATERIALS AND METHODS

Study Area

Study was carried in and around Jammu region, J&K, India for two consecutive years i.e. April 2017-December 2019. Jammu and Kashmir is located in the northern part of the Indian sub-continent in the vicinity of the Karakoram and western mountain ranges. It falls in the great north-western complex of the Himalayan ranges with marked relief variations, snow capped summits, antecedent drainage and complex geological structure. The city of Jammu is situated on a hillock on the banks of river Tawi. The district Jammu is spread over an area of 3097 sq. km (2001 census) with an approximate forest area of 1339 sq. km (2001 census). It is bounded by district Udhampur in the north and north east, Samba district in the east and south east, Sialkot district of Rawalpindi (Pakistan) in west and Rajouri district and parts of district Bhimber (POK) in the North West. Geographically, it lies between 32°27' and 33°30'North latitudes and 74°19' and 75°20' East longitudes. Altitudinally, it extends from 275 meters to 410 meters above the mean sea level (msl). The Jammu city sprawls on both the banks of river Tawi which is a tributary of river Chenab. The old city is confined to the right bank while the later expansions of the city have largely taken place on the left bank with few new colonies on the right bank also. Large scale urbanization and industrialization has given rise to what is now called Greater Jammu, the name given to the old, new and the suburbs of Jammu.

METHODOLOGY

For recording the various aspects of bill deformity, periodic surveys were carried out by adopting systematic field procedures and techniques. Recurrent surveys were performed from 0530 hrs to 1200 hrs in morning and 1300 hrs to 1930 hrs in evening during summers and 0730 hrs to 1300 hrs in morning and 1400 hrs to 1830 hrs in evening during winters. Direct observations were made but in case where the bird was not visible through naked eyes, binoculars were used without disturbing the bird. Besides, photography and video clips were taken to support the data. In order to estimate the number of individuals with bill deformity, the following methods for population estimation were carried out:

Visual census: This was done during the early hours when birds moved to their feeding grounds. In this method, the study area was scanned from appropriate vantage points with the help of binoculars or with naked eye and the number of affected individuals was counted.

Line Transect Method: In this method, the pathway of transect was pre-determined on which the observer walked and recorded the number of birds (under consideration) that were seen and heard in order to estimate the number of individuals. The Line Transect Method was conducted in early morning and afternoon hours. Width of transects depended upon visibility. Greater the visibility, more the width of transects. During the Monsoon season, when the vegetation disrupts the clear visibility of birds, slight deviation was taken from the transect path to get a close view of the bird and after observation, again the permanent path was followed.

Point Transect Method: This can be considered as line transect done at zero speed for short and fixed duration of time [7]. In this method, bird records were made and necessary associated data was taken by standing at a fixed point for 15 minutes. The points were distributed at random in the study area, so as to represent all types of habitat elements of that area. Vegetative conditions of the point, time of observation, number of individuals and different activities of the bird were also taken into consideration.

Tools Used

1. Digital Camera (Sony) DSC-HX 100V;
2. Canon EOS 600D;
3. Digital Camera (Sony) DSC H-55;
4. Binoculars Olympus 10x50 DPS I, Field 6.5º.

RESULTS AND DISCUSSION

During the present piece of work, bill (or beak) deformity was found in six resident bird species belonging to different orders

1. House Crow (*Corvus splendens*): It is also known as the Indian grey necked or Colombo Crow . It is a common bird of the Corvidae family belonging to Order Passeriformes. The forehead, crown, throat and upper breast are richly glossed black whereas the neck and breast are a lighter grey-brown in colour and the legs, wings and tail are black.
2. Jungle Crow (*Corvus macrorhynchos*): The large billed crow (or Jungle Crow) is a widespread Asian species of Crow. It has a large bill and is sometimes known by the common name Thick-billed crow. It also belongs to family Corvidae and Order Passeriformes. It has dark greyish plumage from the back of the head, neck, shoulders and lower body. Wings, tail, face and throat are glossy black. However, the depth of grey shading varies across its range.
3. Red-vented Bulbul (*Pycnonotus cafer*): It is a member of Family Pycnonotidae and Order Passeriformes. It is a resident breeder across the Indian sub-continent. The body is dark brown with a scaly pattern while the head is black or darker in colour. The rump is white while the vent is red. It is easily characterized by its short crest giving the head a squarish appearance.
4. Common Myna (*Acridotheres tristis*): It is also known as Indian myna and is a member of the family Sturnidae, Order Passeriformes. It has a black hooded head, brown body and the bare yellow patch behind the eye. A white patch is present on the wing primaries while the wing lining on the underside is white. The bill and legs are bright yellow.
5. Blue Rock Pigeon (*Columba livia*)- The bird belongs to family Columbidae and Order Columbiformes. The birds are bluish gray with two black bands on the wing and a black tip to the tail. Larger and plumper than a Mourning Dove, with small heads and short legs. Their wings are broad and pointed and the tail is wide and rounded.
6. Indian Pond Heron (*Ardeola grayii*)- It is also called as Indian Paddy bird and belongs to family Ardeidae and Order Pelecaniformes. The Pond Herons are stocky species with a short neck, short thick bill, typically buff or brownish back, and coloured or streaked foreneck and breast. In summer, adults may have long neck feathers. *Ardeola* herons are transformed in flight, looking very white due to the brilliant white wings.

Bill abnormalities were classified into 5 categories viz;
1) Elongated Upper Mandible;
2) Down curved Upper Mandible;
3) Lower Mandible overgrown;
4) Crossed Mandibles
5) Half grown Upper Mandible.
The percentage of observed abnormalities within each category is shown in **Table 1.**

1st Case study: House Crow (*Corvus splendens*): House Crow was commonly found throughout the study area and several cases of bill deformity were observed at both urban and the rural setup. A total of 98 individuals were recorded having different shapes of deformities in their bill. Maximum number of individuals with elongated upper mandible was noticed followed by down curved (overgrown) upper mandible while only one case with cross mandibles was noted down (Table-1, Fig. 1- a & b). Severity of the deformed shape of bill was found to be varied

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between different individuals. Being omnivore, House Crows devour wide variety of food items but due to deformed bill it was noticed that while foraging they instead of pecking properly at the food item, tilted their head sideways with beak wide open and swipe at the ground having food and thereby collect the food material in the lower beak and then returned its head straight way to swallow the food. Furthermore, it was also observed that the individuals with deformed bill don't feed on the dead carcass of animals as they were not able to tear the flesh and play very little role as scavenger in the environment. [8] also reported House Crow with bill deformity feeding on the tit bits of meat near the meat shops.

2nd Case study: Jungle Crow (*Corvus macrorhynchos*): As Jungle Crow is summer migrant of Jammu region, so only single individual of Jungle Crow with elongated upper mandible was observed in the University campus during summer months. The individual was feeding on the food with less difficulty (Fig. 2).

3rd Case study: Red-vented Bulbul (*Pycnonotus cafer*): Only 4 individuals with bill deformity were noted, in which only one individual with elongated upper mandible was seen and three (03) individuals with half grown upper mandible were recorded from sub-urban and rural area (Table-1, Fig. 3). In case of elongated upper mandible they were found to apply the same technique as utilized by House Crow but the individuals with half grown upper mandible were observed to have much difficulty while feeding. These individuals were found to pluck the plant food (like small fruit, berries, flowers, fig etc) from sideways and couldn't carry away the food far away.

4th Case Study: Common Myna (*Acridotheres tristis*): 22 individuals of Common Myna were noticed with the bill deformity in both urban and rural setup. Out of 22 individuals, nine (09) were found to have elongated upper mandible, 06 with down curved upper mandible, one with lower mandible overgrown and four individuals with half grown upper mandible. All affected individuals were found to have difficulty while feeding but being omnivore in their diet, they have positively adapted with the deformed bill by changing their feeding strategies, as it was found to feed by a series of sideways scooping movements.

5th Case Study: Blue Rock Pigeon (*Columba livia*): Two individuals with down curved upper mandible were found in the urban setup of the study area **(Fig. 4)**. Being a granivorous bird, the deformed billed individuals were found to devote maximum time in foraging as they have to collect the grains from the ground. To feed, these individuals forcibly peck against the ground and collect the food grains in the lower part of bill and then straighten up their head to swallow it. [9] recorded that starling in Regent's Park, London, with a decurved bill about two and a half inches long, turned its whole head on one side to pick up food at about the same distance from the base of the bill as it would have done if it had been normal. [10] reported House Sparrow feed by scooping up grain with its head turned to one side and flat on the ground.

6th Case Study: Indian Pond Heron (*Ardeola grayii*): Only one individual with crossed upper and lower mandibles was recorded from open agricultural fields (Fig. 5). Normally Indian Pond Heron feeds on crustaceans, aquatic insects, fishes, tadpoles etc in the wetland habitat and on insects like crickets, dragonflies and bees in terrestrial habitats. But, the criss-cross bill of affected Pond Heron posed a great hindrance in foraging, as it was noted to spend more time in feeding when utilizing terrestrial habitats in an attempt to capture the desired prey which resulted in more number of unsuccessful attempts and lower foraging efficiency owing to its inability to capture small sized of potential prey items with its deformed bill. However, the adult individual with deformed bill survived by feeding on larger prey items in the wetlands which it could hold with its deformed bill. However, the handling time in this case for large prey items was more which resulted in decrease of overall foraging success.

In the present piece of work, it was observed that individuals with abnormal bills were unable to feed in the normal way but instead they have acquired feeding mechanisms appropriate to the beaks which they possess, thus the adaptability enables birds to survive even with abnormal bills. [4] also propound that the acquisition of different

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feeding mechanisms within a species, which may be of importance in enabling birds to take advantage of locally abundant food supplies. Thorpe 11 a & b has shown that individual passerines may acquire unusual feeding methods which are more easily acquired by some species than others. Out of five species, maximum numbers of individuals of House Crow (*Corvus splendens)* were recorded with deformed bill followed by Common Myna (*Acridotheres tristis*) and the intensity of deformed bill was also highest in case of House Crow as compared to other birds owing to their large population. [8] also reported bill deformities in House Crow, Large billed Crow and Yellow-billed Blue Magpie in India. [12] urged monitoring of bill deformities to detect if the incidence of and distribution of defects was changed, especially considering the potential mutagenic, teratogenic and carcinogenic effects of chemical pollutants. Moreover, prevalence appeared higher among adults as compared to young ones as the bill was well developed in case of adults. Probable reason for deformed bill might be due to following reasons.

Accident or Injury: During the nestling period, the nestlings usually fall from the nest as they grow in size and they might often land on their beaks leading to permanent damage which ultimately result in a deformed bill as the nestling becomes adult.

Genetic Defect: Genetic defect during their early developmental stages might have lead to any genetic change which caused abnormal development of the beak. [13] found two out of four nestlings of an American Robin (*Turdus migratorius*) had crossed bills, whilst the other two nestling and parents were normal and suggested recombination in two of the offspring, of recessive factors present in both parents. [14] in an experiment mated a crossed beaked female domestic fowl with a normal male and suggested that this abnormality is inherited.

Toxic Chemicals or Pollutants: Reason for bill deformities may be due to some chemical pollutants such as pesticides, fertilizers etc. The dietary habits of these birds, like House Crow which feeds on dead decaying animals or anything occupies the top position of food chains, make them recipients of concentrating environmental pollutants. Likewise, in case of Indian Pond Heron, the larger prey items they consume such as fishes might have pollutants or other toxic substances accumulated in their body, as the sewage or the runoff water from agricultural fields directly enter the water bodies leading to the accumulation of pollutants in the aquatic organisms. However, in case of Blue rock pigeon which is chiefly granivorous in their diet, might have consumed the grains which were either sprayed with pesticides or fertilizers. The other two species i.e. Bulbul and Myna plunder on both plant as well as animal matter, so any food item may be affected by the pollutant or chemicals. [15] felt that the deformities they found among blackbirds may have resulted from a vicidal chemicals. [16] suggested cause of bill deformities due to industrial contamination. [17] propounded that exposure to stressors, including toxicants, during early developmental stages may result in long-term health effects by altering epigenetic processes. [18] elaborated on the environmental contaminants and chromosomal damage associated with beak deformities in black-capped chickadees (*Poecile atricapillus*). [19] found out that nestlings of the great tit (*Parus major*) have been shown to accumulate high concentrations of organochlorine compounds that reflect contamination from maternal sources and, to a lesser extent, contamination from ingested foods.

Disease: Climate change, urbanization, agricultural practices, pollution have resulted in increasing the spread of infectious avian diseases. Local migration and habitat transition of these birds had exposed them to various pathogens which directly or indirectly affect the overall health. [4] stated that horny covering or rhamphotheca of the bill is produced by the underlying epithelial dermotheca and it is quite possible that diseases of the latter could lead to deformities of the former. Recently, U.S. Geological Survey in Anchorage, discovered a prospective new species of virus "Poecivirus" within the family Picornaviridae leading to Avian Keratin Disorder (AKD) without knowing the transmission. In AKD, accelerated growth of the keratinized outer layer of the beak (the rhamphotheca) causes elongation and crossing of the mandibles of the beak. [20] referring to Budgerigars (*Melopsittacus undulatus*), stated that *Cnemidocoptes pilae* (a mite) may be responsible for damage to the developing beak in the nestlings, resulting in later deformity. [21] said that the avian liver is a major storage organ for iron, an essential element for a

variety of metabolic functions but a potential toxicant at high levels and further stressed that iron is an important micronutrient not only for avian immune response to pathogens but also a requirement for the pathogens themselves. [22] studied black-capped chickadees (*Poecile atricapillus*) and found out that reduced level of iron in the livers of affected adults could have been related to mobilization of stores from the liver in a general elevated immune response of infectious pathogens which were also documented among chickadees with beak deformities.

Nutrition: Food is directly related to the health of the bird and scarcity of any nutrient in the diet of these birds can lead to abnormality. [23] stated that possible reasons for bill deformities includes genetic or developmental causes, injury or disease. These deformities may either have developed slowly over a period of a year or more rapidly as a result of any injury or any reason. It was worthy to note that individuals with deformed bill behaved normally among themselves and other con-specifics also behaved normally with them during various associations in the study area. It was noted that, deformity posed a hindrance while carrying daily activities such as feeding, preening etc, but *Corvus* and *Acridotheres* species have been observed positively living with the deformed bill and has changed their feeding technique owing to their omnivorous feeding. However, [24] stated that X-ray photographs of the deformities of a Scrub Jay (*Aphelocoma coerulescens*), a Red-breasted Sapsucker and a Californian Thrasher suggested that the bony portions of the bill were normal; and that the abnormal growth was limited to the dermatheca.

With regard to breeding, bill deformity though posed less hindrance while carrying out breeding activities as other partner will mainly play the role in nest building and feeding the young ones. Many breeding pairs were encountered in which one partner was normal and other with deformed bill in all above said cases. It was interesting to see that the partner with deformed bill was found to alter the behaviour of his or her partner. During courtship feeding as observed in Crow, Bulbul and Myna, it was noticed that the partner with deformed bill learnt to tilt its head on one side with food in the mouth whereas the other partner took some time to accept the food, but soon altered his/ her behaviour by learning to tilt the head on one side too. Out of the five birds mentioned, the individuals with deformed bill spent more time in nest construction, as it was very much difficult for the bird to carry the nesting material with their deformed bill which ultimately resulted in late breeding and less breeding success. Furthermore, only in case of Red-vented Bulbul, the cup shaped nest was not woven properly whereas in other species the nest was not intricately woven.

One of the interesting things about these birds is that they survive by changing their food and feeding strategies. The way with which these birds adjust their behaviour probably depends on the speed of the onset of the deformity. Presumably it is difficult for a bird to survive if the change is sudden (due to injury) rather than slow. It was also recorded that due to bill (or beak) deformities, these birds were found to suffer from various problems like:

- Foraging efficiency was low in these individuals as they have to spent more time while searching suitable food item and then consuming it.
- Difficulty while foraging due to which chances of weakness leading to starvation was more in case of these individuals with deformed bill.
- Individual with deformed bill were found to have unclean, clumsy and unpreened body feathers as they were not able to preen effectively their feathers properly thus harboring large number of parasites and in turn hampering their flight. [25] said that removal of parasities also requires a normal bill with the tip approximately closely.
- In individuals with severe deformity, some change in behaviour was also noticed as they indulge in more fights both intra and inter specifically.
- Individuals with deformed bill were noted to construct untidy nest and took more time in nest construction, thus lowering their overall breeding success.

Thus, it was concluded that, despite having difficulty in feeding, preening and nest building, these birds were found to survive and adapt with their deformed bill by changing their habits and feeding strategies. Individuals with a slight deformity were found to feed almost normally and those individuals with severe deformed bill were found to have lower chance of survival. The behaviour of most of the birds is affected to a certain extent but sometimes it is quite different from the normal. At present we do not know the exact cause of bill deformities in these individuals

being reported. In this piece of errand, all the possible reasons leading to the bill deformity are discussed but it seems that the bill deformity is becoming a serious avian disease, so further studies at the genetic level are required so that the proper cause of bill deformity can be found and studied. Thus, the present piece of work will help to serve as baseline information for understanding the behavioral aspects of these affected birds while carrying out diurnal activities of their life.

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Table 1: Number (%) of individuals showing different types of bill deformity in six resident bird species of Jammu, J&K, India

Type of Deformity	House Crow *Corvus splendens*	Jungle Crow *Corvus macrorhynchos*	Common Myna *Acridotheres tristis*	Red-vented Bulbul *Pycnonotus cafer*	Blue Rock Pigeon *Columba livia*	Indian Pond Heron *Ardeola grayii*
Elongated Upper Mandible	48 (49%)	01 (100%)	09 (41%)	01 (25%)	-	-
Down Curved Upper Mandible (overgrown)	32 (33%)	-	06 (27%)	-	02 (100%)	-
Lower Mandible overgrown	-	-	01 (5%)	-	-	-
Crossed Mandible	01 (1%)	-	02 (9%)	-	-	01 (100%)
Half grown Upper Mandible	17 (17%)	-	04 (18%)	03 (75%)	-	-
Total	98 (100%)	1 (100%)	22 (100%)	04 (100%)	02 (100%)	01(100%)
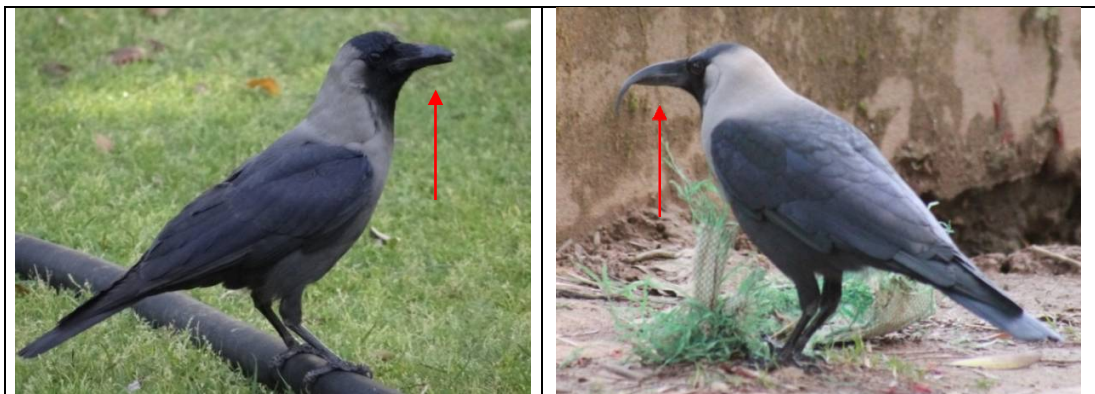
Fig 1:House Crow- a) Half grown Upper Mandible & b) Down curved upper mandible

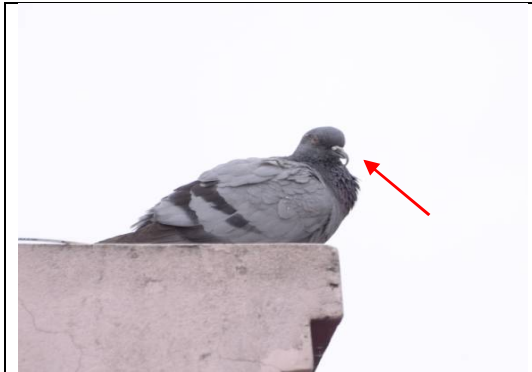**Fig 2: Jungle Crow with Curved Upper mandible**

Fig 3: Red-vented Bulbul with half upper mandible

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Fig 4: Blue rock Pigeon with Curved Upper mandible

Fig 5: Indian Pond Heron with Crossed Mandible

RESEARCH ARTICLE

Cross Sectional Observation of *Kabasura Kudineer* - A Siddha Polyherbal Formulation

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ABSTRACT

Siddha system of medicine is more popular and predominant in southern region of India. This system provides challenging drug to the infectious disease very importantly the current pandemic world. *Kabasura Kudineer* (KSK) is a famous Siddha kudineer formulation promoted by Siddha physicians and accepted by many of the world population. The current review highlight the Vernacular names, Family, Medicinal ingredients, Morphology, Geography, Part used, Phytochemical constituents, and Pharmacological actions of the individual drug of the polyherbal formulation, *Kabasura kudineer*. However, there is a need for further reviews on KSK to study extensively.

Keywords: Siddha system of medicine *Kabasura Kudineer (*KSK), Polyherbal formulation, Phytochemical constituents, Pharmacological actions.

INTRODUCTION

Siddha medicine is one of the oldest medical systems in the world. This system is most commonly practicing in India especially in southern regions. Siddha medicine become popular now a days because of various outbreaks of communicable and infectious diseases like Chikungunya, Dengue, Swine flu etc. These diseases of viral origin are very challenging to the modern world because of lack of ideal anti-viral therapy. COVID-19, which is different from ordinary corona virus because it's a new virus that appears in humans and spreads very quickly from person to person worldwide. Because it is a new virus, no one will have immunity to it and everyone could be at risk of catching it. This includes healthy adults as well as older people, young children and those with existing medical conditions. The polyherbal decoction *Kabasura Kudineer* (KSK) is a well-known one next to Nilavembukudineer (NVK)(fig.1) This KSK is introduced for the prevention and the management of COVID-19. The people of Tamil Nadu are very attentive about this Siddha drug KSK because it is used to prevent and protect from the deadly life threatening diseases. Here, an attempt was made to review the explored ethno pharmacological activities of the ingredients of KSK to strengthen the scientific facts favoring this formulation[1].The preliminary step was to develop the documentary evidence for the medicinal ingredients those are using to prepare the *kabasura kudineer* which is mentioned in selected text books and literatures.

MATERIALS AND METHODS

Study Design: It is a Literature review related study.

Duration of the Study: November 2020 to February 2021

Statistical analysis Collected data were processed and statistically analyzed by simple statistical method using MS. Excel 2010.

RESULTS AND DISCUSSION

Scientific and Selected vernacular Names of the Medicinal Ingredients
Among the 15 ingredients of the KSK, all were identified as herbal materials. These fifteen (15) plant species belonging to 12 families which had been documented as ingredients for the preparation of KSK were used for this review. The botanical names are used worldwide and are established by the "International Code of Botanical Nomenclature" (ICBN). The botanical name is unique to a specific plant. No other plant in the world will have the same botanical name. Common names for a plant, by contrast, will be different in different languages, may differ by region within a country or may be applied to several different plants. Therefore, the scientific name is important to the correct ingredient identification. The scientific and selected vernacular names of the individual plants are summarized in Table 1.

Families of the Medicinal Ingredients of the KSK
The idea of plant families is that plant which has something in common can be grouped together. Users can identify an unknown plant to the correct family by reporting the visible details of the leaves, fruits and other parts. The choice of information to report is entirely up to the user. The order in which information is entered does not be identification. Some families can be identified by single feature. Families of the selected medicinal ingredients. From these 15 ingredients, the species were found in Zingiberaceae, Piperaceae, Myrtaceae, Euphorbiaceae, Asteraceae, Acanthaceae, Combretaceae, Lamiaceae, Costaceae, Menispermaceae, Malvaceae, Cyperaceae families [2-11].

Morphology of the Medicinal Ingredients: It is the study of the various external features of the plants. This is usually considered distinct from plant anatomy, which is the study of the external structure of plants, especially at the microscopic level. Plant morphology is useful in the visual identification of plants. Based on the morphology (Table 3) of ingredients were classified as 2 trees, 10 herbs, 2 shrubs, and 1 perennial plant [12].

Native of the Medicinal Ingredients: This review indicated that natives of the plant species. A plant is considered native if it has occurred naturally in a particular region, ecosystem or habitat without human introduction [7][11][13].

Parts Used for Medicinal Ingredients: Medicinal properties derived from plants may come from many different parts of a plant including leaves, roots, barks, seeds, fruits and flowers. The different part of these plant contain different active constituents. Important parts of plants used for the preparation of KSK are shown in Table 5. This review revealed that parts used for medicinal ingredients were dry fruits, roots and seeds. However, the rhizome, flower bud, leaf and flower were also used [14][15][16][17].

Pharmacological Actions of the Medicinal Plants: Action is the function of drug which mentions the outcome effect of the drug like expectorant, tonic, carminative, stimulant, diuretic, etc. An ingredient can have more than one action. Based on the Table 7, pharmacological actions such as carminative, stimulant and stomachic were possessed by these ingredients [2][5][7-11].

Phytochemical Contents

Phytochemicals also called phytonutrient. Bioactive chemical compounds found in plants, as antioxidants, considered to be beneficial to human health [2][6,7]. It is well-known that plants produce these chemicals to protect themselves but recent researches demonstrate that they can also protect humans against diseases [9,10][13]. There are more than thousand known Phytochemicals [20,21][29]. Therefore, the phytochemical contents seen in these selected ingredients are reported in Table 8.

CONCLUSION

The polyherbal decoction *Kapa Sura Kudineer* (KSK) is a well-known one in the series next to Nilavembukudineer (NVK). This KSK is introduced for the prevention and the management of COVID-19, which is caused by SARS-CoV-2 virus which is a Novel corona virus. The people of Tamil Nadu are very attentive about this Siddha drug KSK because to prevent and protect from the deadly life threatening disease, COVID-19. Here, an attempt was made to review the explored ethno pharmacological activities, native, parts, vernacular name of the ingredients of KSK to strengthen the scientific facts favoring this formulation. This literature review, related research provides useful documentary evidences for medicinal ingredients those are commonly using to prepare the KSK for health management in the Siddha Medicine. However, there is a need for further reviews related to the chemical composition, elemental analysis, qualitative & quantitative analysis of phytochemicals, physicochemical properties and toxic substances. Further extensive scientific studies also should be carried out to justify in future [47].

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Table 1: Scientific and Selected Vernacular Names of the Medicinal Ingredients of KSK [2]

Sl.no	Botanical name	Tamil name	English name	Sanskrit name
1.	*Zingiber officinale*	Chukku	Dried ginger	Ausadha
2.	*Piper longum*	Thippili	Long pepper	Upakalya
3.	*Syzyginm aromaticum*	Kirambu	Clove	Bhadrasriya
4.	*Tragus involucrate*	Sirukanchoriver	Kantakari	Vyaghri
5.	*Anacyclus pyrethrum*	Akkirakaram	Spanish chamomile	Nil
6.	*Hygrophila auriculata*	Neermulliver	Kokilaksha	Gokanta
7.	*Terminalia chebula*	KadukkaiThol	Myrobalan	Abhaya
8.	*Justicia adathoda*	Adathodai ilia	Malabar nut	Arus
9.	*Anisochilus carnosus*	Karpooravalliilai	Mexican mint	Karpuravalli
10.	*Costus speciosus*	Koshtam	Crepe ginger	Kemuka
11.	*Tinospora cordifolia*	Seendhil	Heart-leaved moonseed	Guduchi
12.	*Clerodendrum serratum*	Siruthekku	Blue-flowered glory tree	Bhargavi
13.	*Andrographis paniculata*	Nilavembu	Green chiretta	Bhunimba
14.	*Sidaacuta*	VattathiruppiVer	Common wireweed	Bala
15.	*Cyperus rotundus*	Koraikizhangu	Nut grass	Musta

Table 2: Families of the medicinal ingredients of KSK:

Sl. No.	Botonical name	Family
1.	*Zingiber officinale*	Zingiberaceae
2.	*Piper longum*	Piperaceae
3.	*Syzyginm aromaticum*	Myrtaceae
4.	*Tragus involucrate*	Euphorbiaceae
5.	*Anacyclus pyrethrum*	Asteraceae
6.	*Hygrophila auriculata*	Acanthaceae
7.	*Terminalia chebula*	Combretaceae
8.	*Justicia adathoda*	Acanthaceae
9.	*Anisochilus carnosus*	Lamiaceae
10.	*Costus speciosus*	Costaceae
11.	*Tinospora cordifolia*	Menispermaceae
12.	*Clerodendrum serratum*	Lamiaceae
13.	*Andrographis paniculata*	Acanthaceae
14.	*Sidaacuta*	Malvaceae
15.	*Cyperus rotundus*	Cyperaceae

Table 3: Morphological classification of the medicinal ingredients of KSK:

Sl.no.	Morphology	Frequency	Percentage
1.	Tree	2	13.33%
2.	Herb	10	66.66%
3.	Shrub	2	13.33%
4.	Perennial plant	1	6.66%

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Table 4: Natives of the medicinal ingredients

SI. No.	Botanical name	Geographical area
1.	*Zingiber officinale*	Maritime Southeast Asia
2.	*Piper longum*	India, Malaysia, Nepal, Sri lanka, Vietnam
3.	*Syzyginm aromaticum*	Indonesia, Sri lanka
4.	*Tragus involucrate*	Western Ghats of India
5.	*Anacyclus pyrethrum*	Mediterranean Europe and parts of North Africa
6.	*Hygrophila auriculata*	Tropical Asia and Africa.
7.	*Terminalia chebula*	Sub-Himalayan region of Nepal and northern India to Sri lanka
8.	*Justicia adathoda*	India(Assam, Madhya Pradesh)
9.	*Anisochilus carnosus*	Africa
10.	*Costus speciosus*	South Asia and Southeast Asia
11.	*Tinospora cordifolia*	Northern Western Ghats
12.	*Clerodendrum serratum*	Asia and Africa
13.	*Andrographis paniculata*	South India and Sri lanka
14.	*Sidaacuta*	Central America
15.	*Cyperus rotundus*	Africa, Southern and Central Europe, Southern Asia

Table 5: Parts used for the preparation of KSK

SI. No.	BOTANICAL NAME	PART USED
1.	*Zingiber officinale*	Rhizome
2.	*Piper longum*	Fruit
3.	*Syzyginm aromaticum*	Flower bud
4.	*Tragus involucrate*	Root
5.	*Anacyclus pyrethrum*	Root
6.	*Hygrophila auriculata*	Root
7.	*Terminalia chebula*	Fruit rind
8.	*Justicia adathoda*	Leaf
9.	*Anisochilus carnosus*	Leaf
10.	*Costus speciosus*	Root
11.	*Tinospora cordifolia*	Stem
12.	*Clerodendrum serratum*	Root
13.	*Andrographis paniculata*	Whole plant
14.	*Sidaacuta*	Root
15.	*Cyperus rotundus*	Root tuber

Table 6: Part used for the preparation of KSK

SI.no.	Part used for medicinal ingredient	Frequency	Percentage
1.	Root	8	53.33%
2.	Whole plant	1	6.66%
3.	Stem	1	6.66%
4.	Leaf	2	13.33%
5.	Fruit	2	13.33%
6.	Flower	1	6.66%

Table 7: Pharmacological actions of the medicinal ingredients [14-41]:

Sl. No.	Botonical Name	Pharmacological action
1.	*Zingiber officinale*	Stimulant, Stomachic, Carminative
2.	*Piper longum*	Stimulant, Stomachic, Carminative
3.	*Syzyginm aromaticum*	Stomachic, Carminative, Antispasmodic
4.	*Tragus involucrate*	Diaphoretic, Anti-pyretic
5.	*Anacyclus pyrethrum*	Stimulant, Sialogogue, Rubifacient.
6.	*Hygrophila auriculata*	Diuretic, Refrigerant, Demulcent, Tonic
7.	*Terminalia chebula*	Digestive, Laxative, Tonic, Alterative
8.	*Justicia adathoda*	Anti spasmodic, Expectorant, Diuretic, Germicide
9.	*Anisochilus carnosus*	Stimulant, Diaphoretic, Expectorant
10.	*Costus speciosus*	Expectorant, Tonic, Diaphoretic
11.	*Tinospora cordifolia*	Alterative, Stimulant, Demulcent, Antiperiodic
12.	*Clerodendrum serratum*	Stimulant, Sedative
13.	*Andrographis paniculata*	Stimulant, Tonic, Alterative, Stimulant
14.	*Sidaacuta*	Tonic, Expectorant Demulcent, Diaphoretic
15.	*Cyperus rotundus*	Astringent, Stimulant, Tonic, Demulcent, Diaphoretic

Table 8: Phytochemical contents of the medicinal ingredients of KSK [42-45]:

Sl. No.	Botanical Name	Phytochemical content
1.	*Zingiber officinale*	Beta-sitosterol palmitate, isovanillin, glycol monopalmitate, hexacosanoic acid 2,3-dihydroxypropyl ester, adenine, gingerol, shogaol
2.	*Piper longum*	Coumaperine, piperidine, piperolactam A, pirrolidine, turmerone, aphanamol, bisdemethoxycurcumin, demethoxycurcumin
3.	*Syzyginm aromaticum*	Phenylpropanoids such as carvacrol, thymol, eugenol, cinnamaldehyde
4.	*Tragus involucrate*	Alkaloids, flavonoids, lipids, phenolic compounds, proteins, saponins and triterpenoids
5.	*Anacyclus pyrethrum*	Anacycline, pellitorine, enetriyne alcohol, hyrdocarolin, inulin (c 50%), traces of volatile oil and (+) – sesamin, amides (I, II, III, IV)
6.	*Hygrophila auriculata*	Phytosterols, tannins, carbohydrates, flavonoids, terpenoids, and sterols, lupeol, betulin, and stigmasterol
7.	*Terminalia chebula*	Tannins -gallic acid, chebulagic acid, punicalagin, chebulanin, corilagin, neochebulinic acid, ellagic acid, chebulinic acid, casuarinin, terchebulin, polyphenols such as corilagin, galloyl glucose, punicalagin, terflavin A, maslinic acid. Flavonol, glycosides, triterpenoids, coumarin conjugated with gallic acids called chebulin as well as other phenolic compounds[28]
8.	*Justicia adathoda*	Alkaloids, lignans, flavonoids, and terpenoid, steroids-campesterol, stigmasterol, sitosterol, and sitosterol-D-glucoside
9.	*Anisochilus carnosus*	94.3% of the essential oil. Carvacrol (27.9%), camphor (14.1%) and α-*cis*-bergamotene (10.2%)

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10.	*Costus speciosus*	Diosgenin, prosapogenin B of dioscin, diosgenone, cycloartanol, 25-en-cycloartenol and octacosanoic acid
11.	*Tinospora cordifolia*	Berberine, Palmatine, Tembetarine, Magnoflorine, Tinocordifolin. Octacosanol, Heptacosanol, Furanolactone, Tinocordifolioside, Cordioside, Cordifolioside A, Cordifolioside B
12.	*Clerodendrum serratum*	Serratin along with lupeol
13.	*Andrographis paniculata*	Andrographolide (C20H30O5) is the major diterpenoid. Other diterpenoids are deoxyandrographolide, neoandrographolide, 14-deoxy-11, 12-didehydroandrographide and isoandrographolide, over 20 diterpenoids and over 10 flavonoids
14.	*Sidaacuta*	Beta-phenethylamines, quinazolines and carboxylated tryptamines, in addition to choline and betaine combination of sympathomimetic amines and vasicinone
15.	*Cyperus rotundus*	Cyprotene, acopaene, cyperene, aselinene, rotundene, valencene, cyperol, gurjunene, trans-calamenene, dcadinene, gcalacorene, cadalene, amuurolene, gmuurolene, cyperotundone, mustakone, isocyperol, acyperone, 4,11-selinnadien-3-one and 1,8-cineole
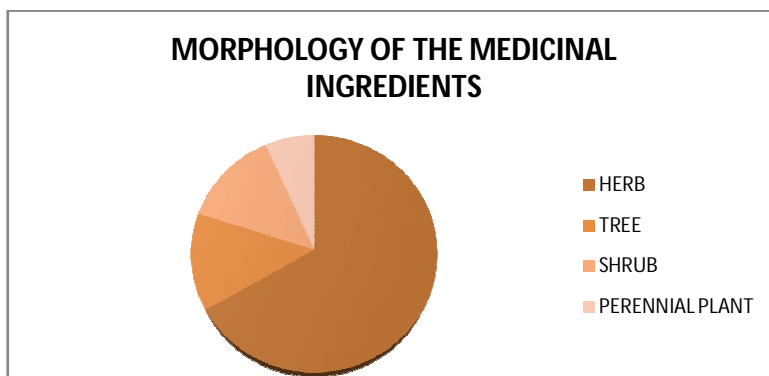
Diagram 1: MORPHOLOGY OF THE MEDICINAL INGREDIENTS
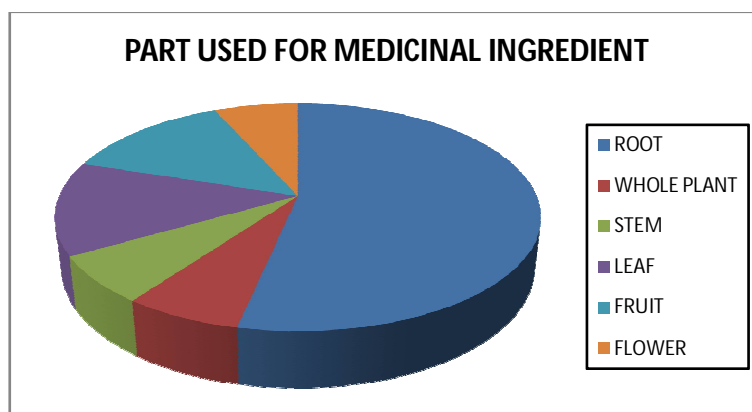
Diagram 2: PART USED FOR MEDICINAL INGREDIENT
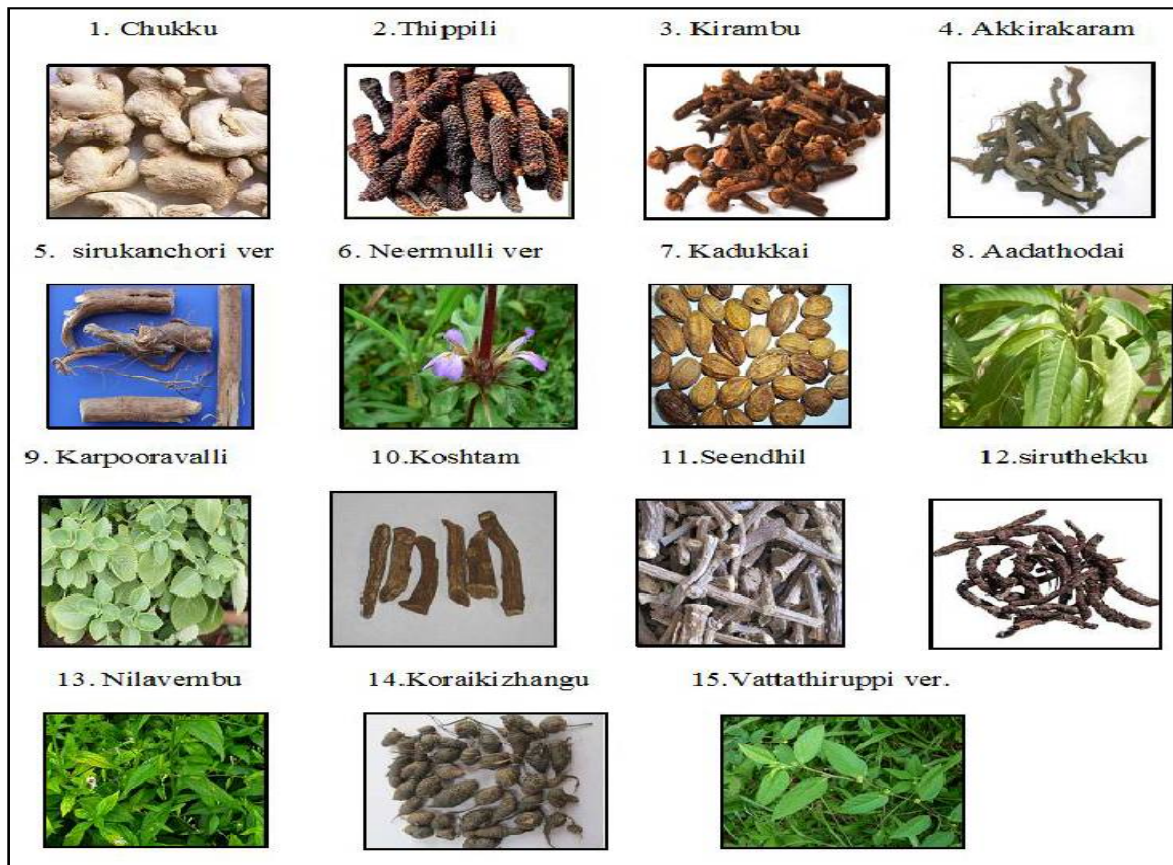
Fig. 1: Photographs of the ingredients of *Kabasura Kudineer* (KSK)

RESEARCH ARTICLE

Molecular Interaction Study in Binary Mixture of Pyridine and Cyclohexane at Different Frequencies by Ultrasonic Technique

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ABSTRACT

Density (ρ), ultrasonic velocities (*U*) and dynamic viscosity (η) for binary mixture of pyridine and cyclohexane is experimented at frequency range 2 MHz, 4 MHz, 6 MHz and 8 MHz at temperature 308.15 K and at atmospheric pressure over various compositions. The density (ρ) and viscosity (η) are calibrated using Specific gravity bottle and Ostwald's glass capillary viscometer respectively. The velocity (*U*) is measured using ultrasonic interferometer. The thermo dynamic parameters such as internal pressure (π_i), free volume (V_f), Gibb's free energy and acoustical parameters such as adiabatic compressibility (β), inter molecular free length (L_f), acoustic impedance (*Z*), relaxation time (τ) and their excess parameters have been calculated. Molecular interaction among the composite liquids explained from these calculations.

Keywords: Ultrasonic velocity, density, viscosity, adiabatic compressibility, free volume, internal pressure, Rao's constant and molecular interactions.

INTRODUCTION

In recent years effort has been made with measurement and interpretation of the ultrasonic properties of liquids and liquid mixtures at different temperatures and frequencies [1-2]. The ultrasonic studies are of great importance in helping to understand the nature and extent of the patterns of molecular aggregation that exist in liquid mixtures, resulting from intermolecular interactions [3-4]. The sign and magnitude of excess parameters have been used to investigate the interactions between the components of the liquid system [5-7].Molecular interactions are interactions between electrically neutral molecules or atoms. Other than atomic bonds these are electrical in nature and consist of attractive forces (orientation, induction, and dispersion forces) and repulsive forces. Ultrasonic waves have their extensive applications in various fields like non-destructive tests for solids and liquids in medical and engineering, pharmaceutical, polymer and chemicals, metallurgical industries etc. Ultrasonic investigations of binary mixtures

have been taking place since decades by so many scholars [8-12]. In the present paper, variation of various parameters of binary mixture of Pyridine and Cyclohexane has been studied for different frequencies.

MATERIALS AND EXPERIMENTS

The chemicals used in the present work were analytical reagent (AR) and spectroscopic reagent (SR) grades with minimum assay of 99.9% were obtained from E-Merk Ltd (India). The solution of the binary liquid mixture of pyridine and cyclohexane were prepared in terms of mole fraction.

Velocity Measurement
The velocity of ultrasonic wave in the binary mixture have been measured using multi-frequency ultrasonic interferometer with a high degree of accuracy operating at 11 different frequencies (Model M-84) supplied by M/s Mittal Enterprises, New Delhi. The measuring cell of interferometer is a specially designed double walled vessel with provision for temperature constancy. An electronically operated digital constant temperature bath (Model SSI-03spl) supplied by M/s Mittal Enterprises, New Delhi, operating in the temperature range -10oc to 85oc with an accuracy of ± 0.1K has been used to circulate water through the outer jacket of the double walled measuring cell containing the experimental liquid.

Density Measurement
The densities of the mixture were measured using a 25ml specific gravity bottle. The specific gravity bottle with the experimental mixture was immersed in a temperature-controlled water bath. The density was measured using the formula

$$\rho_2 = (w_2/w_1).\rho_1$$

where, w_1 = weight of distilled water, w_2 = Weight of experimental liquid,
ρ_1 = Density of water, ρ_2 = Density of experimental liquid.

Viscosity Measurement
The viscosities of the ternary mixture were measured using an Ostwald's viscometer calibrated with double distilled water. The Ostwald's viscometer with the experimental mixture was immersed in a temperature-controlled water bath. The time of flow was measured using a digital racer stop watch with an accuracy of 0.1 sec. The viscosity was determined using the relation,

$$\eta_2 = \eta_1 (t_2/t_1) (\rho_2/\rho_1)$$

Where, η_1 = Viscosity of water, η_2 = Viscosity of mixture,
ρ_1 = Density of water, ρ_2 = Density of mixture,
t_1 = Time of flow of water, t_2 = Time of flow of mixture.

Theory
The following thermodynamic parameters were calculated from Jacobson's relation [**13 – 17**].

Adiabatic Compressibility (β):
The adiabatic compressibility is the fractional decrease of volume per unit increase of pressure, when no heat flows in or out. It is calculated from the speed of sound (U) and the density (ρ) of the medium by using the equation of Newton Laplace as,

$$\beta = 1/U^2.\rho \text{ ------------------ (1)}$$

Intermolecular Free Length (L_f):
The intermolecular free length is the distance between the surfaces of the neighbouring molecules. It is calculated by using the relation

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$$L_f = K_T \beta^{1/2} \text{ ------------------ (2)}$$

where, K_T is the temperature dependent constant and 'β' is the adiabatic compressibility.

Free Volume (V_f): Free volume in terms of ultrasonic velocity (U) and the viscosity (η) of liquid is

$$V_f = (M_{eff}.U/K.\eta)^{3/2} \text{ --------- (3)}$$

Where 'M_{eff}' is the effective mass of the mixture, 'K' is a dimensionless constant independent of temperature and liquid. Its value is 4.281×10^9.

Internal Pressure (π_i):
The measurement of internal pressure is important in the study of the thermodynamic properties of liquids. The internal pressure is the cohesive force, which is a resultant of force of attraction and force of repulsion between the molecules. It is calculated by using the relation,

$$\pi_i = bRT (K\eta/U)^{1/2} (\rho^{2/3}/M^{7/6}) \text{ ------- (4)}$$

Where, 'b' stands for cubic packing, which is assumed to be '2' for all liquids, 'K' is a dimensionless constant independent of temperature and nature of liquids. Its value is 4.281x109. 'T' is the absolute temperature in Kelvin,'M' is the effective molecular weight, 'R' is the Universal gas constant, 'η' is the viscosity of solution in $N.S.m^{-2}$, 'U' is the ultrasonic velocity in $m.s^{-1}$ and 'ρ' is the density in $Kg.m^{-3}$ of solution.

Relaxation Time (T):
Relaxation time is the time taken for the excitation energy to appear as translational energy and it depends on temperature and impurities. The relaxation time can be calculated from the relation,

$$\tau = 4/3. (\beta.\eta) \text{ ------- (5)}$$

Where, 'β' is the adiabatic compressibility and 'η' is the viscosity of the mixture.

Acoustic Impedance (Z):
The specific acoustic impedance is given by,

$$Z = U.\rho \text{ ------- (6)}$$

Where, 'U' and 'ρ' are velocity and density of the mixture.

Gibb's Free Energy:
The Gibb's free energy is calculated by using the relation

$$\Delta G = K_B T.ln(K_B T\tau/h) \text{ ------- (7)}$$

Where, 'τ' is the viscous relaxation time, 'T' is the absolute temperature,
'K_B' is the Boltzmann's constant and 'h' is the Planck's constant.

RESULT AND DISCUSSION

The experimental values of density, viscosity and ultrasonic velocity at different frequencies are presented in table-1. Calculated values of acoustic and thermodynamical parameters are presented in table-2. The excess parameters explaining the nature and strength of molecular interactions, are tabulated in table-3.

When frequency increases, velocity decreases indicating weakening of intermolecular interaction. The molecules in the present mixture are close to each other and the vibrations are transmitted through the molecules to a large distance increasing the apparent free volume.

Free volume decreases very slowly with increase in frequency, indicating weak molecular interaction. Free volume decreases with increase in frequency, which shows the interaction in the mixture being stronger and the expansion is

less. When molecules are subjected to larger frequencies they vibrate rapidly, increasing the interaction between the molecules, which is of dispersive type. This reduces the free volume and hence the above observation.

Internal pressure increases very slowly with increase in frequency. This is because, when frequency increases, molecular motion increases and hence the molecular interaction increases. Gibb's free energy increases slowly with increase infrequency. Increase in ΔG suggests shorter time for rearrangement of molecules in the mixture. This may be due to the fact that, when frequency increases, the energy imparted to the molecules expedites the rearrangement procedure. Excess values of the binary mixtures result from the contributions due to the physical, chemical, and structural characteristics of the component liquids.

Excess free volume is negative and excess relaxation time is negative due to contraction in volume. Excess free length is positive indicates weak interaction. This may be due to the non-polar nature of molecules. Adiabatic compressibility changes in the same way as free length changes. Excess internal pressure and Gibb's free energy is positive at all frequencies, interaction being stronger, shorter time is required for rearrangement of molecules in the mixture.

CONCLUSION

Variation of ultrasonic velocity with frequency in the binary mixture of pyridine and cyclohexane enabled us to study the thermodynamic parameters and their excess values. These variations indicate the nature of the interaction between the components of the mixture. Although cyclohexane is non-polar the intermolecular interaction is evident through the excess values of the thermodynamic parameters. The negative values of excess velocity suggest that the binary liquid mixture is less compressible than the corresponding ideal liquids and the positive values indicate the reverse action. It has been observed that, the change in velocity with change in frequency is conspicuous. This lead to large variation in the parameters and their excess values with change in frequency.

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Table-1: Experimental values of Density, Viscosity and Velocity of binary liquid mixture.

Frequency	Density	Viscosity	Velocity
2 MHz	869.56	0.571	1247.1
4 MHz	869.56	0.571	1238.2
6 MHz	869.56	0.571	1225.1
8MHz	869.56	0.571	1209.7

Table-2: Calculated values of adiabatic compressibility, free length, free volume, Gibb's free energy, Acoustic impedance and Internal pressure of binary liquid mixture.

Frequency	Adiabatic compressibility $(\beta)(x10^{-10}N^{-1}.m^2)$	Free length (Lf) $(x10^{-10}m)$	Free volume (Vf) $(x10^{-7}m^3.mol^{-1})$	Gibb's free energy (ΔG) $(x\,10^{-20}k.J.mol^{-1})$	Acoustic impedance (Z) $(x\,10^6Kg.m^2.s-1)$	Internal pressure (πi) $(x\,106\,N.m-2)$
2 MHz	7.393	0.552	2.663	0.578	1.085	399.6
4 MHz	7.499	0.556	2.635	0.584	1.077	401.0
6 MHz	7.669	0.562	2.591	0.594	1.065	403.2
8MHz	7.859	0.569	2.544	0.605	1.052	405.7

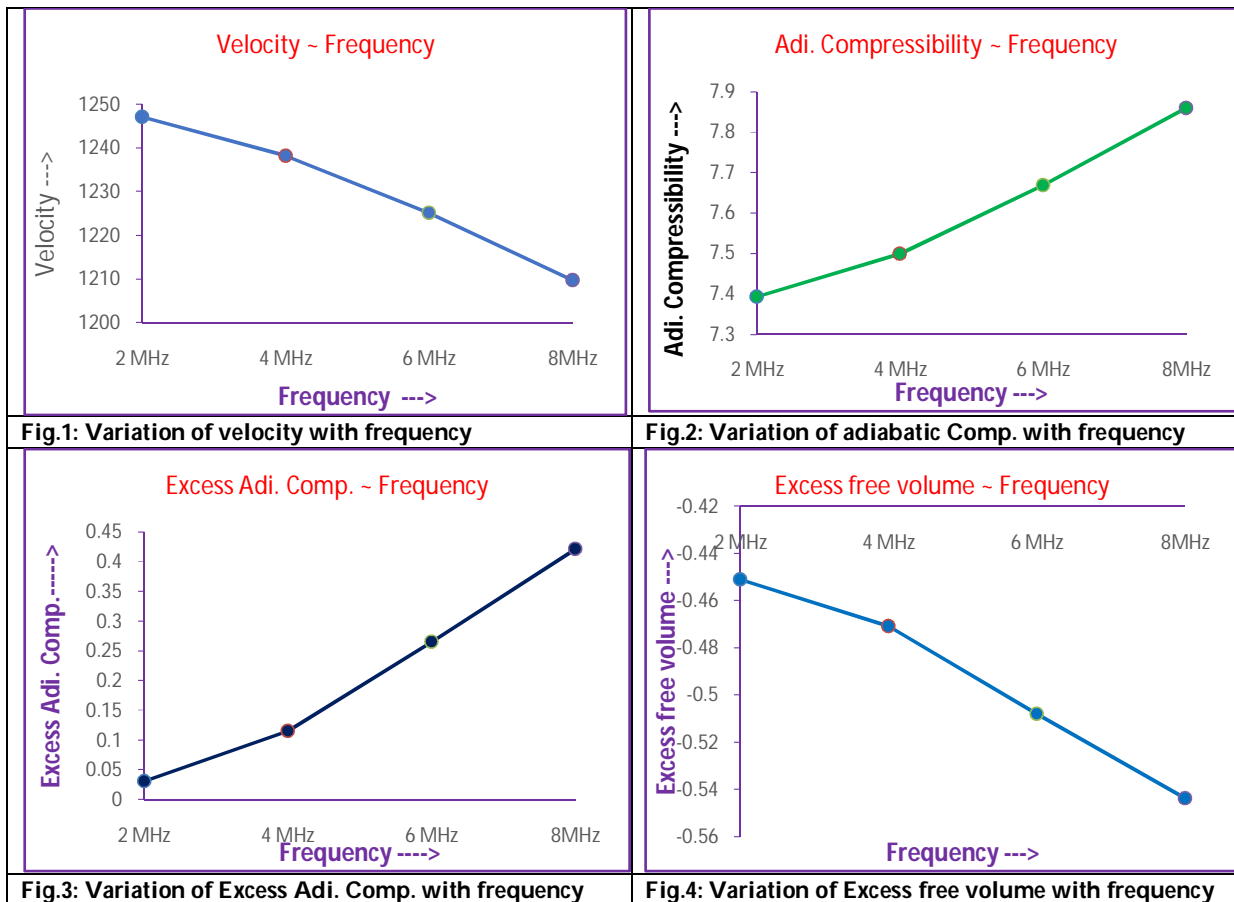**Table-3: Excess values of adiabatic compressibility, free length and free volume, internal pressure, acoustic impedance and Gibb's free energy of binary liquid mixture.**

Frequency	Excess Adi. comp. $(10^{-10}N^{-1}.m^2)$	Excess Free length $(10^{-10}m)$	Excess Free volume $(10^{-7}m^3.mol^{-1})$	Excess internal pressure $(x\,10^6\,N.m^{-2})$	Excess acoustic impedance $(x\,10^6\,Kg.m^2.s^{-1})$	Excess Gibb's free energy $(x\,10^{-20}\,k.J.mol^{-1})$
2 MHz	0.03	0.006	-0.451	16.523	-0.045	0.056
4 MHz	0.115	0.009	-0.471	17.594	-0.050	0.061
6 MHz	0.265	0.014	-0.508	19.571	-0.061	0.069
8MHz	0.422	0.020	-0.544	21.555	-0.071	0.078

Fig.1: Variation of velocity with frequency

Fig.2: Variation of adiabatic Comp. with frequency

Fig.3: Variation of Excess Adi. Comp. with frequency

Fig.4: Variation of Excess free volume with frequency

RESEARCH ARTICLE

Perception about Exercises among Geriatric Population of Tamil Nadu – A Cross Sectional Analysis.

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ABSTRACT

Health belief models always emphasise the need for patient empowerment about disease process and ways to handle them. Geriatric population is no exception for this. Study was carried out to find the perception about exercises among geriatric population of Tamil Nadu, using both offline and online analysis. A cross sectional analysis was conducted both online and offline process using a questionnaire which was specially designed for the study purpose. The questioner involved 6 six questions that were closed ended in nature. 921 subjects participated, 795 online and 126 offline. 46 subject's response were excluded because, 31 were incomplete, 09 were answered without interest (they have clicked option "A" for all the questions) 6 subjects answered more than one option (all of them were offline candidates as this option was not possible on Google page online process). A total number of 875 subjects accounted for the study results with 655 subjects from online survey and 120 subjects from offline mode. The results of the study showed that majority of the people perceived that walking as the best exercises, and thought walking didn't contribute for falling. They perceived that one should exercise daily for better health and didn't consider anaerobic training as a component of geriatric exercises. They stated that even doctors recommended walking as the best exercise and only few were prescribed with a set exercise protocol. This cross sectional study very clearly reflects that no clear understanding about exercise prevailed among the geriatric population in Tamil Nadu, which is the main cause for lack of health and lifestyle disorders.

Keywords: Geriatric, exercise, walking, fall, weight training.

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INTRODUCTION

Aging is commonly associated with progressive deterioration of physical and psychological health. His mismatch leads to many limitations particularly in walking [1]. Fall and fall related complications are the reason for which geriatric population seek medical help predominantly [2]. The fall can be defined as an occurrence *which results in an individual coming to rest unexpectedly on the ground or floor which may injure the individual".* Aged population who suffer from compromised cognitive function may fall twice as often when compared to their age related normal[3].The most common modifiable intrinsic factors that are related to subject fall risk are three in number namely muscle weakness, compromised balance and gait instabilities [4-6]the main tool that is available to alleviate these three factors is exercise. The most advised exercise in the Indian community for geriatric population is walking and the most concerned body component is body weight. Almost all other exercises are not advised and the majority of geriatric population does not consider other forms of exercises also. There may be various reasons for this scenario. The predominant reason is physicians and patients think walking is a safe and simple exercise. Availability of exercise gadgets, environment, lack of group exercise habits and last but not least knowledge about variety of exercises available and its benefits is lacking among general public. With COVID 19 pandemic, the health and fitness levels have to be enhanced for which the perception of the geriatric population have to be objectively studied for any practitioner to gain insight into the management. Hence this study was carried out to find the perception about exercises among geriatric population of Tamil Nadu, using both offline and online analysis. Four senior physiotherapists were included in designing a questionnaire that was used in the study.

METHODS

A cross sectional analysis was conducted both online and offline process using a questionnaire which was specially designed for the study purpose. The questionnaire did not involve rating or ranking so was not evaluated for its validity and reliability prior to application. The questioner involved 6 six questions that were closed ended in nature but also provided opportunity to the participants to explain the scenario if interested. The questionnaire is presented in table 1. A file was created and circulated in these social media platform like Whatsapp, Facebook, Instagram and Twitter, such that one click on the file will land them into the Google forms page which consisted of the questionnaire. The questionnaire was drafted in both English and Tamil languages. We also circulated the forms in the personal email IDs of our previous patients which were gathered from various clinics in Tamil Nadu. The study was conducted from 1st of April 2020 to 30thNovember 2020. Due to public lock down for COVID 19 in India the study was performed offline only after August 1st 2020.

The criteria for participating in this survey was, people from Tamil Nadu who were aged 60 years and above of any gender. Subjects from city, town and villages were allowed to participate if they had interest. The subjects who were medical and paramedical professionals were excluded from the study as they may have a basic idea about exercises from their curriculum. Subjects were requested not to search in the internet for the answers and were advised to answer from their past experience and knowledge. The subject had to fill their demographic data comprising of name, age, sex, educational qualification, place of living. Offline analysis was done through community visits and from physiotherapy clinics with the support of 14 physiotherapists from different parts of Tamil Nadu. The study response size was not fixed earlier as we were not sure about the numbers. But the deadlines for the study were clearly determined as 30th November 2020. Though all potential bias were previously discussed and minimised, a post test analysis was done by three senior physiotherapists and the answers were taken into consideration only after scrutinized.

Statistical analysis - The study results were displayed as percentages as all the data gathered were nominal in nature

RESULTS

In this cross sectional study 921 subjects participated, 795 online and 126 offline. 46 subject's response were excluded because, 31 were incomplete, 09 were answered without interest (they have clicked option "A" for all the questions) 6 subjects answered more than one option (all of them were offline candidates as this option was not possible on Google page online process). A total number of 875 subjects accounted for the study results with 655 subjects from online survey and 120 subjects from offline mode. The details are displayed in figure 1.

The mean age of the participants was 67.4 (2.9) years with 488 male and 387 female participants. The study had response from various parts of Tamil Nadu but predominantly from Salem (29%), Chennai (28%), Coimbatore (12%), Madurai (11%), 12% from other towns and 8 % from rural areas. 73% of the participants were educated till under graduation and 18% till higher secondary school. 9% were illiterate who were interviewed by physiotherapist for answer. The main results of the six questions are displayed in figure 2- 7.

DISCUSSION

The study was performed to analyse the perception about exercise among the geriatric population especially in Tamil Nadu, India. As we were intending to bring about an exercise awareness programme in future to Tamil Nadu, India we limited our study to the same.   Due to the pandemic situation that was prevailing during the data collection period and online method was preferred for data collection and later once the lock down was relaxed offline direct interviews were employed which clearly explains why there is a gross difference among the numbers. Initially it was very difficult for getting the response but the responses was encouraging after we conducted many awareness programs through online. These programs included talks by renowned physiotherapist who spoke on the need for physiotherapist to analyse the social understanding about exercise for better patient empowerment in future. Many physiotherapists approach their old patients using the contact details from the clinic ledger. The questions were   designed as closed ended question in order to facilitate response from the subjects. There were only four options out of which the subject had to select only one.

The motto of the first question was to find out what was the perception of geriatric population regarding how frequently they should exercise. The results show that huge number of population thought it is mandatory for doing exercise every day. Only very few people thought that exercising 2 to 5 days was sufficient. The Centre for disease control and prevention has recommended 3 to 4 exercise session a week as an effective routine for geriatric population [7] Minimizing falls is a common objective of many interventions for geriatric population, as 33%of people aged 65 years and older and 50% of those aged above 85 years sustain falls every year, out of which 10% result in serious complications [8,9].Walking is documented to be the major reason for falls in western countries yet it is described as good among geriatric population. The analysis of this study also showed that people believed that best exercise that geriatric population can prefer was walking, almost half of the participants thought it is true. Aged people with cognitive impairments are vulnerable to falls, even if their motor functions were intact[10,11]. Only one fourth of the people thought that combined exercise program was best.

The third question was designed to identify whether people were aware about shuffling their exercise routine frequently in order to attest to various health components to avoid body getting accommodated to a given exercise patter. The analysis of the results showed that most of the people were inclined towards sticking onto a same exercise program for a long period of time. Many subjects were of an idea that increasing the walking distance can also contribute to better health. The next question assessed whether the subjects were aware about where fall happened. It was very clear that most of the people thought it was during the household activity that fall happens predominantly, with many mentioning toilet and bath rooms as open ended option to answer. With very few people reporting that fall happens during walking. Equal amount of people reported that fall happened during travelling and also during stair climbing. The most ignored aspect of geriatric exercise protocol in South India was

weight training. This was again reflected in our study with majority of the people saying it is not needed or even contraindicated for aged. One fourth of the people thought that it was indicated only when the subject had previous experience of weight training. Very meager amount of population thought it was highly indicated for geriatric population. The final question was asked to understand what was prescribed by doctors for their patients as the prescribed exercise. The results were in favour of walking, which is predominantly prescribed exercise by the doctors and with only one fourth of the people privileged to get an exercise chart to work upon with.

CONCLUSION

This cross sectional study very clearly reflects that no clear understanding about exercise prevailed among the geriatric population in Tamil Nadu, which is the main cause for lack of health and lifestyle disorders. Through this study we recommend large scale surveys to assess the health belief models of people of all age categories particularly concentrating on special population and geriatric population.

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Table 1.Questionnaire used for the online and offline cross sectional analysis.

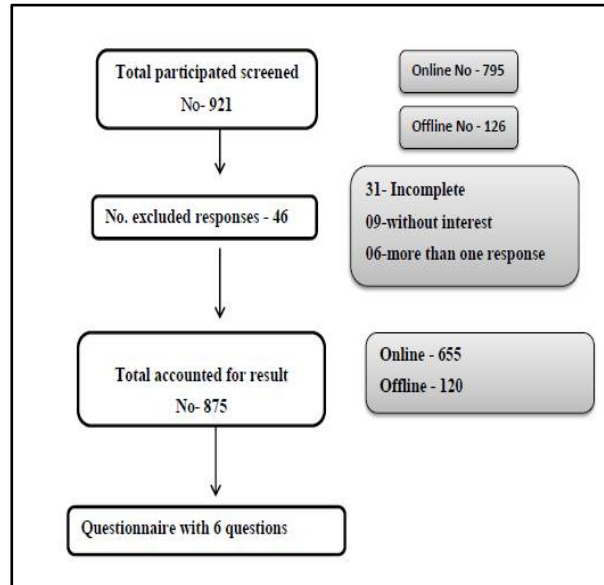| Questionnaire |
|---|
| **Name**   **Age/sex**
Educational qualification   **Place of living** |
| • The below mentioned questions are all pertaining to 60 and above year old subjects health.
• You can also write your opinion on the second page of the document mentioning the question number
• The answers are expected from a logical point of view and the responders are request not to search for answers in different sources, which will influence the intentions of the study.

1. **How frequently one should exercises at 60 and above years?**
a. Every day
b.5-6 days a week
c.4-5 days a week
d.2-4 days a week
2. **Which is the best and safe exercise for aged?**
a.Walking
b.YOGA
c.Weight training
d.A combined weight training and aerobic program.
3. **Which of the following statement are true?**
a.One should keep shuffling the exercises.
b.One should stick on to exercises for long.
c.One should walk for a given distance every day.
d.One should keep increasing the distance of walking every week.
4. **When do fall happens in elderly?**
a.During travelling
b.During stair case
c.During walking
d.During house hold activities
5. **Weight training with dumbbells and barbells are advisable at this age?**
a.May not be needed
b.Highly indicated
c.Can be done if only one has previous experience and practice
d.Highly contra indicated.
6. **What are the most frequent exercises prescribed by your doctor?**
a.Gym workout
b.Walking
c.Yoga
d.A combined exercises guided by a chart |

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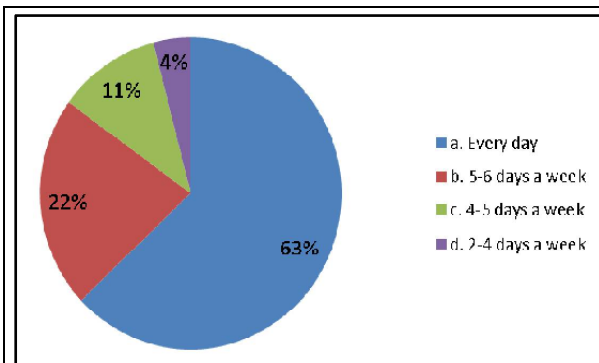

Figure 1.Flow chart of sample selection

Figure 2.Results for question 1 - How frequently one should exercises at 60 and above years?
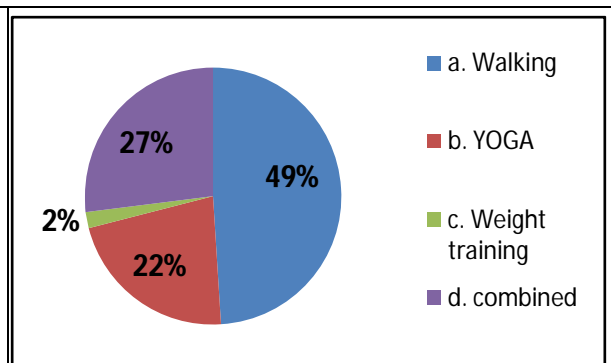

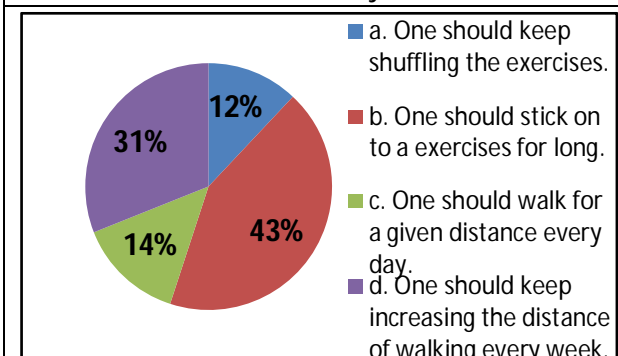**Figure 3.Results for question 2 - Which is the best and safe exercises for aged?**

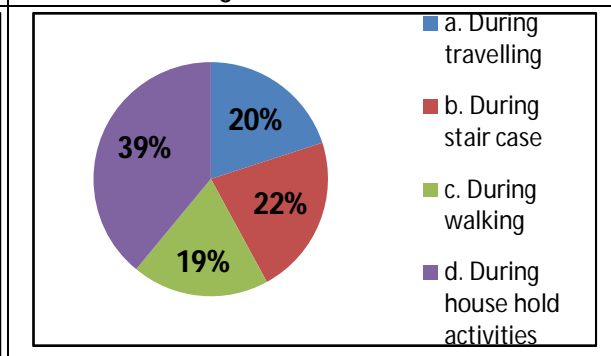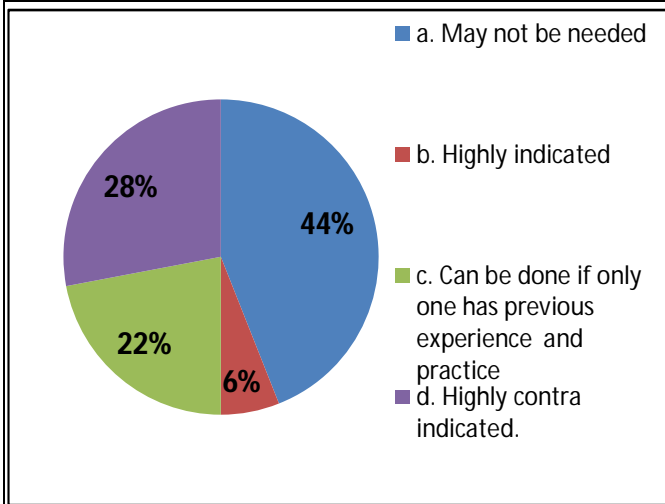**Figure 4.Results for question 3 - Which of the following statement are true?**

Figure 5.Results for question 4 - When do fall happens in elderly?

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a. May not be needed

b. Highly indicated

c. Can be done if only one has previous experience and practice

d. Highly contra indicated.

28%
44%
22%
6%

Figure 6.Results for question 5 - Weight training with dumbbells and barbells are advisable at this age?

a. Gym workout

b.Walking

c. Yoga

d.A combined exercises guided by a chart

18%
24%
16%
42%
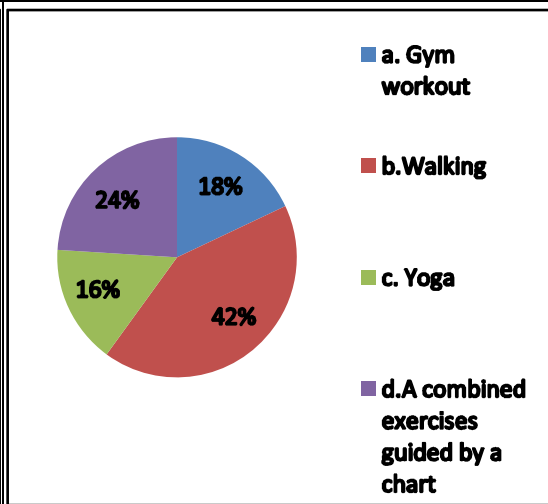
Figure 7.Results for question 6 - What is the most frequent exercises prescribed by your doctor?

RESEARCH ARTICLE

Road Expansion for Urban Sprawl Development- A Case Study

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ABSTRACT

The study was focused on determining the road expansion and network spread of a particular selected area (kalinga institute of social studies) in Bhubaneswar city of odisha state for which the urban development or urban sprawl took place, here the data is observed in between 15 years of gap. The development or creation of road caused different creation of economic zones and helped in socio-economic improvement of people and in GDP growth of that particular area. The software used for analysis is Arc GIS; different satellite datas are captured and mapped. The datas are generated from the spatial data. The dimensions of road network in year of 2005 are marked out and compared with the present scenario of that particular area and the difference is the development graph. Due to road how much of urbanization or growth occurred is also seen and their increase in area is also mapped out. From this entire study it is to be visualized that how a creation of road network causes in increase in growth and development of that particular area in terms what we say is urban sprawl.

Keywords: Urban sprawl, Arc GIS, Road network growth.

INTRODUCTION

Road is a major need for development of an area. If the roads and the network of an area is connected and well distributed then the movement and the spread of urban development occurs. In this study we are observing the road

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networks between 15 years of gap and we are analyzing the effect of development of road and its dimensions with respect to creation of new market places and development of socio-economic standards of the area. The networks are selected in the kiss area of Bhubaneswar and observed through ArcGIS and satellite datas are observed analytically and shape files are created. The geometrical calculations are based over UTM projections and later the difference is observed between the years and visualized through KML. The data of 2005 year is seen as the base data and number of constructed roads are mapped and compared with the constructed and modified roads of 2020. And at end the comparison sheet is seen for better evaluation. As the development of roads occurred thus enhanced the urbanization or creation of new residential and commercial buildings. Thus urban sprawl increased and development happened so on the job creation occurred and GDP of the area increased. When the road network becomes interconnected transportation becomes easier and the traffic movement becomes smooth and easy. Urban sprawl is the result of such development of roads.

Literature Review

F RUBIERA MOROLLON 13 AUG-2020: Urban sprawl has created a new way for sustainable development by which the development will go hand in hand. Through urban sprawl the decision making process and planning and arrangements of buildings and roads can be done in a better way. The reliability over designing and planning will be over now as if the urban sprawl if goes in a right direction the growth will absolutely be in a gentle and smooth process.

NA ROSNI 2016: Urban sprawling is a great topic and interesting layout for the new findings and laying of cities it focuses mainly over the future growth and near analysis. It develops the market, politics, social back ground, natural basins and ecological features of a society. The growth of cities in a right way and direction is very important for proper functioning and regulation for our upcoming future. So urban sprawl and transportation with networking is very important and has to be done in a right way.

DT PRAKASA 5 JUN 2018: Transportation is very important in urban areas so on the smooth regulation of it is highly needed if the planning of a city or town is not proper the transportation and growth of the city is in danger, more the planning and analysis for the growth and spread of network of roads more is the betterment and smoothness in growth of cities and towns . More is regulation of traffic and increase is the development of towns. Hence its always a need to plan a road and its surrounding for development according to need and growth rate.

HS SUDHIRA 2008: Urban sprawl and planning and development can be done but spatial use of satellite datas from where we can classify the datas according to need and can see the road plans and extensions up to our need. Transportation and urban growth goes hand in hand for which each of them area steps for each other. Transportation and planning has to be done in a way that each part of the section of road as well as the urban shaping can go along without interfering the growth of each other.

Need of Study
The general objective of this research is to find out the development due to road networking i.e:
* Analysis of 2005 & 2020 year kiss road.
* Land use and land cover statistics between 15 years of gap.
* Creation on new residential and commercial buildings
* Road network topology.

METHODOLOGY AND EXPERIMENTAL STUDIES

Analysis of 2005 year data: The satellite data of kiss area of 2005 year is obtained and the findings are mapped. In

this analysis the existing roads are mapped and geometrical dimensions are obtained through satellite based analytical data. Along with roads the natural parameters like agricultural lands, barren lands, commercial & residential buildings are captured. Here (figure-1) is the initial 2005 year data which is clearly visible that it is not developed, the building footprints are only at the lower end of the satellite data. This is land use land cover mapped data(figure-2) of 2005 year satellite image to markout the geometrical calculations and dimensions of existing road and builtup residential and commercial zones and natural basins. This step is necessary for analytical calculation,so that the road layout can be traced. The existing road layout (figure-3)and dimensions are calculated in attributes and it is found that the number of existing routes are and the fully developed roads in the kiss area as well as in the residential area is seen and the(table-1) here is attached. With the roads laying in year 2005 the area of commercial and residential zones in the map (figure-4) is found to be 5.763 acre and the total road layout is 11.476 kms in the whole network from which the longest road is layed in bitumen segment with a dimension of length of 6.904

Analysis of 2020 year data: The updated 2020 year data of kiss area is here analyzed and seen for the natural changes and developed commercial and residential zone. The roads are now visible better and the networks are modified to setup the road layout view. Here in the natural satellite image of 2020 year(figure-5) the urban sprawl and development of roads has occurred which is clearly visble and the plots are inter connected with that of inter-connecting pucca roads. The roads expansions have occurred and the lateral growth is clearly visible. Here is the land use land cover map of current 2020 year map(figure-6). As the landuse land cover is mapped, we can clearly see the spread of road network linking all the residential and commercial buildings as well as plots. The road dimensions area geometrically calculated after the digitization process gets completed and the attributes are calculated and new road branches are also mapped. With the formation of new roads the web of roads joins the kiss area with that of colonial areas of commercial & residential zones.Here is the analytical calculated data chart of developed roads and under developed roads. The laid roads and its networks are mapped,digitized(figure-7)and calculated by which their dimensions are being found and is here with attached(table-2). With the roads laying in year 2020 the area of commercial and residential zones in the map(figure-8) is found to be 84.194 acre and the total road layout is 28.887 kms in the whole network from which the longest road is layed in bitumen segment with a dimension of length of 12.947

RESULTS AND DISCUSSIONS

From the above data it can be seen that as the growth of road networks occurred so the urbanization or urban sprawl happened and the area gradually changed to a mass populated area. So the construction of new roads attracted the growth of industries as well as people for new settlement. The development of roads and its networks caused the new settlements and hence the socio-economic upliftment happened. In such manner the road network not only uplifted the area but also helped in creation of new jobs and helped local people for their livelihoods. The road network increased the business opportunities and improved the connectivity to internal areas too. From the excel data sheet the roads are compared with that of their respective years of construction or existence and the results shown by the comparisons are that in year 2005, 23 roads were to be in existence and out of 23 roads only one road is bitumen road which has the longest length of 6.904 and total road network length outcomes to be 11.476 kms. But when the datas are compared with that of 2020 year it is found that about 46 roads are present to final date out of which 27 are bitumen roads and longest length of the road is 12.947 kms and the total network of road comes to be 28.887 kms. Hence it is found that due to increase in road networking there is a rapid growth and development, in terms what we call is urban sprawl developed.

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Table-1

2005 YEAR CONSTRUCTED ROAD DIMENSIONS		
SL.NO	LENGTH IN KM	TYPE
1	1.257	FOOTPATH
2	6.904	BITUMEN
3	0.034	KACCHA ROAD
4	0.178	KACCHA ROAD
5	0.072	KACCHA ROAD
6	0.104	KACCHA ROAD
7	0.158	KACCHA ROAD
8	0.045	KACCHA ROAD
9	0.269	KACCHA ROAD
10	0.22	KACCHA ROAD
11	0.149	KACCHA ROAD
12	0.12	KACCHA ROAD
13	0.281	KACCHA ROAD
14	0.16	KACCHA ROAD
15	0.228	KACCHA ROAD
16	0.3	KACCHA ROAD
17	0.307	KACCHA ROAD
18	0.265	KACCHA ROAD
19	0.048	KACCHA ROAD
20	0.158	KACCHA ROAD
21	0.048	KACCHA ROAD
22	0.098	KACCHA ROAD
23	0.073	KACCHA ROAD
TOTAL ROAD	11.476	

Table-2

Here is the residential and commercial zone builtup area in year of 2020.

2020 YEAR CONSTRUCTED ROAD DIMENSIONS								
SL.NO	LENGTH IN KM	TYPE	SL.NO	LENGTH IN KM	TYPE	SL.NO	LENGTH IN KM	TYPE
1	1.842	KACCHA ROAD	17	2.116	PUCCA	33	0.004	BITUMEN
2	0.021	KACCHA ROAD	18	2.275	PUCCA	34	0.009	BITUMEN
3	0.043	KACCHA ROAD	19	2.153	PUCCA	35	0.033	BITUMEN
4	0.04	KACCHA ROAD	20	2.255	PUCCA	36	0.03	BITUMEN
5	12.947	BITUMEN	21	2.255	BITUMEN	37	0.008	BITUMEN
6	1.126	FOOTPATH	22	2.212	BITUMEN	38	0.038	BITUMEN
7	1.14	FOOTPATH	23	5.563	BITUMEN	39	0.02	BITUMEN
8	1.135	FOOTPATH	24	5.182	BITUMEN	40	0.44	BITUMEN

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9	1.164	FOOTPATH	25	5.559	BITUMEN	41	0.004	BITUMEN
10	0.056	FOOTPATH	26	1.171	BITUMEN	42	0.348	BITUMEN
11	0.04	FOOTPATH	27	1.121	BITUMEN	43	0.328	BITUMEN
12	0.4	FOOTPATH	28	1.111	BITUMEN	44	0.021	BITUMEN
13	2.273	FOOTPATH	29	1.129	BITUMEN	45	0.058	BITUMEN
14	2.169	FOOTPATH	30	1.113	BITUMEN	46	0.047	BITUMEN
15	2.256	PUCCA	31	1.111	BITUMEN	TOTAL		28.887
16	2.235	PUCCA	32	0.009	BITUMEN			

Table-3 Road statistics and dimension comparison

2005 YEAR CONSTRUCTED ROAD DIMENSIONS		
SL.NO	LENGTH IN KM	TYPE
1	1.257	FOOTPATH
2	6.904	BITUMEN
3	0.034	KACCHA ROAD
4	0.178	KACCHA ROAD
5	0.072	KACCHA ROAD
6	0.104	KACCHA ROAD
7	0.158	KACCHA ROAD
8	0.045	KACCHA ROAD
9	0.269	KACCHA ROAD
10	0.22	KACCHA ROAD
11	0.149	KACCHA ROAD
12	0.12	KACCHA ROAD
13	0.281	KACCHA ROAD
14	0.16	KACCHA ROAD
15	0.228	KACCHA ROAD
16	0.3	KACCHA ROAD
17	0.307	KACCHA ROAD
18	0.265	KACCHA ROAD
19	0.048	KACCHA ROAD
20	0.158	KACCHA ROAD
21	0.048	KACCHA ROAD
22	0.098	KACCHA ROAD
23	0.073	KACCHA ROAD
TOTAL ROAD	11.476	

2020 YEAR CONSTRUCTED ROAD DIMENSIONS								
SL.NO	LENGTH IN KM	TYPE	SL.NO	LENGTH IN KM	TYPE	SL.NO	LENGTH IN KM	TYPE
1	1.842	KACCHA ROAD	17	2.116	PUCCA	33	0.004	BITUMEN
2	0.021	KACCHA ROAD	18	2.275	PUCCA	34	0.009	BITUMEN
3	0.043	KACCHA ROAD	19	2.153	PUCCA	35	0.033	BITUMEN
4	0.04	KACCHA ROAD	20	2.255	PUCCA	36	0.03	BITUMEN

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5	12.947	BITUMEN	21	2.255	BITUMEN	37	0.008	BITUMEN
6	1.126	FOOTPATH	22	2.212	BITUMEN	38	0.038	BITUMEN
7	1.14	FOOTPATH	23	5.563	BITUMEN	39	0.02	BITUMEN
8	1.135	FOOTPATH	24	5.182	BITUMEN	40	0.44	BITUMEN
9	1.164	FOOTPATH	25	5.559	BITUMEN	41	0.004	BITUMEN
10	0.056	FOOTPATH	26	1.171	BITUMEN	42	0.348	BITUMEN
11	0.04	FOOTPATH	27	1.121	BITUMEN	43	0.328	BITUMEN
12	0.4	FOOTPATH	28	1.111	BITUMEN	44	0.021	BITUMEN
13	2.273	FOOTPATH	29	1.129	BITUMEN	45	0.058	BITUMEN
14	2.169	FOOTPATH	30	1.113	BITUMEN	46	0.047	BITUMEN
15	2.256	PUCCA	31	1.111	BITUMEN	TOTAL	28.887	
16	2.235	PUCCA	32	0.009	BITUMEN			

Figure-1

Figure-2

Figure-3

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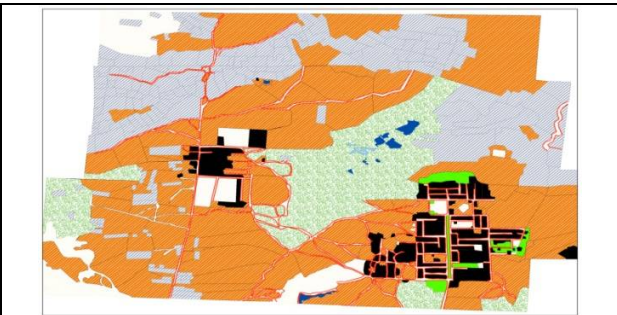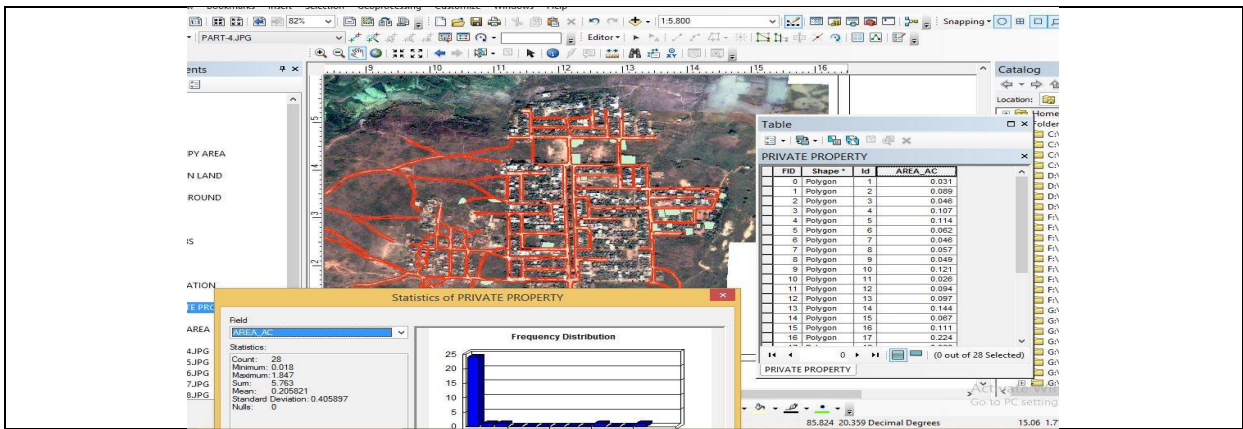
Figure-4

Figure-5

Figure-6

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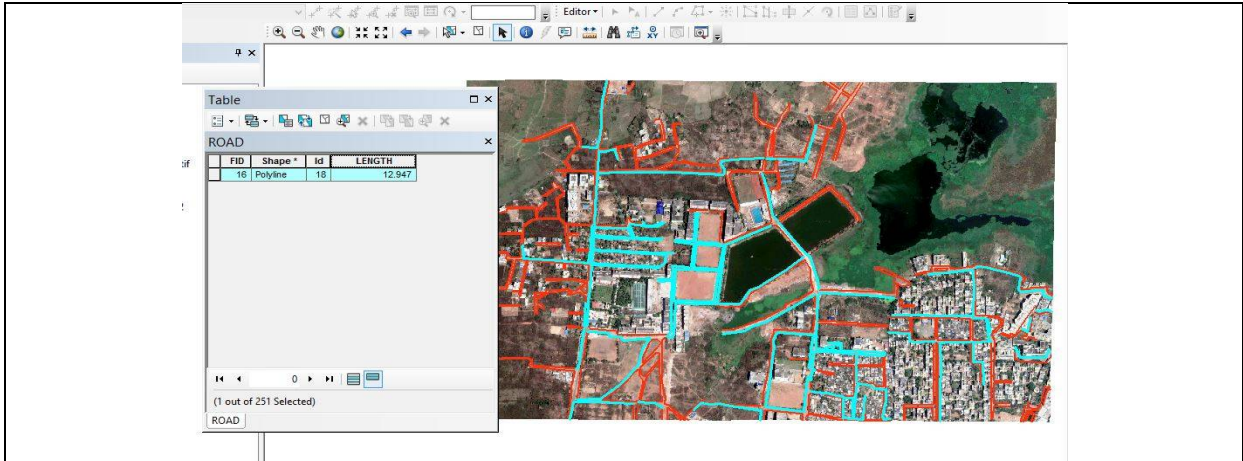
Figure-7
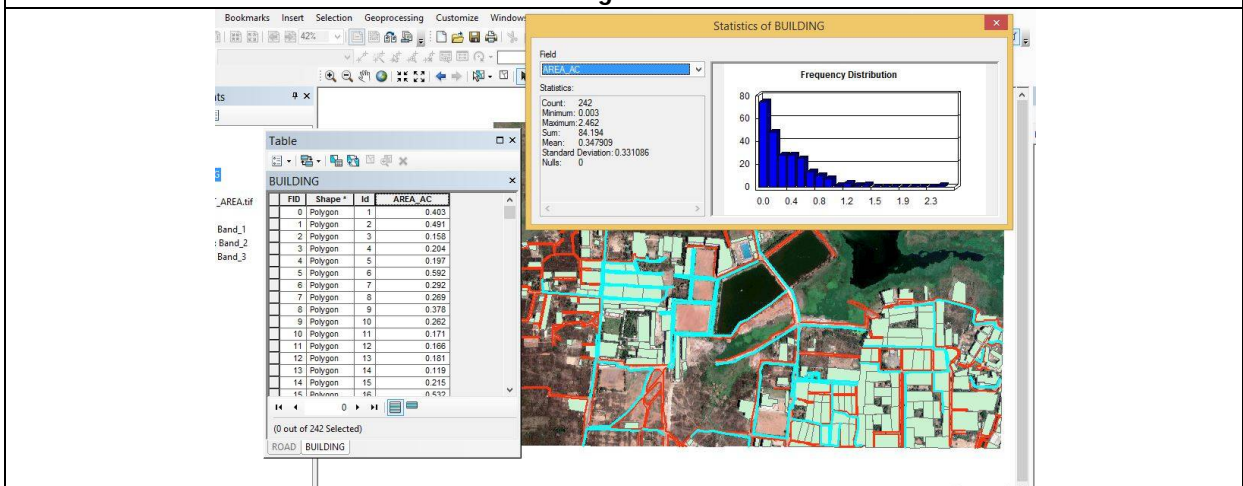
Figure-8

Figure-9 2005 year data

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MAP SHOWING LAND USE AND LAND COVER IN 5KM RADIUS OF KISS COLLEGEBHUBANESWAR

Figure-10 2020 year data

RESEARCH ARTICLE

Performance Enhancement in Associative Pattern Mining by Transaction Reduction using Fuzzy Trapezoidal

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ABSTRACT

Association rule mining is one of the efficient methods to find the correlations among various items of transactions known as associative patterns. They are used to make more valuable decisions and strategies. It deals with crisp partitioning and precise properties of membership and also considers Boolean two-valued logic that leads to the problem of uncertainty and vagueness. In order to overcome these issues, fuzzy logic is combined with association rule mining which uses many or multiple-valued logic to reduce the number of transactions in this paper. It is accomplished by fuzzy partition method which partitions the data repository into various fuzzy regions. It adopts trapezoidal membership function of fuzzy logic for each quantitative value of each transaction item. As the number of transactions is reduced, the requirements of more processing time and more memory space are minimized. The experimental results clearly revealed that the considerable number of transactions is reduced.

Keywords: Association rule mining, fuzzy, frequent itemsets, minimum support, minimum confidence, associative patterns

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INTRODUCTION

Association rule mining (ARM) has become the area of growing significance as voluminous amount of information flows in the information era of today. The main challenge is to analyze these data and summarize it into useful potential information to discover knowledge patterns from them to make valuable decisions [1]. Various techniques are used for mining large item set and reducing it into smaller item set [2]. ARM [3] is one of the most imperative research areas in the concept of data mining (DM) that facilitates the mining of concealed recurrent patterns based on frequencies in the association rules from any item set or datasets containing entities to represent the most recent trends in the given dataset [4-6]. The main aim of extracting knowledge from databases is to generate a large frequent itemset that is iterative. However, more number of iterations requires more time and incurs heavy computation cost for generating candidate itemsets and for calculating the occurrence of a candidate set in a transaction set and subsequently in a database involved [6]. Classical ARM uses sharp partitioning of dataset to transform numerical attributes into boolean attributes [7]. Various algorithms for mining association rules have already been proposed. Apriori algorithm is one among them which is easy to execute and very simple to mine all frequent itemsets in a database [8] [9]. Moreover, it provides important information in assessing significant correlations of items by only considering if an item is present or not in the database [10] without considering the weight of the items. It is computationally inefficient in terms of processing time, accuracy, prevention of redundant rule generation, resource requirement etc [11]. The issues with classical ARM regarding the sharp partitioning are

- It creates the problem of uncertainty. Information is lost at the boundaries of these ranges. Even at the small changes in determining these intervals may create very unfamiliar results which could be also wrong [11].
- These partitions do not have proper semantics attached with them [11].

Zadeh proposed the fuzzy set theory [11] which divides an attribute of data into sets covering certain ranges of values to engage the sharp boundary problem and to deal with cognitive uncertainty of vagueness and ambiguity. The concept of Fuzzy ARM (FARM) approach is for from the need to efficiently mine quantitative data frequently occurs in databases. Additionally, with regard to the matter of decision making, one has to take user's perception and cognitive uncertainty of subjective decisions into considerations [12]. The aforesaid problems can be resolved by the use of fuzzy logic. The transformation of numerical attributes into fuzzy attributes is performed using the fuzzy logic concept in FARM. In classical rule mining, attribute values are represented by just 0 or 1. But, in FARM, they are represented within a range between 0 and 1. Fuzzy attributes are converted from crisp binary attributes. FARM methods treat each item of a transaction database as a linguistic variable and each linguistic variable is partitioned based on its linguistic value [12].

FARM is a variant of classical ARM which discovers meaningful knowledge in fuzzy expert systems [13]. The collection of fuzzy membership functions [13] and rules used in fuzzy expert systems deal with range of values instead of boolean logic [14]. There are many fuzzy logic techniques which are applied in FARM to discover frequent items and rules. Fuzzy systems are based on fuzzy logic and fuzzy set theory which provide a consequential addition to traditional logic [14]. The fuzzy logic deals with approximates rather than precise modes of reasoning which implies that the chains of reasoning in fuzzy logic are short in length and rigor does not play as important role as it does in classical logical systems [15]. Linguistic variables and their values are handy in carrying out analysis of decision making, as they can be described with fuzzy concepts to subjectively correspond with the possible cognition of a decision maker [15]. In fuzzy logic, each attribute can be viewed as a linguistic variable. It is characterized by a quintuple denoted by $(x, T(x), U, G, M)$ where x is name of the variable, $T(x)$ is set of names of linguistic values or terms which are linguistic words or sentences in a natural language, U is a universe of discourse, M is a semantic rule for associating a linguistic value with a meaning and G is a syntactic rule for generating values of x. The meaning of a linguistic value is denoted by x and it is characterized by a compatibility function $c: U \rightarrow [0, 1]$. It associates each u in U compatibility with x [16]. Using a fuzzy partition method, each attribute can be partitioned by various linguistic values. Fuzzy partition methods have widely been used in pattern recognition and fuzzy reasoning in which various linguistic values K is defined in each quantitative attribute. K is also pre specified.

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Singleton, Gaussian, R-functions, L-functions, Triangular and Trapezoidal are membership functions used for the linguistic values. The proposed Performance Enhancement in Associative Pattern Mining by Transaction Reduction using Fuzzy Trapezoidal methodology (PEAPMTRFT) partitions each item of transactions in the data repository into linguistic value using trapezoidal membership function. It partitions the data repository into four grid regions namely Low, Moderate, High and Very High. It calculates fuzzy values of each data item to form a fuzzy data repository. It scans data repository only once to discriminate relevant and irrelevant items by determining the appropriate grid regions by considering the maximum fuzzy value of them. Then, 2, 3, 4,…, m itemsets are constructed. The frequent itemsets are constructed based on minimum support and associative patterns are constructed based on minimum confidence threshold values. The rest of this paper is organized as follows. Various works related to fuzzy partition methods are discussed in section 2. The proposed PEAPMTRFT methodology is discussed in section 3. An example for the proposed methodology is illustrated in section 4. Section 5 discusses the results and finally, section 6 ends with conclusion.

RELATED WORK

The performance of student data on the basis of Apriori-like algorithm to apply on fuzzy set was predicted by Sushil Kumar Verma and R.S. Thakur [1]. It showed the potential of the fuzzy ARM algorithm for enhancing the effectiveness of academic planners and level advisers in higher institutions of learning to identify those students who needed individual attention to decrease the failure ratio and taking suitable action for the next semester examination. Ayush kumar Agarwal et al. [2] presented a literature review in the field of fuzzy mining association rules techniques. They stated that fuzzy mining approaches gets smoother results rather than conventional mining technique and these approaches make fuzzy mining promising in real applications and it provided better performance by the reduction of large itemsets. Hemant Kumar Soni [3] described different techniques and compared their performance with regard to single objective evolutionary and other non-evolutionary techniques. They also proved that the association rules were based on multi objective parameters and multi objective evolutionary algorithm based efficient technique that could be used for real life application in the areas like text mining, temporal database, financial data, biological data, web mining and others. Dhiraj Kapila [4] presented a fresh FARM algorithm, called EFAR-HD, for the smaller and crisp datasets methods such that patient and sales or marketing datasets as a viable and proficient option to fuzzy apriori and FAR-Miner was designed for the smaller datasets to enhance accuracy and execution speed. In [5], Mimanshu Gupta et al. presented the literature review in the field of fuzzy mining association rules using different technologies. In [6], Iyad Aqra et al. presented an ARM approach where a new item set format structure was adopted to address the problem of threshold that necessitates rescanning the entire database. To obtain the final instance of the frequent itemset, it prepared frequent itemset with all possible itemsets occurring in the database as an intermediate step. Moreover, the approach applied a threshold to extract categorical frequent item sets with diverse threshold value and created an intermediate itemsets. The algorithm was no longer to rescan the entire database and also helped to extract many frequent itemsets according to a pre-determined value. T.Lakshmi Surekha et al. [7] dealt with the profit and quantity factor given to each item in each transaction to find the weight of each frequent set. The weights were obtained by multiplying profits and quantities and the marketers were more interested in the profit than the frequency of a set. Profitable patterns were obtained using Profitable Apriori and Profitable FP-Growth algorithms. Results of both the algorithms were compared based on the parameters viz., memory usage, time taken to produce the frequent patterns and the number of frequent patterns. Mohammed Al-Maolegi and Bassam Arkok [8] proposed an improved Apriori to reduce the number of transactions to be scanned. Whenever the k of k-itemset was increased, the gap between the original Apriori and improved Apriori was increased from view of time consumed and whenever the value of minimum support was increased, the gap between improved Apriori and the original Apriori was decreased from view of time consumed. The improved Apriori consumed less time to generate candidate support count than the same in the original Apriori.

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An application of a method for creating prediction models utilizing fuzzy ARM to extract relationships between epidemiological, meteorological, climatic, and socio-economic data from Korea was proposed by Anna L. Buczak et al[9].It was modified and extended to build models for predicting malaria. In [10], S. P. Syed Ibrahim and J. Shanthalakshmi Revathy developed a framework for generating association rules. First, the Hits model algorithm was used to derive the weights from the transactions in a database by considering binary attributes of the item and a new measure wq-support and wq-confidence were defined to find the significance of itemsets based on these weights of the item. It differed from the traditional support by considering the quantity of the items in the transactions. The weight and quantity were used together in mining rules and focused to the item sets with significant weight and high utility. The algorithm was developed by modifying WUARM with weighted quantity setting. It was finally mentioned that in future, the weight of the item in the transaction could be calculated by using hybrid method. Aritra Roy and Rajdeep Chatterjee [11] stated that as classical ARM used the concept of crisp sets, it has several drawbacks. Mainly, the use of crisp partitions created the problem of uncertainty in which valuable data might become inconsistent over these sharp partitions. The concept of fuzzy ARM could be used to overcome those drawbacks. In [12], Chien-Hua Wang *et al.* proposed a fuzzy DM technique to find fuzzy association rules by using the fuzzy partition method and FP-growth. It was demonstrated that it did not need to generate candidate itemsets and it improved the efficiency of repetitious database scanning. It has achieved better executive efficiency. It was also stated that managers could select their own preferences and refer to past experiences and relate cognitive abilities to design a number of linguistic values and shapes such as Gaussian distribution and trapezoidal membership functions. Hence, it would correspond with manager's subjective cognition. Additionally, the proposed method needs to improve its storage space. In handling the algorithms, the linguistic number of each quantitative attribute and recordable number of transaction data affects the size of the storage space in the FP-tree. In [13], Chuan-Kang Ting et al. proposed memetic algorithm to optimize the membership functions for fuzzy ARM. They stated that a chromosome representation consisting of the parameters and structure type of membership functions were used. The consideration of structure in the representation gained three advantages. It facilitated the design of heuristics and constraints for appropriate cover age and overlap and local search could be performed on the structure types. The memetic algorithm achieved significantly higher   fitness than genetic algorithm (GA). S. Sharmila and S.Vijayarani [14] described four well known algorithms namely GA, SLAVE algorithm, Fuzzy Frequent Itemset (FFI) algorithm and Multiple Fuzzy Frequent Items (MFFI) algorithm. They were applied on different datasets. The GA and SLAVE algorithm were based on GA that extracted fuzzy association rule. MFFI was the extension of FFI and these four algorithms were compared with four performance measures i.e. number of frequent items, number of fuzzy rules, execution time and memory space. Finally, it was concluded that MFFI algorithm gave better performance than other algorithm. In [15], Jinal J. Shah and Lokesh P. Gagnani defined fuzzy set concepts, multiple level taxonomy and different minimum supports for each level with multi objective function, rule based mechanism and found association rules in a given transaction data set. The proposed MOGA fuzzy mining algorithm with multiple support value with rule based generated large itemsets level by level and then got final rules in Rule Base from non-dominated solutions of non-dominated set. In [16], Chien-Hua Wang *et al.* proposed a fuzzy DM algorithm which combined fuzzy set theory and FP-Growth to deal with quantitative values and find interesting patterns among them. It was stated that the rules mined represent quantitative regularity for large transaction database and the experimental results provided better mining results due to the characteristic of FP growth. It owned feature of without candidate generation and scanned twice for all process.

PROPOSED METHODOLOGY

The proposed Performance Enhancement in Associative Pattern Mining by Transaction Reduction using Fuzzy Trapezoidal methodology (PEAPMTRFT) is fuzzy logic based to enhance the performance of associative pattern mining. It combines fuzzy set theory and ARM to deal with quantitative values of items and finding the interesting associative patterns among them. The given quantitative data repository is distinguished into four grid partitions. To form a grid partition, input space is divided into several fuzzy slices based on a membership function with

linguistic values where the number of linguistic values depends on the chosen membership function. Trapezoidal membership function is normally used to form four grid regions to determine each linguistic term of each quantitative item. It is defined by a lower limit a, a lower support limit b, an upper support limit c, and an upper limit d, also $a < b < c < d$. Let k and g_k be the linguistic value of each partition and grid region respectively for quantitative item I. then, the trapezoidal membership function $\mu_{k,g_k}(I, a, b, c, d)$ is defined as

$$\mu_{k,g_k}(I, a, b, c, d) = \begin{cases} 0, & I < a \\ \frac{I-a}{b-a}, & a \leq I \leq b \\ 1 & b \leq I \leq c \\ \frac{d-I}{d-c}, & c \leq I \leq d \\ 0, & I > d \end{cases} \tag{1}$$

A quantitative item I of jth item of ith transaction I_{ijk} is represented using fuzzy membership partition k as

$$I_{ijk} = \frac{\mu_{k,g_1}}{I_k, g_1} + \frac{\mu_{k,g_2}}{I_k, g_2} + \frac{\mu_{k,g_3}}{I_k, g_3} + \cdots + \frac{\mu_{k,g_k}}{I_k, g_k} \tag{2}$$

where μ_{k,g_k} is the kth fuzzy grid of k linguistic terms.

The proposed PEAPMTRFT consists of three phases. They are

- Creation of Fuzzy Data Repository
- Determination of Fuzzy Grid Region
- Generation of Fuzzy Associative Patterns

Phase I: Creation of Fuzzy Data Repository

Fuzzy logic is applied to the quantitative data repository and each quantitative item I_j is partitioned by trapezoidal membership function μ_{k,g_k} using eqn. (1) into a fuzzy grid region $G_{K=1}^k$ with an appropriate linguistic value and k is maximum number of grid regions (here k =4). The trapezoidal membership function divides each item into k grid regions viz., *low, moderate, high* and *very high*. They represent four fuzzy values I_k, g_k for each quantitative item and creates fuzzy data repository using eqn. (2).

Phase II: Determination of Fuzzy Grid Region

The fuzzy values I_k, g_k of each grid region $G_{K=1}^k$ are accumulated for each quantitative item I_{ij} using eqn. (3). The grid region $G_{K=1}^k$ for each item is determined based on the maximum value using (4). The chosen grid region g_k is used for the determination of the relevant items I_{ijk} and transactions T_i.

$$\text{Count}_{k,j,g_k} = \sum_{j=1}^{m} \mu_{k,j,g_k} \quad \forall \, T_1, i=1, 2, 3, \ldots, n \tag{3}$$

$$\text{Count}_{g_k}^{max} = \max_{K=1}^{k} \left(\text{Count}_{k,j,g_k} \right) \tag{4}$$

Phase III: Generation of Associative Patterns

Relevant items and transactions from the fuzzy data repository are chosen according to the chosen grid region to organize 1, 2, 3, …, m fuzzy itemsets using the minimum support α where $0 \leq \alpha \leq 1$ and are generated accurate associative patterns using minimum confidence β where $0 \leq \beta \leq 1$. The algorithm for the proposed methodology is as follows.

Algorithm: PEAPMTRFT

Input

- A quantitative data repository with Transactions T_i where i= 1 to n, Items I_j where j = 1 to m
- Four parameters of grid region boundaries a,b,c,d

Output

- Fuzzy Data repository
- Associative Patterns.

// **DB**: Data Base, **FDB**:Fuzzy Data Base, I$_j$: jth item, T$_i$: ith transaction

a,b,c,d: grid region boundaries, **x$_{ijk}$**: Fuzzy item of jth item in ith transaction for kth grid,

α: min_support, **β**: min_confidence, **FIS**: Fuzzy ItemSets,

FFIS: Fuzzy Frequent ItemSets, **FAP**: Fuzzy Associative Patterns //

Begin

α= 0.3; β=0.6;

For each item I$_j$ in T$_i$ in DB

FDB=$\varnothing$

if (q(I$_j$)< a) then μ$_k$ = 0

else if ((q(I$_j$) ≥ a) && (q(I$_j$) ≤ b)) then μ$_k$ = (x-a)/(b-a)

else if ((q(I$_j$) ≥ b) && (q(I$_j$) ≤ c)) then μ$_k$ = 1

else if ((q(I$_j$) ≥ c) && (q(I$_j$) ≤ d)) then μ$_k$ = (d-x)/(d-c)

else if (q(I$_j$)> d) then μ$_k$ = 0

$$I_{ijk} = \frac{\mu_{k,g_1}}{I_{k'}\,g_1} + \frac{\mu_{k',g_2}}{I_{k'}\,g_2} + \frac{\mu_{k',g_3}}{I_{k'}\,g_3} + \cdots + \frac{\mu_{k'}\,g_k}{I_{k'}\,g_k}$$

FDB = FDB U I_{ijk}

End For

For each item I$_j$ in T$_i$ in DB

$$Count_{k,i,j,g_k} = \sum_{j=1,i=1}^{j=m,i=n} \mu_{k,ijg_k}$$

End For

$Count_{g_k}^{max} = \max_{K=1}^{k}\left(Count_{k,i,j,g_k}\right)$

$G_{K=1}^{k} = Count_{gk}^{max}$

FIS=$\varnothing$;

For each $I_{ijk}\in$ FDB

If $I_{ijk} \geq G_{K=1}^{k}$ Then FIS$_1$=FIS$_1$UI_{ijk}

End For

SC = 0

For each $I_{ijk}\in$FIS$_1$

If $I_{ijk}\in$ FIS$_1$ then $SC = SC + \mu_{k,ijg_k}$

End For

For each $I_{ijk}\in$FIS$_1$

If SC ≥ α then FFIS$_1$ = I_{ijk}

End For; z= 2; SC = 0

Repeat

FIS$_z$= FIS$_{z-1}$⋈FIS$_{z-1}$

If $I_{ijk}\in$ FIS $_z$ $SC = SC + \mu_{k,ijg_k}$

If SC ≥ α then FFIS$_z$ =FFIS$_z$UI_{ijk}

until (FIS$_z$≠ $\varnothing$)

For each $I_{ijk}\in$FIS$_z$

$I_{ijk} \rightarrow I_{ijk}UI_{ijk}$

If Conf (α(I_{ijk}UI_{ijk}) / α(I_{ijk})) ≥ β then

FAP = $I_{ijk} \rightarrow I_{ijk}UI_{ijk}$

End For

End

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PROPOSED METHODOLOGY-AN EXAMPLE

To show the relevance of the work, a sample of 11 transactions with 5 items and for each item, the quantities taken are shown in table 1. Fuzzy value for four regions is calculated for each item, using eqn. (1) and eqn. (2). The constructed fuzzy data repository is exhibited in table 2 along with fuzzy values and fuzzy regions based on the quantities of the itemsets. Fuzzy values of each quantitative item for each grid region are accumulated for all transactions using eqn. (3) which is shown in table 3. Fuzzy value of each quantitative item that satisfies $Count_k^{max}$ is only considered to choose the item. The maximum among $Count_{k,i_k}$ which is $Count_k^{max}$ for each grid G_k is calculated using eqn. (4). They are 3.332 for I_1, 4.334 for I_2, 3.999 for I_3, 3.332 for I_4, 5.001 for I_5. The relevant fuzzy itemsets are chosen based on it. In this case, I_1_Moderate, I_2_High, I_3_Moderate, I_4_Moderate and I_5_Low are the chosen grid regions, because they have the maximum values and are shown in table 4. Based on table 4, I_2 and I_4 are eliminated from T_1, I_2 and I_5 from T_2, none from T_3, I_2 and I_4 from T_4, none from T_5, I_2 from T_6, I_2 and I_4 from T_7, all from T_8, I_3 from T_9, I_3 from T_{10} , I_2 from T_{11}. As it removes all items from T_8, the entire T_8 is completely removed from fuzzy data repository. Suppose α = 0.03 and β = 0.6, the associative patterns generated by the proposed method are shown in table 5.

RESULTS AND DISCUSSIONS

It is observed that PEAPMTRFT spawns associative patterns by efficiently utilizing both the processor and memory, as only these two determines the performance, they are focused in this study. Associative pattern mining scans many more transactions to derive associative patterns. The efficacy of processor and memory are enhanced by reducing the number of transactions. It is accomplished by combining fuzzy logic in associative pattern mining. It eradicates uncertainty of vagueness and ambiguity by discriminating items into relevant and irrelevant. Irrelevant items and transactions are detected using fuzzy logic in the first phase itself by constructing fuzzy data repository. It eliminates irrelevant items of transactions and consequently irrelevant transactions too. They are ignored for the generation of $1,2,3,…,m$ frequent itemsets. As only relevant items and transactions are in search space during processing, it greatly reduces the required search space as well as the processing time. Thus, the required memory space is minimized and also the processor is efficiently utilized. Besides, as data repository is scanned only once for the generation of associative patterns, the efficacy of the processor is enhanced considerably. Thus, the use of fuzzy logic generates associative patterns with the eradication of vagueness and ambiguity by reducing the number of transactions. When PEAPMTRFT methodology was executed on foodmart, mushroom, retail and chainstore datasets of SPMF tool with 4141, 8416, 88162 and 1112949 instances respectively, it reduces 376, 765, 8814 and 10268 instances respectively. The number of transactions is reduced approximately by 9%. Only 3765, 7651, 79348 and 1102681 instances are only scanned and processed in place of 4141, 8416, 88162 and 1112949 instances which results in reducing the size of memory requirement and processor time. Table 6 shows the number of instances reduced by the proposed method PEAPMTRFT. Figure 1 shows the comparison of the original data set with the same after applying PEAPMTRFT on the four datasets.

CONCLUSIONS

The proposed PEAPMTRFT combines both fuzzy logic and ARM to reduce the number of transactions in the data repository. It greatly enhances the performance by efficiently utilizing both memory and processor to generate interesting associative patterns. It removes uncertainty of vagueness and ambiguity by eliminating irrelevant items and transactions. As only relevant items and transactions are taken into account for the generation of associative patterns and also the data repository is scanned only once which results in efficient utilization of memory, processor and also generates association rules with appropriate ranges.

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Table 1: Transactions

TID	Items				
	I_1	I_2	I_3	I_4	I_5
1	5	4	3	10	1
2	0	3	4	2	10
4	2	9	0	4	3
4	0	7	5	10	0
5	3	8	0	4	3
6	2	3	2	0	3
7	0	2	3	9	1
8	7	2	8	9	10
9	6	0	8	3	4
10	2	7	8	2	0
11	0	3	4	2	1

Table 2: Fuzzy Data Repository

TID	Items with Fuzzy Values
1	1.0/Moderate+0.0/High, 0.0/Low+1.0/Moderate, 0.667/Low+0.333/Moderate, 0.0/High+1.0/Very High, 1.0/Low+0.0/ Moderate
2	0.0, 0.667/Low+0.333/Moderate, 0.0/Low+1.0/Moderate, 0.667/Low+0.333/Moderate, 0.0/High+1.0/Very High
3	0.667/Low+0.333/Moderate, 0.667/High+0.333/Very High,0.0, 0.0 /Low+1.0/Moderate, 0.667/Low+0.333/Moderate
4	0.0, 1.0/High+0.0/Very High, 1.0/Moderate+0.0/High, 0.0/High+1.0/Very High, 0.0
5	0.667/Low+0.333/Moderate, 0.667/High+0.333/Very High, 0.0,0.0/Low+1.0/Moderate, 0.667/Low+0.333/Moderate
6	0.667/Low+0.333/Moderate, 0.667/Low+0.333/Moderate, 0.667/Low+0.333/Moderate, 0.0,0.667/Low+0.333/Moderate
7	0.0,0.667/Low+0.333/Moderate, 0.667/Low+0.333/Moderate, 0.667/High+0.333/Very High, 1.0/Low + 0.0/Moderate
8	1.0/High+0.0/Very High, 0.667/Low+0.333/Moderate, 0.667/High+0.333/Very High, 0.667/High+0.333/Very High, 0.0/High+1.0/Very High
9	1.0/Moderate+0.0/High, 0.0, 0.667/High+0.333/Very High, 0.667/Low+0.333/Moderate, 0.0 /Low+1.0/Moderate
10	0.667/Low+0.333/Moderate, 1.0/High+0.0/Very High, 0.667/High+0.333/Very High, 0.667/Low+0.333/Moderate, 0.0
11	0.0, 0.667/Low+0.333/Moderate, 0.0/Low+1.0/Moderate, 0.667/Low+0.333/Moderate, 1.0/Low + 0.0/Moderate

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Table 3: Fuzzy Values for Four Grids

Item-Grid	Count
I_1_Low	2.668
I_1_Moderate	3.332
I_1_High	1.000
I_1_Very High	0.000
I_2.Low	3.335
I_2_Moderate	2.665
I_2_High	4.334
I_2_Very High	0.666
I_3_Low	2.001
I_3_Moderate	3.999
I_3_High	2.001
I_3_Very High	0.999
I_4_Low	2.668
I_4_Moderate	3.332
I_4_High	1.334
I_4_Very High	2.666
I_5_Low	5.001
I_5_Moderate	1.999
I_5_High	0.000
I_5_Very High	2.000

Table 4: Grid Regions

Item	Max Count
I_1_Moderate	3.332
I_2_High	4.334
I_3_Moderate	3.999
I_4_Moderate	3.332
I_5_Low	5.001

Table 5: Associative Patterns

I_1_Moderate=>I_2_High
I_1_Moderate=>I_2_HighI_4_Moderate
I_1_Moderate=>I_2_HighI_5_Low
I_1_Moderate=> I_2_HighI_4_ModerateI_5_Low

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Table 6: No. of Transactions Reduced using PEAPMTRFT

Dataset	Original No. of Transactions	No. of Transactions Reduced	No. of Transactions Processed	% of transactions
FoodMart	4141	376	3765	0.0907
Mushroom	8416	765	7651	0.0908
Retail	88162	8814	79348	0.0999
ChainStore	1112949	10268	1102681	0.0902
		Average		0.090=9%

Fig. 1: Comparison of the Number of Instances on Foodmart, Mushroom, Retail and Chainstore Dataset with Original and Fuzzy Data Repository
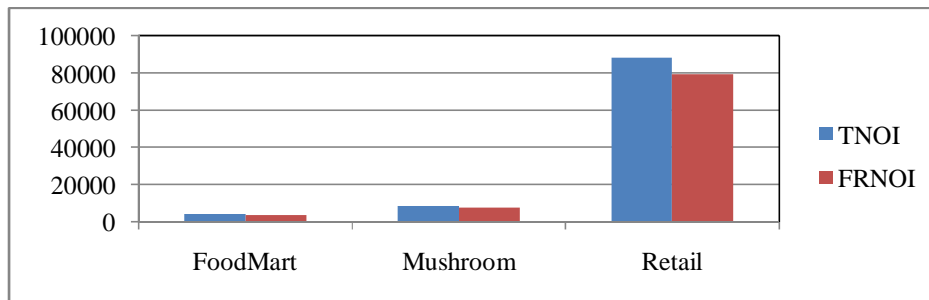
TNOI - Total Number of Transactions, FRNOI - Fuzzy Relevant Number of Transactions

Fig. 1(a): Comparison of the Number of Instances on FoodMart, Mushroom and Retail Dataset with Original and Fuzzy Data Repository
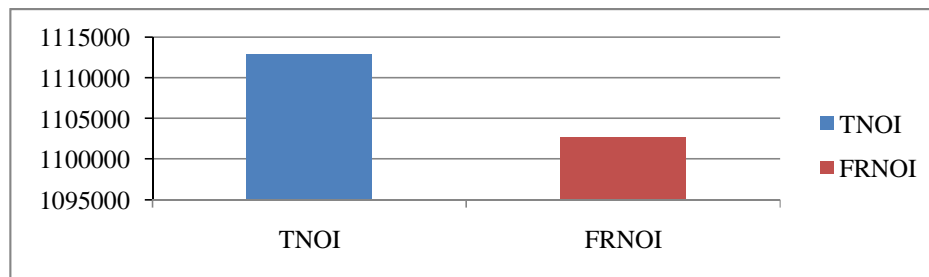
TNOI - Total Number of Transactions, FRNOI - Fuzzy Relevant Number of Transactions

Fig. 1(b): Comparison of the Number of Instances on Chainstore Dataset with Original and Fuzzy Data Repository

RESEARCH ARTICLE

The Effect of Myofascial Release Technique on Pain and Neck Disability Over Conventional Neck Exercises on Patients with Cervicogenic Headache

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ABSTRACT

Purpose: The purpose of the study is to determine the effect of Mysofascial Release Technique (MFRT) on pain and Neck disability over the conventional neck exercises in patients with Cervicogenic Headache. Methods : Thirty six females between 35- 45 years of age with atleast two to three episodes of Cervicogenic headache(Unilateral headache) in the past three months were selected randomly and assigned to two groups – Control Group (Conventional Exercise) and Experimental Group (MFRT). Both the groups underwent a Pretest and Post test for assessment of Pain and Neck Disability by VAS scale and Neck Disability Index (Self Questionnaire). The treatment sessions were 4 times a week on alternate days for a total of 15 sessions. Results: The results of the study showed a significant decrease in pain and disability with the group that received MFRT. Conclusion: MFRT of cervical muscles significantly decreases the pain and reduces the disability in neck. Hence it is recommended to be given clinically to reduce pain and disability in patients with cervicogenic headache.

Keywords: Cervicogenic Headache; Myofascial Release; Conventional neck exercise; Craniocervical flexion exercise; Sub occipital Muscle Pain

INTRODUCTION

Cervicogenic Headache is a common clinical condition affecting women more than men and is characterized by headache (often unilateral) with a kind of throbbing pain starting at the back of the neck. It is defined as secondary and unilateral headache that is known by referring pain from soft or hard cervical structures to occipital, temporal,

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frontal and sometimes pre-orbital regions [1-3]. The pain is usually steady with a dull feel and sometimes or other associated with vomiting and blurring of vision, but has a clear distinction from migraine headaches. The prevalence of CGH is estimated to be 0.4%-2.5%in general population and is more in women by 4 times than in men. It accounts for 15%-20% of all chronic headaches [4,5].The clinical signs vary from individual to individual and often pain with neck movement and sustained improper neck posture, stiffness in neck and tenderness over suboccipital areas with limited range of motion in cervical spine. The patient often complains of severe headache, reduced ROM in neck, reduced work performance, lack of concentration which may last for hours together. The underlying pathology is due to the convergence of primary sensory afferents from cervical nerve root C1 – C3 with the afferents from occiput and trigeminal nerve. Hence the potential risk structures are those innervated by cervical roots C1 – C3, the Suboccipital muscles in particular [6]. The treatment ranges from cervical joint mobilization, facet joint manipulation, Ultrasound therapy, Laser therapy, trigger release therapy, posture correction and exercise sessions including cervical and upper thoracic strengthening. Myofascial release therapy to the sub occipitalis muscles primarily aims to reduce the restrictions in the myofascial connective tissue and eliminate pain and restore motion. A low level load will elongate the viscoelastic medium, the fascia and ensures smooth gliding of the underlying structures. The Cranio-flexion exercise to activate the deep neck flexors and shoulder strengthening exercises were administered to reduce the pain and to correct the posture. The primary aim of the study is to evaluate the effectiveness of myofascial release technique on cervicogenic headache patients in reducing pain and disability in neck.

MATERIALS AND METHOD

The study is a randomized control trial with 2 groups – a Control group and an Experimental group. The subjects are human volunteers, females in specific residing at Salem district. Thirty-six females between age group 35 – 45 years of age with a history of at least two to three episodes of cervicogenic headache in the past 3 months were randomly selected and assigned to two groups. Subjects with headache and vision problem or sinusitis and those who have obliterated cervical lordosis and those who have similar history but who had undergone various other treatment for the same are excluded.

The purpose of the study was explained and an informed consent was taken. Both groups underwent a pre-test assessment for pain and Neck disability through Visual Analog Scale and Neck Disability Index respectively. Visual Analog scale consists of numerical value from 0 to 10, where 0 indicates no pain and 10 is maximum pain. Neck disability index is a questionnaire designed to give us information on how neck pain affects the ability in day to day life. It has a total of 10 sections with each section having 6 answers of which first is marked 0 which indicates there is no disability and the last answer is marked 5 which indicates maximum disability. The maximum scoring is 50. The total scoring is the sum of individual scores in each section. After the pre- test is over Group 1 (Control Group) received the conventional neck exercises which included the Cranio-cervical neck flexion performed in supine position. The subject's head is positioned in slight upper cervical neck flexion and examiner places the hand on the table just below the subjects occiput. She is given a verbal cue to gently flex the upper neck and lift the head off the examiner's hand but retaining the neck flexion. Verbal cue includes "Tuck your chin in or hold your head up." This exercise enhances the deep neck flexors and strengthens it. A set of 10 exercises with a gap of 1 minute in between is given. The entire treatment is for 15 days on alternate days for 1 month.

The group 2 (Experimental group) was given myofascial release for Sub occipital muscles for 15 days on alternate days for a period of 1 month. The subject lies supine and the examiner is seated at the head end. The examiner's finger pads are placed palm up beneath the patient's sub occipital region, in contact with the trapezius and its immediate underlying muscles. A slow and gentle pressure is applied ventrally and obliquely into the tissues for 3 – 5 seconds and then the pressure is released. The procedure is repeated for 2 – 3 minutes. It aims mainly at restoration of normal cervical lordotic curve, remodel the myofascial tissue, improves blood flow, improves lymphatic drainage and thereby reduces pain. Both the groups underwent a post test and the scoring were recorded.

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Data Analysis

The collected data were analyzed using SPSS 22 software with Wilcoxon Signed Rank test for within group analysis and Mann Witney's U test for between group analysis. Normal distribution of collected data were scrutinized by Kolmogorov- Smirnov test (K-S).

RESULTS

The result of Wilcoxon Signed Rank test showed a mean difference of 3 and -4.06 (Table 1) for the outcome measure VAS and NDI respectively for the control group. And the experimental group showed a Z value of -3.72 and p value 0.0001 for both outcome measures which is statiscally significant.(p < 0.05) (Table 2).

The comparative analysis of results of both the groups showed insignificant value in pre tests. At the same time the results of both outcome measures showed a Z value of 2.75 and 2.45 with p value – 0.002 and 0.007 which are highly significant statistically.(Table 3).

DISCUSSION

The aim of the study was primarily to find the effectiveness of Conventional neck exercises performed widely over the recently developed Myofascial Release Technique in reducing pain and Neck disability in Cervicogenic Headache in females. Though there is prevalence of disease in male too, the study focused only on females which is a limitation to the study. But it was aimed at reducing the disability in females thereby improving the quality of their life which has a significant contribution to the entire society. In future studies the authors aim to reach a wide population.

CONCLUSION

The results of the study make the authors to conclude that Myofascial Release therapy for sub occipital muscles is more effective than conventional neck exercises.

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Table 1. Within Group Analysis – Control Group

Variable	Group	Mean	SD	MD	Z value	p value
VAS	Pre	7.5	1.38	3	-3.72	0.0001
	Post	6.77	1.39			
NDI	Pre	33.38	5.57	-4.06	-3.51	0.0002
	Post	32.33	5.50			

Table 2. Within Group Analysis – Experimental Group

Variable	Group	Mean	SD	MD	Z value	p value
VAS	Pre	8	1.64	3	-3.72	0.0001
	Post	5.27				
NDI	Pre	31.22	6.02	3.22	-3.72	0.0001
	Post	27.72	5.69			

Table 3. Between Group Analysis – Control vs Experimental

Variable	Group	U value	Z value	p value
VAS	Control Pre			
	Experimental Pre	129	-1.028	0.15
	Control Post			
	Experimental Post	74.5	2.75	0.002
NDI	Control Pre			
	Experimental Pre	130	0.99	0.15
	Control Post			
	Experimental Post	84	2.45	0.007

RESEARCH ARTICLE

Green Synthesis, Characterization and Antibacterial Activity of Zinc Oxide Nanoparticles (ZnONPs) Produced using *Ipomoea pes-caprae* (Kuthirai kulambu)

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ABSTRACT

In the recent decades, nanotechnologies have been in that act of significant study field of current materials science. A biosynthesis nanoparticle has been a mass wide activity owing to it's in herent feature rapidly, environmentally-friendly and low cost-effective. The first time, ZnO NPs using *Ipomoea pes-caprae* leaves aqueous are the introducing method. There morphological in Zinc Oxide nanoparticle abide characterized on SEM with EDAX. They X-ray diffraction (XRD) study do thus crystal nature and exposed of purity of ZnO NPs. FTIR spectroscopy are using to analyses the identified the functional groups responsible for reduction. Stabilisation and capping agents present throughout the NPs. Further, the synthesized ZnO NPs are tested for antibacterial activity. The nanoparticles was found to has antibacterial activity against pathogenic bacterial strains *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Klebsiella pneumoniae* by the observation of inhibition around each well.

Keywords: Green synthesis, *Ipomoea pes-caprae*, X-Ray Diffraction techniques, Antibacterial activity

INTRODUCTION

The advanced and evolution of nanoscience in the 21st century, nanoparticles has using to produced diverse productions, that has led to the induction evolution of nanomaterials therein diverse aspects of life this industrial products, environmental material, biomedical by agricultural industries (1). The synthesis of nanoparticles with particular morphology and properties is one of the more foremost aspects of nanoscience they studies materials from size lies with the nano size (1nm=10^{-9} nm). Applications of has been nanostructures is viewed in catalysis, sensors, water purification, antibacterial and nanoelectronics while chemical synthetic procedures may lead to the creation of toxic chemicals by – products or required high temperatures and pressure, biosyntheses of nanoparticles used plant extracts providing a facile and 'green' method of nanoparticles synthesis (2). Zinc Oxide nanoparticle (ZnONPs) were the most considerable that due to of them specific more used in difference of products such as cosmetics, pigments, electronic equipment, bioimeging, drug delivery, waste water treatment, and environmental remediation (3,4,5,6,7,8). Before, more study has notified for the green synthesis of ZnO NPs used different leaf extracts such as Eucalyptus globules (9), *Aloe barbadensis miller* (10),*strychnos nux- vomica* L.(11),*Cassia fistula* (12), *Rose indica* L. (13), *Laurus nobilis* (14) *Nephelium lappaceum* L(15). Hence in the now *Ipomoea pes -caprae* was consider due the accepted medicinal properties for the synthesis based on ZnO NPs used leaf extracts.

Ipomoea pes- caprae (Beach morning glory) of the family Convolvulaceae is a perennial creeping vine with milky sap. Roots is produced it's the modes leaves is alternate, oval shaped and notched in the end, akin the footprint of a goat stalks, flowers is seductive, bell-shaped, pink, purpie or violet with deeper colour in the centre. The species is pan-tropical and common along sandy beaches for Asia and the pacific *Ipomoea pes-caprae* are useful sand –binder, burgeoning under conditions since sand blast and salt (16). Antibacterial resistances have expanded internationally as a main threat therefore it is required in that us need therapeutic agents to substitutive antibiotic in saving bacterial infections. Biosynthesis nanoparticles of antibacterial source due has an extra properties of individual functionalization about molecular drugs to the nanoparticles as indicated by they improved antibacterial effect. This method have operated on the current studies that have been revealed the manufacturing of best antimicrobial nanoparticles due to farther extending during antibacteria therapies. This study have used *Ipomoea pes-caprae* a using medicative special plant to synthesis and characterization of zinc oxide nanoparticles from the leaf extracts

MATERIALS AND METHODS

Collection there of the Plant Sample
Ipomoea pes-caprae leaf is collection from paragipettai, cuddalore, Tamil Nadu, India. The leaf was identified whilst authenticated by Department of Botany, Annamalai University at Tamil Nadu in India.

Leaf Extract of Preparation
The *Ipomoea pes-caprae* are collected and rinsed efficiently first with the tap water followed with by distilled water to this remove all the dust and unwanted visible particle .When the leaves got completely dried, they were chopped into fine pieces. Each 20 gm in chopped leaves of *Ipomoea pes-caprae* were boiled with 100ml double distilled water at 60^0 c with constant stirring up to 30mins. The extract obtain be filtered through Whatman no .1 filter paper and finally extract were collection for further experiment.

Synthesis of ZnO NPs:
Extraction of leaf was carried out using different amount of the *Ipomoea pes-caprae* leaf extraction (5, 10, and 15 ml) added IM Zinc nitrate hexahydrate solution in a 50ml Erlenmeyer flask for bioreduction process. The flask was then kept overnight at room temperature. The bio-reduction of Zinc nitrate hexahydrate into Zinc Oxide nanoparticles can be confirmed by visual observation. The *Ipomoea pes-caprae* leaf extract, changed to deep yellow colour upon mixing

reduction of Zinc nitrate hexahydrate solution strongly indicates the synthesis of zinc oxide nanoparticles. The colour change was compared with control aqueous leaf extract solution precipitates finally, this paste was then collected in a ceramic crucible and annealed at 400^0 C for 2 hours in the muffle furnace light yellow colored powder was obtained and this powder was carefully collected. The materials were powder using a mortar and pastel so, that got a fine powder, that is easy for further characterizations.

Characterization of ZnO NPs:

The green synthesized nanoparticles were characterized by FT-IR, XRD and SEM with EDAX. The particle size of the purity was observed by X-ray diffraction (XRD) and Fourier transform infrared (FTIR) spectrum in the range 4000-400cm-1.The morphology and size of the nanoparticles were determined by scanning electron microscopy (SEM). Energy Dispersive X-ray (EDAX) analysis were studied for analyzing the purity of ZnO nanoparticles.

Antibacterial Activity

Antibacterial assay were carried out by ager disk diffusion method for the zinc oxide nanoparticles synthesis from the leaf extract of *Ipomoea pes-caprae* pathogenic organisms such as *Escherichia coli, Pseudomonas aeruginosa, Staphylococcus aureus, Klebsiella pneumonia* was obtained microbial type culture collection from Rajah Muthiah Medical college, Annamalai University, Chidambaram, Tamil nadu. The ZnO nanoparticle and positive control disc with using to the compare the result. The antibacterial activity were evaluation by the measuring the zone of to inhibition against the test organisms. Finally we are measured diameters (mm) of the zone of inhibition of the control strain and test with the a ruler and callipers.

RESULT AND DISCUSSION

Structural Analysis

The particles size and structural properties from ZnO nanoparticles are revealed with used powder X-ray diffraction. The XRD pattern was recorded in the 2θ range from 20 to 80 (Fig-1) Show the XRD patterns of the ZnO NPs obtained of the three different concentrations. The seven diffraction peaks appeared at positions 31.76, 34.43, 36.25, 47.54, 56.56, 62.82 and 67.90 represent to the 100, 002, 101, 102, 110, 103 and 112 lattice planes of hexagonal crystal structure confirms the crystalline nature (JCPDS NO 89-1397). Similar patterns of the XRD for zinc oxide nanoparticle have been reported. Also, three unassigned peaks in XRD pattern of zinc oxide nanoparticles appeared all the concentrations, which may be related to phytochemical compounds in the leaf extract as capping and stabilizing agent. The average particle size of zinc oxide nanoparticles was calculated by Debye-Scherrer's formula

$$D=K\lambda/\beta\cos\theta$$

Where, D-the average crystallite size in Å, λ-is the wavelength of the X-ray radiation (λ=0.15406mm) Cu Kα radiation, K-usually taken as the shape factor (0.9).θ-the Bragg angle, and β-is the line width at half-maximum height. The Scherrer formula was used to calculated the crystalline size in the range of 37nm.

FT-IR Analysis

The FT-IR spectra and *Ipomoea pes-caprae* leaves extract (Fig-2) has show absorption bands at 3477 and 2910 cm-1 representing C-H and O-H stretching alcohols and phenols group. The absorption peak are located it around 1609 cm-1 representing C=C stretching vibration of primary amines. Bending vibration present at 1390 cm-1 is associated with C-H ,stretch vibration present at 1133cm-1 are associated with C-H, stretch vibration present at 1133cm-1 are associated with C-F vibration of alkyl and aryl halides, bend vibration are located at 831cm-1 representing of aromatic rings. This bands indicate polyols (phenolic and flavonoids), terpenoids, by protein compounds that is abundant on *Ipomoea pes-caprae*. FTIR spectra of green synthesis ZnO nanoparticles has show absorption band at, 3477cm-1,

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2910cm^{-1}, 1609cm^{-1}, 1390 cm^{-1}, 1133cm^{-1}, 831cm^{-1} and 475cm^{-1} approximately. The peaks in the region between 600 and 400cm^{-1} are allotted to M-O (Zn-O) (17). At band at 475cm^{-1} the confirms stretching vibrations from zinc oxide NPs, at the participation of polyols, terpenoids, and proteins hazing functional groups of amines, alcohols, phenols, alkanes, alcoholic , carboxylic, acids, esters, ethers, aromatics on bioreduction reactions. The terpenoids are poorly water-soluble and therefore cannot be among prime moieties involved on the bioreduction reaction the nanoparticles as reported proteins seen to exhibit little the importance in biosynthesis (18) the, water soluble phenolic acid and flavonoids compounds are considered reaction because the probable mechanism are still unclear and need further investigation. Therefore, it can be-so assumed that is biological molecules should possible perform the function in the formation of capping and stabilizing the ZnO NPs.

SEM with EDAX

The morphology and the synthesis has been zinc oxide nanoparticles are characterized at the SEM in technique. The SEM image show the surface nature of zinc oxide nanoparticles used of the green have under studies because show in the (fig-3). SEM image of zinc oxide nanoparticles prepared using *Ipomoea pes-caprae* at different magnification, which, revealed the shape of particles is agglomeration. The EDAX spectrum free from impurities and it is seen in the limit of the EDAX (fig-3).Identifying lines for the emission field energy the zinc oxide oxygen and this correspond to peak on the spectrum, therefore zinc oxide have been identify correctly.

Antibacterial Activity

The antibacterial activity of biosynthesized zinc oxide nanoparticles was investigated with two different concentrations (50,100μm/ml). Antibacterial activity is investigated against (*Escherichia coli, Staphylococcus aureus Pseudomonas aeruginosa, Klebsiella pneumoniae*) by well diffusion method on the results are shown in (Fig- 4). The diameter of inhibition zones (mm) around each well with zinc oxide nanoparticles synthesized by *Ipomoea pes-caprae* extract are found to have the highest antibacterial activity against *Staphylococcus aureus* (15mm) and *Pseudomonas aeruginosa* (14mm), respective and comparative lesser antibacterial activity of zinc oxide nanoparticles is found against *E.coli* (12mm) and *K. pneumoniae* (13mm) for every concentration increasing of ZnO nanoparticles, pathogenic strain was decreased in the inhibition of zones.

CONCLUSION

At present analysis concludes that, ZnO nanoparticles that is quickly at the green synthesis used Ipomoea pes-caprae leaves extract has been inexpensive, non-toxic, environmentally with the size of 37nm showing hexagonal crystalline structure. The zinc oxide nanoparticles synthesis with green leaf extract was found to has very excellent antibacterial *E. coli* and *K. pneumoniae* a very excellent against antibacterial activity into *S. aureus and P. aeruginosa.*

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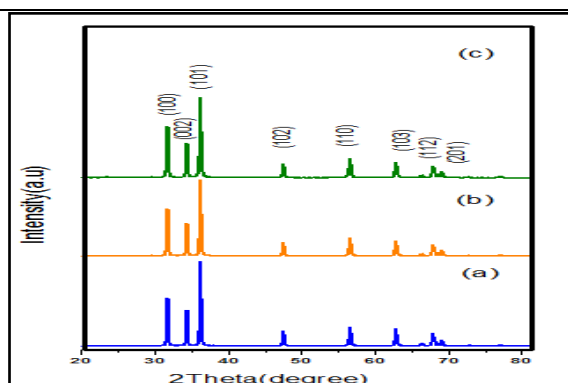
Fig.1 XRD patterns of zinc oxide nanoparticles using *Ipomoea pes- caprae* leaf extract (a) 5 ml (b) 10 ml (c) 15 ml. (show unassigned peaks)
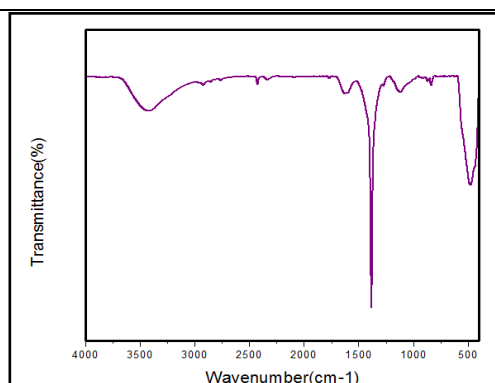

Fig .2 FTIR spectrum of synthesized zinc oxide nanoparticles using *Ipomoea pes-caprae* leaf extract
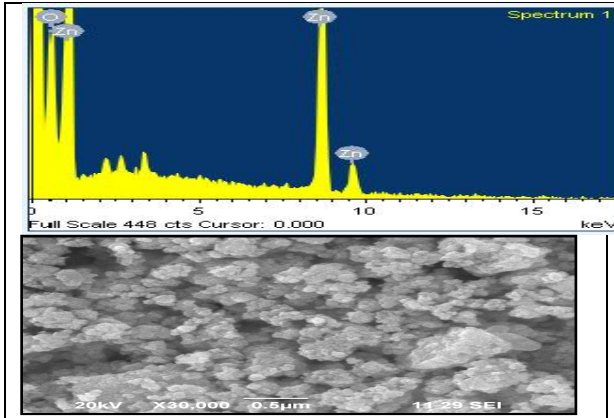
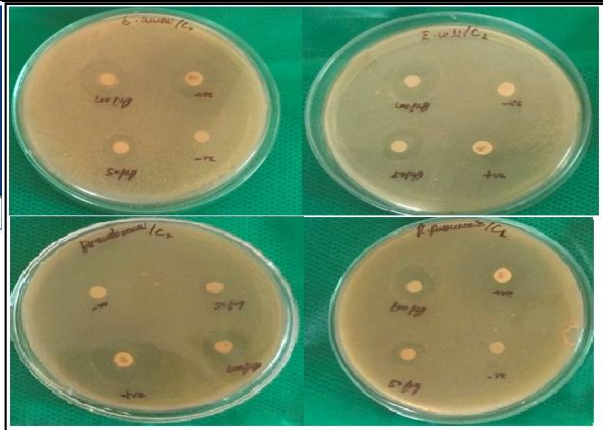**Fig. 3 SEM with EDAX spectrum of zinc oxide nanoparticles using *Ipomoea pes- caprae* leaf extract**

Fig.4 Antibacterial activity of synthesized zinc oxide nanoparticles using *Ipomoea pes-caprae* leaf extract

RESEARCH ARTICLE

Generation of Power using Bladeless Wind Turbine

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ABSTRACT

This paper gives a comprehensive analysis of an environmentfriendly wind turbine without any blades. Vortex bladeless wind is a latest buzz in the field of wind generation which generates electricity from vibration initiated by wind pressure that rotary motion is converted to electricity. The vortex wind generator constitutes a cylindrical shaped structure which is placed on the ground. Over this conical shaped body it experiences a vibration. This vortex wind turbine phenomenon is use to convert a linear oscillation to rotational motion. This generator produce eloquent amount of power in less time. In this paper we try to give a new concept of renewable energy which is ecofriendly and environmentally and giving maximum voltage regulation as a result. It is totally new approach of this renewable sources. This concept based on that wind strikes to rigid body and turn linear motion to kinetic energy and then electrical power.

Keywords: Wind Energy, Bladeless Wind Turbine, Vortex Induced Vibration, Generator, Rectifier

INTRODUCTION

In India easily available sources of non-conventional energy is wind power and it found plentifully in the environment. Now a day, there are various possibility of obtaining different types of energy in which wind power is one of them, resulting from the movement of large volume of air that is the use of wind energy [1, 2]. Wind energy is friendly for environment and it is inexhaustible energy which we used for various purposes. Wind is the essential part of nature which is used by the people for a long time. Production of electricity on industrial level generally wind power plant can be installed. Few years ago, the conventional wind mill are mostly known for killing a lot of birds. That resulting, we found lot of skeleton of birds near the turbine area. These conventional turbines produce more noise, so that it can harmful for the people who live near these conventional wind mills. This device can be

destructive with waste flying in every direction in the wind blows and having tendency to break. A lot of money is required for regular maintenance and to keep running. The vortex wind generator which convert renewable energy into electrical energy is one of the future of the turbine. This senior project investigate how a similar bladeless wind turbine is made as shown in fig (1). The spinning motion of air or other fluids harnessed by bladeless turbine. When wind passes from one of the cylindrical turbines, the down side wind of the cylinder in spinning whirlpool or vortex is sheared off. That vortex exerts force on that cylinder which cause to vibrate. Wind energy is the term which is used very long time ago when people discover how to generate or utilized electricity wind energy power plant mostly create by industrial purposes and for the agriculture also. And we also store power in batteries for the future scope [1-6]. Apart from all above mentioned points it also shows vast variation in structure, mode of operation, safety requirements and such various performance parameters. Table 1 depicts the comparative analysis of bladeless wind turbine and the traditional wind turbine [6-11].

METHODOLOGY

The main principle of this wind turbine is conversion of linear oscillation of mast to rotational motion. As the mast is subjected to wind energy, it tends to oscillate due to the vortices formed around the structure of the mast, which can be converted to rotational force to generate electricity [5]. In the bladeless wind turbine the mast is fixed with respect to the center base and the rib structure at the top of the mast contain the thread arrangement is used for pulling the threads attached to it. Energy is obtained by continuously oscillation of the mast. The mast utilizes wind power to pull the threads along with the chain attached to the sprockets which drive the shaft and rotates the alternator to generate power. Proper planning and execution of the workflow decides the successful completion of the bladeless wind turbine [12-18].

Working Principle

The energy conversion happens in the mast, in which the wind strikes the column mast to vibrate. This vibration is converted into mechanical energy and then into electrical energy [14]. When the wind affected on the surface area of the mast from one specified direction, stream lines of the wind tend to depart and get sheared off. Further passage results into the formation of wind currents called vortices or eddies. When they are strong enough to overcome the internal resistance offered by the mechanism (crank shaft or direct linear alternator), the mast vibrates due to spring connected at outside surface of the mast. Then spring is connected to the center base. The connecting rod is bound to transmit this vibration to the crank. The sprockets can be connected to a generator. We can also connect the lower end of the mast with the linear alternator directly. Also, we can use a rectifier circuit to transform this A.C. current to D.C. current and charge a battery or connect it the load [5, 6, 17, 21, 22, 25].

Components Used

In the available literature [7, 8, 22, 23, 26, 27,28,29] the details of the structure of the turbine is given. The unique structure of this newly invented wind turbine consists of various components as shown in fig. 2

1. The Components of vortex wind turbine which is help to generate electricity and used for the operation of the machine as given in fig.2
2. Centre base- The row material used for the center base is iron and having angular rigid structure. The center base provides equally distant point for the mast position. It capable to toleration of the mechanical stress acting on the base. For mast and spring base gives the strong foundation to stand straight.
3. Mast- The mast is having conical shaped body and having rigid structure which Rotates according to wind flow. Mast is having lighter in structure spick and span increase in the Rotational Motion also decrease in the mechanical stress on the base as well as suspension on the spring**.**
4. Rectifiers-A rectifier is a device that converts an oscillating two-directional alternating current(AC)into a single-directional direct current(DC) .

5. Boost converter-A boost converter is a DC to DC power converter that step up voltage from its input to its output. It is a class of switched –mode power supply containing at least two semiconductor and at least one energy storage element.
6. Battery –A battery can be defined as an electromechanical device which can be charged with an electrical current and discharge whenever required.
7. AC load- AC loads are devices which received alternating current (AC) electrical power source in an electrical system.
8. Voltage sensor-A voltage sensor is a sensor used to calculate an monitor the amount of voltage in an object. Voltage sensor can determine the AC voltage or DC voltage.
9. Voltage regulator-A voltage regulator is a system designed to automatically maintain a constant voltage. A voltage regulator may use a simple feed-forward design or may include negative feedback.
10. Aurduino- Aurduino is an open source hardware ,project user community that design an manufacture single board microcontrollers an microcontroller kit for building digital devices.
11. LCD display- LCD is a flat panel display or other electronically modulated optical device that uses lights modulating properties of liquids crystal combined with polarizers.

Circuit Diagram

In the above circuit diagram is a use for Bladeless wind Turbine. We used G1 and G2 is a Generator or Alternator for generate electricity from bladeless wind turbine mechanism. Generator or Alternator is generating AC power source. The alternator power is AC and AC power cannot store to the battery, so BR1 and B2 to is Bridge rectifier to convert AC power to DC pulse-setting current. DC pulse-setting current filter by capacitor C5 and C6, and we get pure DC Power. The Output DC power not stable but battery required stable power source for charging so we use DC-DC converter or Boost Converter to increase stable output Voltage provide to battery for charging. INV1 is inverter circuitry that changes direct current (DC) to alternating current for AC load devices. The V1 is a voltage Sensor which is use to read Battery Voltages and provide to the Arduino and Arduino display battery voltage to the LCD display. The LCD1 is a 16x2 Liquid Crystal Display for display 16 alphanumeric character with 2 lines. The Voltage Read operation performed by the Arduino SIM1, The Arduino is Smart Board or Ture Computer Board in which used advance microcontroller Atmega328P. which is capable to read Analog, digital and write Analog and digital, store data into EPROM, Serial communication and INPUT, OUTPUT pins, etc. All the components of Arduino required 5V supply. So, we design 5V power supply we used U2-7805 voltage regulate IC. The capacitor C1 and C2 is a voltage stability capacitor and capacitor C4 and C3 is a disk capacitor or ceramic capacitor used for remove of spike of voltages AC noise, etc.

Applications
This novel type of wind turbine shows wide applications due to its unique structure [17, 20, 22]. Noticeable applications are as follows
• The vortex wind generator are used in various purpose and applications
• Agriculture- For the various purpose in agriculture field power is used for water pumping, lighting on the frames, etc.
• Operating machinery and equipments to heat and cool the building for all these purposes energy required for the small as well as large scale.
• Turbine energy for domestically- The bladeless wind turbine is the minor type of production which is used for the generate power and used to bring it with solar plant generation and off grid plant which helps to store energy in battery and provide to useful places and rural areas where electricity is the essential need .
• Energy for telecom- Now a days electronic communications and broadcasting technology are provided in rural areas for the telecom but it is really headache and complicated system for that we suggest and provide off grid solutions for these such areas

- Vortex wind generator for off grid lighting- The bladeless wind turbine is the solution to provide renewable lighting for the off grid .the produces energy which is stored in battery for the future use and useful use for the sport ground street lights and rural public areas or marketing areas.
- Signage and signaling- These are the essential need for signage and signaling in those areas where electricity is the initial need and bladeless wind turbine is the easiest way to generate power and supply off grid .
- Off grid power for rail signaling- There are lack convenient main electricity for rail signaling bladeless wind turbine is the useful source to provide electricity and we also can store in battery and used it further as required.

CONCLUSION

The vortex wind generator (bladeless wind turbine) is a new concept to generate power by using conventional wind turbine. This concept is not harmful for the environment and wildlife also it helpful for nature. The bladeless wind turbine are easily available source and easy to maintain and occupying less space. This topic is proved that same level it is create a power using without blade it generate power but less than the conventional wind turbine. But either it generate less power but it is beneficial. This concept use on the basic level or in individual level which is useful for the small industries or small industrial level. These vortex wind turbine is one of the best or suitable or an essential way to generate power in present conditions. It will help to increase percentage of renewable energy for electrical power generation and provides economically efficient power to the consumers. Hence for the survival of renewable energy source in coming future we have to spread this bladeless wind turbine concept and for that wind energy is the efficient option.

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Table 1. Comparison of conventional wind turbine and Bladeless wind turbine

| Parameters	Conventional Wind Turbine	Bladeless Wind Turbine
MODE OFOPERATION	Generation of electricity is done by the using blades	Generation of electricity is done without using of blades
MODE OFGENERATION	It catches wind power by using Rotational motion of the blades.	It catches wind power by using "Vortices"
STRUCTURE	It is well built & having maximum wear& tear.	It is well built & having minimum wear & tear.
SAFETY	For birds it is not safe and having possibility to Collision with blades	For birds it is safe and no possibility to collision with blades
MAINTENANCE	It required high maintenance cost	It required low maintenance cost and easy to maintain.
CONSTRUCTION	It having many moving parts	It having some or very few moving parts
EFFICIENCY	It having high efficiency.	It having low efficiency.

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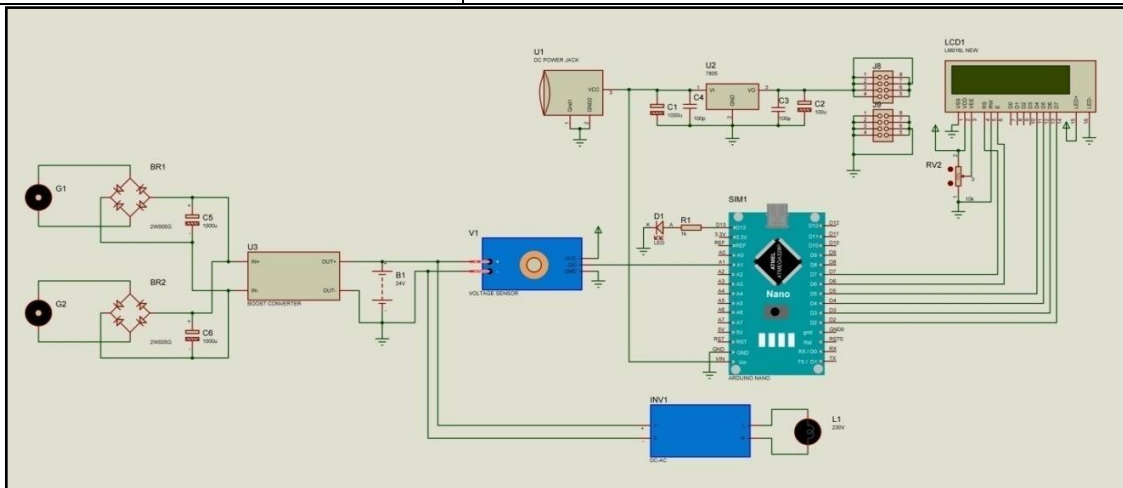

Fig.1. Bladeless Wind Turbine
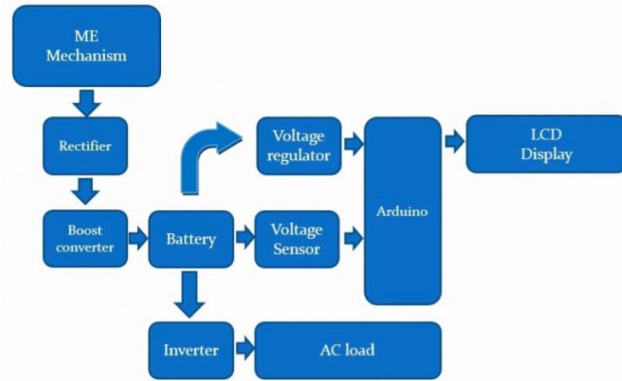
Fig.2. Block Diagram of Bladeless Wind Turbine

Circuit Diagram

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RESEARCH ARTICLE

Mathematical Modeling of Antigens and Antibodies

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ABSTRACT

The evolution of Antigen and Antibody molecules is discussed in this paper. In particular, I tried to derive the expression for the contiguous fraction which provides the idea of how much matching takes place between antigen and antibody molecules. Though it is well known that the contiguous fraction *r* is very small, there is not much clear idea available to know about the measure of smallness of *r*. In this paper, I had proved that *r* is of logarithmic scale thereby showing the continuous evolution of antigen and antibody complements.

Keywords: Antigens, Antibodies, Conditional Probability, Generating Function, Logarithmic Scale.

INTRODUCTION

Throughout this paper, I shall denote Antibodies by Ab and Antigens by Ag. The mammalian arsenal of Ab molecules has to recognize and bind $N \sim 10^{16}$ foreign Antigens Ag's, but had better not recognize $N' < N$ self-antigens. There are only $n \sim 10^{11}$ antibody types that can possibly be constructed from permutations and combinations of an even smaller number of genetic fragments, so an Ab can only be required to match some contiguous fraction—an epitope—of an antigen molecule to maintain binding and initiate activation. But how large that contiguous fraction would be? Perhaps quite small, say *r* discernible units, a unit being a number of amino acids producing a definite local configuration. In this paper, I shall develop a model which addresses the evolution of Ag – Ab complement by deriving an expression for *r*.

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Describing the Model

I now address of choosing r to maximize the probability P that any of the N randomly selected environmental antigens matches at least one of the antibodies, but none of the N' self-antigens match any of the n antibodies The fact is that although a successful solution of this problem will be evolutionarily maintained and strengthened by specific mechanisms that we will describe later, we have first to arrive at an adequate solution.

Solution to the Model

Let us fix the n antibodies. Let P_F be the probability that a random Antigen (Ag) fails to match a given Antibody (Ab). Hence,

P(that each of N foreign Ag's matches at least one Ab)

= [P(that a given foreign Ag matches at least one Ab)]^N

= [1 – P(that a given foreign Ag matches none of the n Ab's)]^N

$= \left(1 - P_F{}^n\right)^N$ (3.1)

On the other hand, P(none of the N' self-antigens matches any of the n Ab's) $= P_F{}^{nN'}$

So, $P = \left(1 - P_F{}^n\right)^N \times P_F{}^{nN'}$ (3.2)

I will now find value of P_F such that P in (3.2) attains maximum. Taking logarithm on both sides of (3.2), we get

$$\frac{dP}{dP_F} = nP\left[\frac{-NP_F{}^{n-1}}{1 - P_F{}^n} + \frac{N'}{P_F}\right] (3.3)$$

Equating fist derivative to zero, we get $P_F{}^n = \dfrac{N'}{N' + N}$ from which $P_F = \left(1 + \dfrac{N}{N'}\right)^{\frac{-1}{n}}$ (3.4)

For the value of P_F obtained in (3.4) we find that the second derivative is negative. Hence P in (3.2) attain maximum

for the value of P_F given in (3.4) The maximum value of P is given by $P_{\max} = \left(\dfrac{N}{N' + N}\right)^N \left(\dfrac{N'}{N' + N}\right)^{N'}$ (3.5)

From (3.5) w see that the maximum value of P is very small since both the terms in the product of (3.5) are less than 1. In this case a natural question that would arise is that with this very small probability how the evolutionary process would happen for long time? To answer this, I proceed to the following argument.

Conditional Probability

Let us define P(N, N') = P(an Ab is chosen to match N and N')

It is reasonable to assume that P(N, N' | an Ab is chosen to match $N + \Delta N$ and $N' + \Delta N'$) = 1.

Now from the definition of conditional probability $P(A \mid B) = P(B \mid A) \times \dfrac{P(A)}{P(B)}$, we have

$$P\left(N + \Delta N, N' + \Delta N' \mid \text{match for } N, N'\right) = \frac{P\left(N + \Delta N, N' + \Delta N'\right)}{P\left(N, N'\right)} \quad (4.1)$$

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From (4.1), we see that it is only the logarithmic increment that is responsible for maximization of evolution. Hence this answers the question posed in previous section.

Generating Function

Imagine m types of unit with, for convenience, equal probability of occurring, and each with a complementary Ag unit. Hence, two units complement each other with a probability of $1/m$. Suppose tentatively assume that matching means at least r complementary pairs in sequence for two molecules, each of length l and in register. Denote complementary pairs by x, non-complementary by y, so that a pair of molecules is represented by a string of x's and y's.

Failure to complement means that all contiguous x-sequences have length $< r$. To start, let us write down the generating function $G_r(x,y)$ for all non r-matching x, y sequences of any length. We do this by noting the locations of the y's and the condition that they are separated by 0, 1, 2, 3, . . . , $r-1$ x's. These intervening x's are then represented

by the sum $1 + x + x^2 + \cdots + x^{r-1} = \dfrac{1-x^r}{1-x}$

Hence, we have

$$G_r(x, y) = \frac{1-x^r}{1-x} + \frac{1-x^r}{1-x} y \frac{1-x^r}{1-x} + \frac{1-x^r}{1-x} y \frac{1-x^r}{1-x} y \frac{1-x^r}{1-x} + \cdots$$

$$= \left[\left(\frac{1-x^r}{1-x}\right)^{-1} - y\right]^{-1}$$

Thus the generating function is given by $G_r(x, y) = \left[\left(\dfrac{1-x^r}{1-x}\right)^{-1} - y\right]^{-1}$ (5.1)

Generating Function for Comparison Sequences

In this section, I try to derive the generating function for comparison sequences, which are weighted by their probabilities, and by z^l for length l. Assuming $x \to \dfrac{1}{m} z, y \to \left(1 - \dfrac{1}{m}\right) z$, from (5.1) and simplifying we get

$$G_r(z) = \frac{1 - \left(\dfrac{z}{m}\right)^r}{1 - z + (m-1)\left(\dfrac{z}{m}\right)^{r+1}} \quad (6.1)$$

We now have to find $P_F^{(l,r)}$ which is the coefficient of z^l in $G_r(z)$.

From denominator of (6.1) we see that the optimal value of z is given by

$$z* = 1 + (m-1)\left(\frac{z*}{m}\right)^{r+1} \quad (6.2)$$

For large values of r, we see that there is a root close to 1. By Iteration we find that such a root is given by

$$z_1 = 1 + (m-1)\frac{1}{m^{r+1}} + (r+1)\left(\frac{m-1}{m^{r+1}}\right)^2 + \cdots \quad (6.3)$$

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Thus, if I is very very large compared to r, then we get $P_F^{(l,r)} = \left(1 + \dfrac{m-1}{m^{r+1}}\right)^{-l} \left(1 + O(m^{-r})\right)$ (6.4)

Now using (3.4) in (6.4), we get

$$1 + \frac{m-1}{m^{r+1}} = \left(1 + \frac{N}{N'}\right)^{\frac{1}{nl}} = 1 + \frac{1}{nl}\log_e\left(1 + \frac{N}{N'}\right) + \cdots$$

From this, we see that the optimal value of r is given by

$$r = \log_m(nl) + \log_m(m-1) - 1 - \log_m\left(1 + \frac{N}{N'}\right) + \cdots \quad (6.5)$$

From (6.5), we see that the value of r is in logarithmic scale to base m.

CONCLUSION

This paper is mainly concerned about addressing the contiguous fraction r which is a measure of antigen molecule to maintain binding and initiate activation. It is well known that r is small but don't much about how small it is. This paper through (6.5) address this question and proves the fact that r is indeed in logarithmic scale. We know that logarithmic functions are very slowly increasing functions and that characteristic is reflected in this paper through six sections.

Through the value of r obtained in (6.5), we see that r is essentially the total number of m-bits in n and l, and consequently a quite small number, in anecdotal agreement with observation. The model I had used can readily be modified by assuming unequal molecular lengths not in register, requiring more than one r-sequence, and so forth—but none of this changes the qualitative result.

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RESEARCH ARTICLE

Record of Entomofauna in Hundred Years Old Lentic Water Ecosystem in Mysore, Karnataka, India

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ABSTRACT

Systematic investigations were made by following standard methods to record aquatic insects in >100 years old Kukkarahalli Lake, Mysore, India during 2020. Aquatic Entomofauna was observed using different standard methods including visual count method that revealed variation in population density, abundance and distribution of different aquatic insect species. Both mature (imago) and immature (e.g. larva or naiad) staged aquatic insects belong to five orders viz., Coleoptera, Diptera, Hemiptera, Odonata and Trichoptera was observed and their population distribution significantly (F=4.217; P>0.01) differed. Four species of Hemipterans, three species of Coleopterans, few Dipteran larvae belong to the families Chironomidae and Muscidae, imago and naiad forms of Odonates and the Caddisfly larvae of Trichoptera were found during different months. The Damselflies were more predominant compared to Dragonflies and the Wilcoxon paired rank test indicated significant difference (T=10 $X^2_{0.01}$) between these two groups. The correlation coefficient of naiad and imago population of dragonfly and damselfly showed a positive correlation ('r'=0.361). Further, imago population of different aquatic insect species distribution indicated a significant variation (F=6.161; P>0.01) in Kukkarahalli Lake. Further, the aquatic insect population density was high during April and it was followed by March and February. Since, aquatic insects are generalists in their feeding habits, obtain food by shredding, scraping, collecting, gathering, filtering and piercing methods. Therefore, it is important to study the aquatic Entomofauna which is highly diversified within their communities and their abundance to assess the quality and restoration of freshwater Lake Ecosystems.
Keywords: Aquatic Entomofauna, Lentic water, Mysore, India

INTRODUCTION

India is one of the mega biodiversity countries and possesses good number of freshwater habitats including both lentic and lotic ecosystems, which are more diverse in their existence. The freshwater lentic ecosystems include lakes, ponds, springs, rivers, wetlands, reservoirs and ditches [1], provided suitable habitat for different insect groups. It is presumed that the lentic water habitats provide suitable shelter for 3 to 5% aquatic insect species. Of all, nearly 3% insects initiate their life cycle in aquatic habitat before emerge as winged imago to lead life on terrestrial ecosystem [2]. Many aquatic insects spend their different stages under aquatic conditions, which may take few weeks to several years [3]. Presently, most of the lentic ecosystems in urban areas have been subjected to increased pollution. Consequently, the physico-chemical properties of lentic ecosystems are impaired. The altered lentic ecosystems greatly affect the distribution patterns of insects in the water due to their sensitivity to changing physico-chemical properties [4]. Further, aquatic insects play a crucial role while maintaining the stability in aquatic habitats [5]. Impaired water qualities limit the diversity and restricting many species [6] and suffer with lesser biotic components. Different species of aquatic insects live in benthic, limnetic and littoral zones, which improve the nutrients status and habitat quality [7 & 8] due to their differential responses [8]. Thus, aquatic insects are considered as model organisms; used to analyze the structure and function of the freshwater ecosystems [9 & 10]. The abundance, birth rate with short duration of life cycle, large biomass and rapid colonization, filter feeding activity play a pivotal role to upkeep the aquatic habitat clean [4, 8 & 11]. The filter feeding activity of few insect species in the water greatly help to sort out fine particles suspended in water and it would help allow enough light to penetrate to the bottom of the water, where algae and other plants are growing and depended on light source to synthesize their food [11].

More than 45,000 insect species are known to live in freshwater ecosystems [12]. Several insect species are involved in nutrient recycling and form an important component of natural food chain and food web in aquatic ecosystems [8 & 11]. The ponds, pools, lakes and other freshwater habitats provide suitable shelter to many surface hunters (e.g. water striders, water skates and beetle species) and divers (e.g. mayfly, stonefly, dragonfly, caddisfly and mosquitoes) [13 & 14]. Several researchers have published the reports on aquatic insects at different parts of the world. [15] have reported the aquatic insects and their sensitivity to the changing water quality and anthropogenic disturbance. [16 & 17] have assessed the aquatic insect's role as bio-indicators of water quality. [18 & 19] have studied the tropic structure, water quality and eutrophication of the aquatic ecosystems by indicating the importance of aquatic insects. Hence, aquatic insects study could help understand the tropic structure, water quality and status of Lakes and Ponds [4 & 14]. [20] have revealed the importance of aquatic insect's identification, taxonomic status and their diversity for fish population. [4] have reported the ecological integrity of Mayflies. [21] have recorded the aquatic insects of North America. [22 & 23] have reported on Mayflies. [24] have reported the ecological integrity of aquatic insects in upper Niger Delta. [25] have studied the diversity and distribution of aquatic insects in streams of the Mae Long Watershed area in Thailand. [26] have published the report on distribution, abundance and diversity of aquatic insects in Keniam River in National Park of Pahang in Malaysia. [27] have studied the aquatic insects in Joyasagar Tank in Assam. [28] have reported the aquatic insects in Himachal Pradesh. [8] have reported the stoneflies, mayflies, caddisflies role in decomposition of leaf litter and colonized microbial degradation of organic matter in Kurangani stream of Western Ghats in south India. Das *et al.* (2014) have conducted the preliminary investigations on 70 species of aquatic insects which belongs to 59 genera of 24 families in five insect orders at Acharya Jagadish Chandra Bose Indian Botanical Garden in West Bengal. [29] has reported the aquatic insects of Lakes in and around Hyderabad. [30] has studied the aquatic insects of Vellayani Lake in Kerala. [31] have recorded the aquatic insects from Midnapore Pond in West Bengal. [32] have studied the diversity and distribution of aquatic insects in Sothuparai Reservoir in Periyakulam, Tamilnadu. [33] have studied the aquatic insect species in Bakuamari stream of Assam. [34] have reported the aquatic insects in Yettinahole in Karnataka. All these published clearly revealed the importance of aquatic insects study.In India, Mysore is one of the clean cities and called Heritage city of Karnataka State. It is housed with Kukkarahalli Lake, locally called Kukkarahalli Kere, which is located in the heart of Mysore city. It was constructed by the then Mummadi Krishnaraja Wodeyar (1794–1868) of the Mysore Dynasty

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(Kingdom of Mysore) in 1864 [35]. The lake has historical heritage and attracting hundreds of bird species every year. Many migratory bird species are nesting in this lake ecosystem. Published reports on aquatic insects of Kukkarahalli Lake are scanty. Aquatic insects are important food sources for several local and migratory birds, which reside few weeks to months amidst Kukkarahalli Lake [36]. Published literature on aquatic insect species composition, distribution, abundance and the mitigation measures undertaken to protect the biotic components in Kukkarahalli Lake are meagre. Hence, the present investigation has necessitated.

MATERIALS AND METHODS

Study Area: The Kukkarahalli Lake is located amidst Manasagangtri Campus, University of Mysore, Mysore. It is constructed by the then Mummadi Krishnaraja Wodeyar (1794–1868) of the Mysore Dynasty in 1864. It is situated between 12.3° N latitude and 76.63°E longitude at 2,479.4 ft above msl [37] with 62 ha of water catchment area. The open water surface area is occupied with >50% of the Lake and with five to eight ft depth. Moreover, 4.5 km walkway is established on the periphery of Lake with good plantation and becomes lung space of Mysore city. The Lake is housed with vast variety of microorganisms, plants and animals which help to maintain the local biodiversity [36].

Methodology: Observations were made by following random sampling method at different places of Kukkarahalli Lake to record aquatic insects on weekly basis during 2020. The Lake was divided into four segments and in each segment one sampling site was earmarked permanently by fixing sign board. At each sampling site, one square meter sized four quadrates were selected randomly by leaving 10 meters gap between the quadrates. Every week, two observations were made during morning i.e., 0900 to 1130 AM and evening 0300 to 0530 PM in a day. The aquatic insects were sampled in all the quadrates by using a fine quality EISCO aluminium insect collecting net which was immersed in the water and dragged gently for one to two minutes and taken out from the water for visual count and identification of insects. After taking a photograph, insects were released back to the water after visual count. Only moribund and dead insects were collected and preserved in 70% alcohol for identification by using Stereo zoom microscope and with the help of standard taxonomic keys [37]. Maximum five counts were made in each and every quadrate. Percent occurrence, population density, abundance and distribution of different aquatic insect species were calculated by following standard methods. Percent occurrence = Number of aquatic insect species / Total number of all the aquatic insect species x 100. The population density = Individuals of a particular insect species recorded during the observation x Number of sampling sites x Number of visits made in a month. The abundance = number of particular insect species/number of species recorded. Collected data was statistically analyzed by using standard methods as per [38].

RESULTS

Different species of mature (imago) and immature (e.g. larva or naiad) staged aquatic insects were recorded in Kukkarahalli (Tables 1 and 2). Aquatic insects belong to five orders viz., Coleoptera, Diptera, Hemiptera, Odonata and Trichoptera (Table 1). Three species of Coleopterans namely: water scavenger beetle (*Hydrophilus piceus*) (Family: Hydrophilidae), water diving beetle (*Dytiscus marginalis*) (Family: Dytiscidae) and Whirligig beetle (*Gyrinus* sp.), (Family: Carabidae) were found in Kukkarahalli Lake (Table 1). The Dipteran larvae belong to the families Chironomidae and Muscidae were recorded during the present study (Tables 1 and 2). The adult Hemipterans such as *Ranathra elongata* (Family: Nepidae), giant water bug (*Belostoma* sp.) (Family: Belostomatidae), water strider (*Aquarius remigis*) (Family: Gerridae) and common backswimmer (*Notonecta* sp.) (Family: Notonectidae) were commonly found in Kukkarahalli Lake (Tables 1 and 2). Moreover, both adult and naiad forms of Odonates such as ground skimmer dragonfly (*Diplacodes trivalis*) (Family: Libellulidae) and golden dartlet damselfly (*Ischnura* sp.) (Family: Coenagrionidae) and the Caddisfly larva of *Polycentropus* sp. (?) (Order: Trichoptera) were recorded during

the present study (Tables 1 and 2). Table 3 shows the immature staged aquatic insects such as Chironomous larva (*Chironomous* sp.) (Family: Chironomidae), flies maggots, caddisfly larva and naiads of dragonfly and damselfly were recorded in this Lake. Of all, damselfly naiads were more (2,679) followed by dragonfly naiads (1819), and chironomous larvae and flies maggots (1350). The caddish fly larvae were <1000 and indicated significant variation (F=4.683; P>0.01) existed between immature forms of aquatic insects in Kukkarahalli Lake (Table 3). Table 4 shows the imago population of dragonfly and damselfly. The damselflies were more predominant (967) compared to dragonflies and the Wilcoxon paired rank test indicated significant difference (T=10 $X^2_{0.01}$) between these two groups. Moreover, the correlation coefficient of naiad and imago population of dragonfly and damselfly in Kukkarahalli Lake showed interesting facts. Between the naiad and imago population there was a positive correlation ('r'=0.361) existed that indicated significant correlation coefficient ('t'=3.79) between them (Table 5). Similarly, naiad and imago population of damselfly showed positive correlation ('r'=0.450) that indicated significant correlation coefficient ('t'=3.55) existed between naiad and adult damselfly population (Table 5). Occurrence of beetle species during different weeks in Kukkarahalli Lake is depicted in Table 6. The adult beetles such as whirligig beetles were more (2972) and it was followed by scavenger beetle (1136) and predacious diving beetle (860) and there existed a significant difference (F=58.07; P>0.01) between these species (Table 6). Table 7 shows the imago population of Hemipterans namely *Belostoma* sp., *Notonecta* sp., *Ranathra* sp., and water striders found during different weeks in Kukkarahalli Lake. Among the Hemipterans, water striders population was very high (4,337), followed by *Belostoma* (3,011), *Ranathra* (1,975) and *Notonecta* (1,880) and there existed a significant variation (F=6.161; P>0.01) between these species (Table 7). Table 8 shows the imago population of Coleoptera, Hemiptera and Odonata during different weeks in Kukkarahalli Lake. Comparatively, Hemipteran population was very high (11,203) and it was followed Coleopterans (4,968) and Odonates (1,700) and there existed a significant variation (F=17.121; P>0.01) between these groups (Table 8). Overall, 24,814 individuals of both immature and imago or adult aquatic insects belong to five orders were recorded during the present study (Table 9). Of all, Hemipterans were more predominant (11,203) compared to other aquatic insect groups. It was followed by Odonates (6,296), Coleopterans (4,968) and Dipterans (1,350). However, Trichopterans were less in number (997) and there existed a significant difference (F=4.217; P>0.01) between these aquatic insect groups (Table 9). Further, the aquatic insect population density was high (10,941) during April and it was followed by March (8,442) and February (5,451). This indicated the gradual increase of aquatic insects from February onwards in Kukkarahalli Lake (Table 9).

DISCUSSION

In India, many cities are housed with small to medium sized freshwater Ponds, Lakes amidst their environment. These freshwater habitats in cities become congenial sites for innumerable species of flora and fauna [39], provided source of recreation to local people. More than 45,000 insect species are known to live in freshwater ecosystems [13]. Mysore is a heritage city of Karnataka has >100 years old Kukkarahalli Lake, which is located at the centre of Mysore city [35 & 36]. It has provided suitable habitat of aquatic insect species, which belong to Coleoptera (e.g. water scavenger beetle (*Hydrophilus piceus*), water diving beetle (*Dytiscus marginalis*) and whirligig beetle (*Gyrinus* sp.), Diptera (e.g. Larval/maggot forms of Chironomidae and Muscidae families), Hemiptera (e.g. Nepa (*Ranathra elongata*), giant water bug (*Belostoma* sp.), water strider (*Aquarius remigis*) and common backswimmer (*Notonecta* sp.), Odonata (e.g. naiads of ground skimmer dragonfly (*Diplacodes trivalis*) and golden dartlet damselfly (*Ischnura* sp.) and Trichoptera (e.g. Larva of Caddisfly, *Polycentropus* sp.). Overall, 24,814 individuals of both immature and imago or adult aquatic insects were observed during the present investigation. This indicates the healthy status of Lake even after 100 years of its establishment. The Odonates, Coleopterans and Dipterans were found moderately and Trichopterans were less in number. Among the immature staged aquatic insects, damselfly naiads were more (2,678) followed by dragonfly naiads (1,819). The dragonflies and damselflies are closely associated with freshwater habitats and act as indicators of the health of freshwater ecosystems [10, 23, 24 & 37]. During their early stages, dragonfly and damselfly are fully aquatic and depends on freshwater habitat conditions. The naiad and imago population of dragonfly and damselfly in Kukkarahalli Lake showed a positive correlation between them. Interestingly,

dragonflies and damselflies emerged from naiads into adults respectively 51.2 and 36.1% respectively. Remaining 48.8 and 63.9% naiads didn't reach to imago stage. Perhaps, the considerable size of dragonfly (48.7%) and (63.9%) damselfly population might have eaten up by fishes, aquatic birds and other animals in the Lake and served as one of the food sources to higher vertebrates like fishes, frogs, water snakes and water birds [19, 20 & 37]. Hence, immature staged Odonates satisfied the hungry mouth of few species of vertebrates in freshwater habitats. Notwithstanding the naiads of dragonflies and damselflies sensitivity to polluted water, naiad's population declined considerably. Because, during winter and summer seasons, the water level in Kukkarahalli Lake decreased considerably. Perhaps, decreased water level might have altered the physico-chemical properties of Lake Water [19] and might have disfavored the emergence of dragonflies and damselflies from their naiads. Hence, dragonfly and damselfly population is under threat, needs protection. On this line further in depth studies are necessitated.

Further, the flies maggots and Chironomous larvae population size was 1,350. Moreover, few Dipterans are able to intercept, ingest and assimilate dissolved organic carbon and also act as carnivorous piercers, which feed on the fluids of their prey. The Chironomous larva are saprophagous organisms, act as shredders feeds on leaf tissues, leaf-litter detritus after colonized by aquatic fungi and bacteria. Moreover, Chironomous larvae collect and gather bio-film with the help of radula like structure and feeds on coarse particulate organic matter after converting it into fine particulate settled at Lake bottom. The primary sources of nutrition for collector-gatherers are bacteria and benthic invertebrates that colonize organic particles [8]. The entire organic matter is ingested and egested every few days and help cycling the fine particulate matter that are higher than rates of actual organic matter production [12]. Thus, larval forms of Chironomidae family act as collector-gatherers and extremely important in the structure and function of freshwater Lakes food webs. The caddish fly larvae were <1000 and indicated less population in Kukkarahalli Lake. Trichopterans (e.g. Caddisfly larvae) actively forage in benthic and water column habitats, act as both shredders and piercer, feeds on leaf tissues, leaf-litter detritus and animal fluids by puncturing algal cells and ingesting the cytoplasm. In most cases, leaf-litter detritus is consumed after conditioned by aquatic fungi and bacteria. The suspended food particles, small invertebrates, fecal pellets, detritus particles and fragments of detached bio-film are delivered directly to the Lake water through rainfall runoff or through sewage water runoff. Due to the specialized feeding adaptations of Trichopterans (e.g. Larva of Caddisflies), all these organic particles, films of organic material and substrata in Lakes, diatoms and other algae, cyanobacteria, heterotrophic prokaryotes and eukaryotes and the exudates produced by such assemblages are cleared by Trichopterans and Dipterans. Therefore, Trichopterans and Dipterans contribution is greatly important for maintaining the benthic quality of freshwater habitats.

Furthermore, Hemipterans were more predominant (11,203) and represented by *Belostoma, Ranathra, Notonecta* and *Aquarius* species. These species act as primary and secondary consumers in freshwater habitats, feed on microscopic organisms on the surface water, periphyton and detrital particles include fecal to decomposing leaf litter of various sizes in the column and bottom water respectively [12]. The Hemipterans feeds also on phloem or xylem sap from a different array of plant species. Few species predate on small invertebrate and vertebrate forms such as fishes, amphibian tadpoles, snakes and tissues of living macrophytes [40]. The Coleoperans were represented by *Hydrophilus* sp., *Dytiscus* sp., *Gyrinus* sp. The *Gyrinus* species population was more and it was followed by *Hydrophilus* sp., and *Dytiscus* sp. Adult beetles tear larger prey into smaller pieces, while larvae pierce and pump digestive juices into their prey. Water diving beetle occasionally eat small tadpoles, fishes, whereas water scavenger beetles feeds on small insects, aquatic invertebrates, decomposing organic material that comes from aquatic vegetation, animal faeces etc. Coleopterans (e.g. adult *Dytiscus*) actively foraging in benthic and water column habitats in freshwater Ponds and Lakes. These species are predacious aquatic insects capture prey by actively foraging in benthic and water-column habitats [8 & 40]. All these observations evinced that species of aquatic insects live in benthic, limonitic and littoral zones improve the nutrients status and habitat quality [7] in freshwater Lakes due to their differential responses [9]. Thus, certain species of insects in freshwater Lakes play a crucial role while maintaining the stability of aquatic habitat [5]. Impaired freshwater quality limits their diversity and restricts the species composition [6]. Therefore, emphasis should be given to study such freshwater ecosystems periodically to

updates their biotic components so as to upkeep the afresh conditions of freshwater Lakes amidst urban environment [15 & 21]. Further, Mysore is one of the clean cities in India, where the Kukkarahalli Lake has historical heritage [36]. The Lake is >100 years old, built by the Wodeyar Dynasty in 1864 amidst Mysore city. Now, it is being maintained by the Mysore University administration [41 & 42]. Hundreds of local resident and migratory bird species are visiting this Lake [37].The societal benefits and ecological role played by aquatic insects such as Dragonflies, Damselflies, Water Bugs, Caddisflies and Beetles is greatly important [13 & 43]. Hence, preservation is essential to restore the Lakes and in turn help conserve local biodiversity in urban areas. Our observations are on par with the observations of [8, 13, 15, 17, 18, 20, 21, 32, 42, 44, 45, 46, 47 & 48].

CONCLUSION

Aquatic insects are generalists in their feeding habits. They obtain food by various means using different methods such as shredding, scraping, collecting and gathering, collecting and filtering and piercing. Accordingly, different species of aquatic insects live in freshwater habitats behave has shredders, scrapers, collector-gatherers, collector-filterers, predators and piercers. Therefore, it is important to understand the feeding habits of aquatic insects which are highly diversified within their communities and their abundance can be used to assess the quality of different freshwater ecosystems. That is why aquatic insects are considered as model organisms while analyzing the structure and function of the freshwater habitats. Because, they show high abundance, high birth rate with short generation time, large biomass and rapid colonization in freshwater habitats. Moreover, they have adapted to live in freshwater for few weeks to several years by having variously modified body parts. Hence, they can be considered as bio-indicators of freshwater habitats to estimates the degree of environmental impact and potential effects on other living organisms. Moreover, tropic structure, water quality and level of eutrophication can be measured by using aquatic insects in freshwater habitats. During the present investigation, few insect species belongs to the order Coleoptera, Diptera, Hemiptera, Odonata and Trichoptera have evinced their role as shredders, scrapers, collector-gatherers, collector-filterers, predators and piercers. The occurrence, abundance and distribution of these species revealed their importance to maintain quality of freshwater Lakes in urban environment. Thus, present investigation provided an insight on the insect fauna living in freshwater habitats in urban area and indicated the importance in freshwater Lake System.

Mitigation Measures Suggested

The Kukkarahalli Lake is located amidst Mysore, its conservation is essential by protecting its biotic components especially the aquatic insects. Following mitigation measures are necessitated to restore the afresh condition of water of Kukkarahalli Lake and in turn support the food chain and food webs established between various organisms at different tropic levels amidst this Lake.
- Prohibit the removal of wild grass and grazing of cows
- Clearing the weeds and toxic algae
- Avoid the release of residential sewage to Lake
- Establish the inlets for proper rainwater harvesting
- Maintain quantity and quality of water in the Lake during different seasons.
- Periodic analysis of physcio-chemical and biological parameters of Lake water during different seasons
- Conduct location specific silting to remove dead and toxic algae
- Create awareness on importance of aquatic insects
- Install display boards with the images of importance of freshwater habitats
- Establish Lake Management Board
- Using the expertise of local people, biologists and entomologists to solve burning issues of freshwater Lakes in urban areas.

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Table 1. Aquatic insects recorded in Kukkarahalli Lake

Sl. No.	Order	Sl.No.	Family	Sl.No.	Common name	Scientific name
1.	Coleoptera	1.	Hydrophillidae	1.	Water scavenger beetle	*Hydrophilus piceus*
		2.	Dytiscidae	2.	Water diving beetle	*Dytiscus marginalis*
		3.	Carabidae	3.	Whirligig beetle	*Gyrinus* sp.
2.	Diptera	4.	Muscidae	4.	Dipterans larva	-
		5.	Chironomidae	5.	Chirnomus larva	*Chironomous* sp.
3.	Hemiptera	6.	Nepidae	6.	*Ranathra*	*Ranatra elongata*
		7.	Belostomatidae	7.	Belostoma	*Belostoma* sp.
		8.	Gerridae	8.	Water strider	*Aquarius remigis*
		9.	Notonectidae	9.	Back swimmer	*Notenecta* sp.
4.	Odonata	10.	Libellulidae	10.	Dragonfly	*Diplacodes trivalis*
		11.	Coenagrionidae	11.	Damselfly	*Ischnura* sp.
5.	Trichoptera	12.	Hydroptilidae	14.	Caddiesfly	*Polycentrapus* sp.

Table 2.Nymph/naiad/larval forms recorded in Kukkarhalli Lake

Sl.No.	Order	Sl.No.	Aquatic insect species	Nymph	Larval	Imago
1.	Coleoptera	1.	*Hydrophilus piceus*	-	-	+
		2.	*Dytiscus marginalis*	-	-	+
		3.	*Gyrinus* sp.	-	-	+
2.	Diptera	4.	Dipterans maggotes	-	+	-
		5.	*Chironomous* sp.	-	+	-
3.	Hemiptera	6.	*Ranatra elongata*	-	-	+
		7.	*Belostoma* sp.	-	-	+
		8.	*Aquarius remigis*	-	-	+
		9.	*Notonecta* sp.	-	-	+
4.	Odonata	10.	*Diplacodes trivalis*	+	-	+
		11.	*Ischnura* sp.	+	-	+
5.	Trichoptera	12.	*Polycentrapus* sp.	-	+	-

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Table 3. Immature forms of aquatic insects recorded in Kukkurahalli Lake (n=48)

Week	Larval forms		Odonate Naiad forms	
	Diptera	**Trichoptera**		
	Chironomous Larva & flies maggot	**Caddisfly larva**	**Dragonfly**	**Damselfly**
1.	-	37	56	70
2.	-	24	32	107
3.	-	02	123	224
4.	-	04	125	209
5.	-	06	198	298
6.	125	07	322	341
7.	93	41	161	185
8.	244	148	181	180
9.	341	201	270	308
10.	279	262	220	206.
11.	268	265	131	231
12.	-	-	-	319
Total	1350	997	1819	2678
'F' value	4.683*			

*Value is significant at 1% level.

Table 4. Imago population of Odonates (n=48)

Week	*Diplacodes trivalis*	*Ischnura* sp.
1.	-	-
2.	38	-
3.	53	-
4.	23	15
5.	34	24
6.	122	67
7.	49	56
8.	55	58
9.	180	120
10.	169	196
11.	209	261
12.	-	170
|Total	932	967
$X^2_{0.01}$	10.0*	

*Value is significant at 1% level.

Table 5. Correlation coefficient of naiad and imago population of dragonfly and damselfly (n=48)

Week	*Diplacodes trivalis*		*Ischnura* sp.	
	Naiad	**Imago**	**Naiad**	**Imago**
1.	56	-	70	-
2.	32	38	107	-
3.	123	53	224	-

4.	125	23	209	15
5.	198	34	298	24
6.	322	122	341	67
7.	161	49	185	56
8.	181	55	180	58
9.	270	180	308	120
10.	220	169	206	196
11.	131	209	231	261
12.	-	-	319	170
Total	1819	932	2678	967
%	48.8	51.2	63.9	36.1
'r' value	0.361		0.45	
't' value	3.79*		3.55*	

* Values are significant at 1% level.

Table 6. Imago population of aquatic beetle species recorded in Kukkarahalli Lake (n=48)

Week	*Gyrinus* sp.	*Dytiscus* sp.	*Hydrophilus* sp.	Total
1	334	49	95	478
2	325	82	161	568
3	375	-	200	575
4	352	135	188	675
5	383	118	172	673
6	350	120	110	580
7	322	168	118	608
8	301	110	42	453
9	230	78	50	358
Total	2972	860	1136	4968
'F' Value	58.07*			

*Value is significant at 1% level.

Table 7. Imago population of Hemipterans in Kukkarahalli Lake (n=48)

Week	*Belostoma* sp.	*Notonecta* sp.	*Ranathra* sp.	*Aquarius* sp.	Total
1.	56	-	58	-	114
2.	89	67	65	350	571
3.	154	131	118	256	659
4.	266	109	97	197	669
5.	236	101	154	417	908

6.	280	112	222	100	714
7.	294	50	126	91	561
8.	244	63	152	471	930
9.	368	474	281	572	1695
10.	284	344	242	688	1558
11.	419	166	215	427	1227
12.	321	263	245	768	1597
Total	3011	1880	1975	4337	11,203
'F' value	6.161*				

*Value is significant at 1% level.

Table 8. Imago Population of aquatic insects in Kukkarahalli Lake

Week	Coleoptera	Hemiptera	Odonata
1.	478	114	-
2.	568	571	38
3.	575	659	53
4.	675	669	38
5.	673	908	58
6.	580	714	189
7.	608	561	105
8.	453	930	113
9.	358	1695	300
10.	-	1558	365
11.	-	1227	370
12.	-	1597	170
Total	4968	11203	1799
'F' Value	17.121*		

Note: Data is based on Tables 4, 6 and 7. *Value is significant at 1% level.

Table 9. Month-wise occurrence of aquatic insects in Kukkarahalli Lake (n=48)

Month	Coleoptera	Diptera	Hemiptera	Odonata	Trichoptera	Total
February	2296	-	2013	1075	67	5451
March	2314	462	3113	2331	202	8422
April	358	888	6077	2890	728	10941
Total	4968	1350	11203	6296	997	24814
'F'Value	4.217*					

*Value is significant at 1% level.

RESEARCH ARTICLE

Production of Biofilm Destructing Enzymes from Agro Wastes by using Yeast and Bacteria

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ABSTRACT

Mosambi peels and rice bran samples were collected for biofilm destructing enzyme production. Isolated and identified bacteria and yeast samples were inoculated into mosambi and rice bran fermented media. From the fermented media, the phytochemical screening such as phenols, flavonoids, steroids, tannins, cardiac glycosides, terpenoids, reducing sugar and saponins were studied. The protease and amylase enzyme separated from media and studied their biofilm destruction activities. In this study amylase and protease exhibited more biofilm destructing.

Keywords: Mosambi peels, rice bran, biofilm**,** amylase, protease.

INTRODUCTION

Biofilms are communities of microorganisms, which develop on surfaces in natural and artificial environments [**1,2**] and that the microorganisms are encased in a self-synthesized extracellular polymeric matrix (EPS) and grow attached to biotic or a biotic surface [3]. Biofilm formation is a sequential process where one by one microorganisms come and adhere to the substratum [4]. A biofilm comprises any syntrophic consortium of microorganisms in which cells stick to each other and often also to a surface [5].Enzymes can be used for degradation of biofilm [6-9], but due to the heterogeneity of the extracellular polysaccharides in the biofilm, a mixture of enzyme activities may be necessary for a sufficient degradation of bacterial biofilm. Nowadays it is obvious that enzymes are used as key

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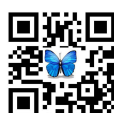
active components in many cleaning agents, disinfectants and detergents [10,11].Microbial biofilms are populations of microorganisms that are concentrated at an interface and typically surrounded by an extracellular polymeric slime matrix [12]. Microorganisms present in biofilms are protected against disinfection products and medicine by the EPS matrix and by enzymes produced by the microorganisms themselves [13-16]. Basically, biofilm formation can also be termed as continues process and its take days to weeks to months to forms and eventually detachment, erosion and sloughing of the biofilm occur post maturity. The ability of sloughing of the biofilm occur post maturity. The ability of bacteria to form biofilm has various impacts on its members. The enzymes preparations typically prevent adhesion and remove adhered biofilms by degrading the EPS produced by biofilms. Therefore, nature of enzyme becomes important for the detachment and removal of biofilm [17]. Bacterial proteases and amylases can remove biofilm completely instead of killing only planktonic organisms [18]. Enzymes are proteins, which act as catalysts. Enzyme lower the energy required for a reaction to occur without being used us in the reaction fermentation involves the use of microorganisms, like bacteria and yeast produced of enzymes bio cleaners are an organic solution produced by the simple fermentation of fresh agro waste with addition of brown sugar and water using the selective microorganisms like yeast and bacteria. Enzymes bio cleaning solution by using the residues and waste of agricultural product like agro waste with help of yeast and bacteria.

MATERIALSANDMETHODS

Collection and preparation of samples
Fruit waste such as mosambi peels are collected from various fruit juice stall and rice bran sample are collected in shop. The samples werebrought to laboratory using sterile plastic bags for further analysis. The fruit waste was cut into small pieces by using knife are further fermentation process.

Preparation of fermentation medium

Fermentation process of mosambi
Take fruit peels (150g) and add 50g of molasses, add 500ml water and 1 ½ teaspoon full of yeast, 5ml bacterial culture suspensions whichever the fermentation is to be carried out.

Fermentation process of rice bran
Take (150g) of rice bran and 50 g of molasses and add 500 ml of water and 1 ½ teaspoon full of yeast and 5ml of bacterial culture whichever the fermentation is to be carried out. Estimation of bacterial from fermented sample, isolation of microorganisms, spread plate method Gram staining, Biochemical test ; Indole, Methyl red, Vogesproskauer, Citrate, urease, Catalase, and TSI and phytochemical screening of the solvent extracts of agrowastes foralkaloids,flavonoids,cardiacglycosides,phenols,reducingsugars,aminoacids,carbohydrates,monosacharides,saponins,steroids,tannins and terpenoids.

Biocleaner activity
The enzyme solution will be checked over biofilm coated pet bottles the bioleaching efficiency will be compared will each enzymes preparation.

Production of Biocleaning solution
The agro wastes samples were fermented in conical flask with other ingredients, clean the mosambi peel and 1 liter of water, soak the peels in a small pieces for faster decomposition and add ½ kg brown sugar, close the lid keep it away from the sunlight and do not close tightly. The gap open for the gases to escape from the flask, you should open it next time shake the flask in 2-3 days to give it a nice mix, keep stirring the solutions once 7-10 days and keep a check on white worms the peels with start break down and after 3 months used the solution to clean a pet bottles.

Biofilm formation

Bottle is filled with water and tightly closed after 3 months the layer are formed corner neck of the bottle.

Primary screening for Amylase Production

The strains (*Escherichia coli)* isolated were inoculated on starch agar media and incubated at 30°C in an incubation the plates were flooded with grams iodine solution. It was allowed to stand for few minutes and poured off. Clear distinct zone around the amylase producing colonies were observed.

Primary Screening of Protease Production

The strains (*Escherichia coli*) isolated were inoculated on skim milk agar media and incubated at 30°C in an incubator for 3-4 days. Distinct clear zone appears around the protease producing colonies.

Partial Purification of Enzymes

Purification steps were carried out with the help of ammonium per sulphate precipitation method.

Ammonium per sulphate precipitation

A volume of crude enzymes was taken separately added slowly the required quality ammonium per sulphate. The addition of ammonium per sulphate was done under constant stirring at 4° C for 30 mins and then stirring was continued for another 30 mins and then allowed for settlement for 3 hours at 4° C the precipitated protein were separated by centrifugation at 8000rpm at 4° C for 20 mins the separated proteins were dissolved in minimum amount of 0.05M citrate buffer (pH-5) and refrigerated for further analysis.

RESULT AND DISCUSSION

The test organisms used in this study include *E. coli* bacteria isolates were maintained in nutrient agar medium for identification of microorganisms in mosambi and rice bran sample were serially diluted and plates on suitable medium. One of the such method is using the agro wastes into produce the enzymes and organic acids in substrate by using yeast and bacteria will be better in use of replacing the cleaning materials .The scientific way of doing the above enzyme cleaner production from microbial organisms in the agro wastes is the main concept of the project. Enzyme bio cleaners are an organic solution produced by the simple fermentation of agro wastes with addition of brown sugar and water by using the selective microorganisms like yeast and bacteria. This has been developed simple cleaning solution for pet bottle. This product is nature and safe, extraordinary remove of biofilm. Many industries are dependent on enzymes for production of their goods, fermentation is a method of generating enzymes for industrial purpose. Fermentation involves the use of microorganisms like yeast and bacteria to produce the enzymes. Hence, we found interest to produce bio cleaning enzyme in this study. In present study biofilm coated was formed at the neck and bottom of the pet bottle as thin, slimy layer. Enzyme produced as a bio cleaner an organic solution produced by simple fermentation agrowastes with addition of brown sugar and water by using the selective microorganisms like yeast and bacteria

Protease Enzymes

After fermentation the protease enzyme assay was carried out to determine the amount of tyrosine, the enzyme assay was confirmed with skim milk agar zone fermentation.

Amylase Enzymes

After inoculated the amylase enzymes assay was carried out determine the amount of maltose, the enzymes assay was confirmed with starch agar zone formation.

CONCLUSION

In the present study amylase, protease was investigated for biofilm inhibition. Therefore, mentioned enzymes have been the safest commercial use to inhibit biofilm formation in the processing units, as depolymerase poses no threat of any kind of contamination of the product the formation of harmful by products. Based on above scientific on technical information, the present work is proposed to carry out the production and analysis of fermentation parameters of cleaning solution by using the residue and waste of agricultural produces like fruit and rice bran with help of yeast and bacteria with addition of cheaper carbohydrates sources like brown sugar and water medium. This has been developed for people to make simple cleaning solution at home, this cleaning solution help to displace unknown, potentially pathogenic(disease causing)bacteria with known, healthy microorganisms and in this way contribute to better human health.CIP and cleaning solution are currently been used for of biofilm in pet bottle. The solution is used to clean the pet bottle avoid dirt from pet bottles, remove biofilm from the bottles. The microbial biofilm communities colonizing single use pet drinking bottles, determine their plastic specificity of bottle .The solution has be proved to have no corrosive or otherwise adverse effects on equipment, to patient can even be harmless consumed biofilm time take to grow in bottle for 3 months.

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Table 1: Phytochemical analysis of various extracts of Mosambi and Rice bran

COMPOUNDS	PETROLIUM ETHER		CHLOROFORM		METHANOL		ETHANOL		AUQEOUS	
	MI	RBS	MI	RBS	MI	RBS	MI	RBS	MI	RBS
Steroids	-	-	-	-	-	-	-	+	-	-
Terpenoids	+	+	-	-	+	+	+	+	+	-
Flavonoids **i)Alkaline reagent**	-	-	+	-	+	+	+	+	+	-
ii)Led acetate	-	-	+	-	+	+	+	+	+	-
Saponins	+	+	-	+	+	+	+	+	+	+
Tannins **i)Led acetate**	+	-	-	+	-	+	-	-	+	+
ii)Ferric chloride	-	-	-	-	-	+	+	-	-	-
Cardiac glycosides	-	-	-	-	-	+	-	-	-	-
Amino acid	-	+	-	-	+	+	-	-	+	+
Proteins	-	-	-	-	-	-	-	-	-	-
Carbohydrates	-	-	-	-	+	-	-	+	+	-
Reducing sugar	-	-	-	+	-	+	+	+	+	-
Aalkaloids	-	-	-	-	-	-	-	-	+	-

MI- Mosambi Sample, RBS- Rice Bran Sample

RESEARCH ARTICLE
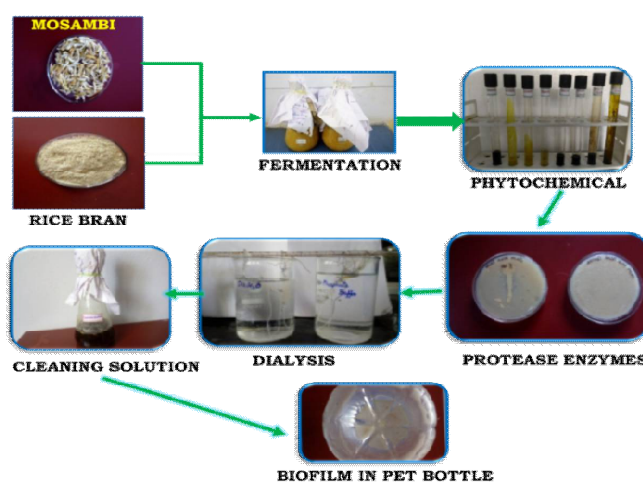
Mathematical Modeling of Tumor Dynamics

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ABSTRACT

In this paper, I had tried to determine the number of free lymphocytes and number of tumor cells that a particular person can have when affected with tumor disease. This is done by forming a mathematical model comprising of two ordinary differential equations. One can solve for the associated variables by reducing the complexity of the differential equations by suitably converting them to first order simultaneous differential equations.

Keywords: Free Lymphocytes, Tumor Cells, Total Free Cells, Tumor Cell Population, Simultaneous Differential Equations.

INTRODUCTION

In this paper, I will describe the deterministic dynamics of a solid tumor in the presence of a reactive lymphocyte population stimulated by, and antagonistic to, the tumor through a simple mathematical model. The main idea is to determine the number of free tumor cells located at its surface. This will provide a good idea of treating with that particular tumor.

Describing the Model

Assume that only the surface of the tumor is accessible (neglecting effect or penetration via vascularization). Let *L* be the free (unbound) lymphocytes, and $\overline{C}$ be the number of free tumor cells at the tumor surface. Assume as well first-order lymphocyte attrition, but stimulation proportional to the exposed tumor cell population, together with a standard logistic saturation factor for lymphocyte production governed by the following differential equation

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$$\frac{dL}{dt} = -\lambda_1 L + \alpha_1' \overline{C} L \left(1 - \frac{L}{L_0}\right) \quad (2.1)$$

Furthermore, the total number of tumor cells C, will increase by proliferation of the total free cells C_f, but decrease by binding of those not already bound given by the differential equation $\frac{dC}{dt} = \lambda_2 C_f - \alpha_2' \overline{C} L \quad (2.2)$

I now try to solve differential equations (2.1) and (2.2)

Solving the Model

The binding of the surface tumor cell population, say C_s, to the lymphocyte population L is imagined as equilibrium controlled in the usual way by the equation $\overline{C} + L \rightleftharpoons (\overline{C}L) \quad (3.1)$

Through (3.1), we get $(\overline{C}L) = K\overline{C}L \quad (3.2)$

But we know that $\overline{C} + (\overline{C}L) = C \quad (3.3)$. Hence from equations (3.1), (3.2), (3.3) we get

$$\overline{C} = \frac{C_s}{1 + KL} \quad (3.4)$$

Before proceeding further, I now present the following Figure for better understanding.
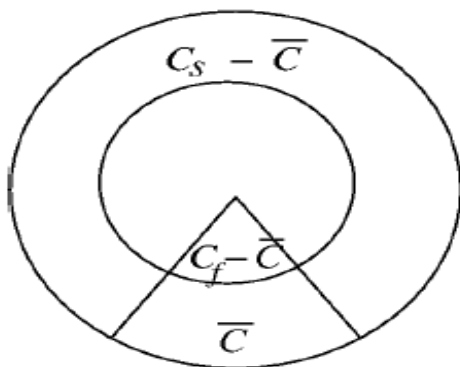
Now if we consider the tumor to be roughly spherical in shape as shown in the above Figure, then we can write $C_s = \gamma C^{2\beta} \quad (3.5)$ where β is the measure of bathing lymphocyte population.

Since we know that $C_f = C - (\overline{C}L)$ we can eliminate $\overline{C}, C_s$ and C_f and substituting in the differential equations (2.1) and (2.2), we get

$$\frac{dL}{dt} = -\lambda_1 L + \frac{\alpha_1' C^{2/3} L}{1 + KL} \left(1 - \frac{L}{L_0}\right) \quad (3.6)$$

$$\frac{dC}{dt} = \lambda_2 C - \frac{\gamma C^{2/3} L}{1 + KL} \left(\lambda_2 K + \alpha_2'\right) \quad (3.7)$$

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Equations (3.6) and (3.7) represent first order simultaneous differential equations in *L* and *C* and so can be solved either by integration or by numerical methods.

CONCLUSION

By forming a simple mathematical model with two differential equations as in (2.1) and (2.2), I had introduced other variables which reduce the complexity of original differential equations providing simultaneous differential equations as derived in (3.6) and (3.7). Knowing the appropriate constants, we can readily solve the simultaneous differential equations for *L* and *C*. Further, using (3.3), we can determine $\overline{C}$ be the number of free tumor cells at the tumor surface. These values will provide a good idea of how to treat a patient who is affected by tumor ailments.

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RESEARCH ARTICLE

Online Buying Behaviour of NetiZens: A Study with Reference to Madurai, Tamilnadu

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ABSTRACT

This study attempts to analyze the features related to the buying behaviour of online shoppers. Consumer buying behaviour in respect of online shopping was studied using different socio-economic variables. It also provides a support that helps researchers understand the drivers of consumers' attitude and goal to shop on the Internet, and consumers' perceptions regarding ease of use and usefulness. Conclusions derived from the analysis can be used as useful guide for market orientation. The outcomes of the study suggest that assessment of consumer buying behaviour can contribute to a better understanding of consumer buying behaviour in respect of online shopping.

Keywords: Consumer, Behaviour, E-Marketing, E-commerce, Online, Internet

INTRODUCTION

The Internet is an open worldwide communication network, linking countless number of computer networks throughout the world, through an intensive network of telephone lines. The increased availability of Internet is influencing the growth of Internet users around the world. The popularity of e-marketing has been increased tremendously in last 15 years. Companies are investing heavily in promotion of their products & services via internet based marketing. But its growth rate is relatively slower as compared to other emerging technologies. The prominent reason of slower growth than expected may be due a large proportion of population in India as well as other developing & under developed countries that people are still not aware of computers & internet technology also security concern regarding personal information on websites. Companies need to create buying behavior of the consumers.

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Studying buying behaviour, motives and intention along with the attitude of the online buyers is within the theoretical constructs of the Theory of Reasoned Action. The Theory of Reasoned Action (Fishbein, 1980) examines the relationship between attitudes and future intention to participate in these buying behaviors. The behaviors include: when they click on banner ads (with which site and age group), response to e-mail advertisements, way in which product information is searched using search engines and within the site, use of comparision engines, attention and time to customer review and reaction toward them, product basket, online support services, use of e-mail service, feedback form, checkout. According to Cheung et al (Online Consumer Behavior: A Review and Agenda for Future Research, 2003), a base model called Model of Intention, Adoption, and Continuance (MIAC) for the development of an online consumer behavior framework. This model predicts that behavior is governed by intention. Satisfied consumers are most likely to continue hence adoption and continuance are connected to each other through several mediating and moderating factors such as trust and satisfaction. There are Individual/Consumer characteristics, Environmental Influences, Product/Service Characteristics, Medium Characteristics, and Online Merchants and Intermediaries Characteristics which affect the consumer behaviour.

LITERATURE REVIEW

According to Sabarirajan et al (2020), retailing in India has been increasing at a rapid speed over the last decade. More significantly it witnessed most important changes in terms of retail mix, quality and scale of retailing, varieties of retail formats and over above change in consumer preferences and shopping habits. Dramatic technology changes have contributed vastly to improve the shopping experience of customers. The customers as postmodern subjects pursue the belief system of the market out of no decision (P.S. Venkateswaran et al, 2019).Culture can also be considered as one of the critical factors in both adopting and the success of E-marketing. The first element in the adoption of E-marketing is the availability of a supportive atmosphere and environment. In order for the E-marketing tools (i.e. the Internet) to serve as an effective marketing tool, all parties in the relationship or the transaction must be familiar with PCs and appreciate the benefits and the potential applications of the Internet and WWW. Without a supportive culture, technology may not be able to replace fully the enterprise-customer relationships. If the culture place more value on strong relationships in business and people prefer informal and personal relationship based communication there will be either no or low implementation for E-marketing. This strong human orientation can make the self-service mode of many E marketing based activities somewhat unattractive. On the other hand, there are a lot of cultural aspects that can affect E-marketing adoption enterprises. These aspects include: people attitude towards E-marketing activities, trust, and security, lack of social acceptance for electronic economic activity and customer acceptance and participation in the E-marketing transaction. Venkateswaran et al., (2015) studied the influence of perceived quality, purchase intention and customer satisfaction towards brand loyalty for branded coffee powders in Madurai. Organizational learning is the organizations capability to maintain and improve performance and interests (A. Sabarirajan et al, 2015). David Rajesh et al (2015) studied the influence of service loyalty on service quality, information, functional quality, technical quality, services cape quality, service convenience and satisfaction for commercial banks. They found customer satisfaction is the most significant predictor of the service loyalty.

RESEARCH METHOD

Research on the effect of consumer behaviour towards e-marketing is a descriptive research. Here population represents residents of Madurai (Tamil Nadu, India) city. Sample selected comprises of business professionals, students & other educated people of urban area only. Study undertaken use stratified sampling i.e. population is divided into a 3 strata according to age, income & occupation. For each stratum, 106 respondents were picked by random means from different areas. Sample size of research is arbitrarily taken as 106 for the convenience of research. Questions were prepared using Nominal scale & Ordinal scale as attributes studied were non parametric.

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After checking the validity & reliability of the questionnaire primary data was collected from respondents in city malls (Vishal, Mega Mart, Inox) , cyber cafes including Reliance Web World, Sify internet cafe. Since scale used in the questionnaire was non- parametric in nature therefore data was coded in order to analyze data. SPSS (Statistics Packages of Social Software) 17.0 was used as analysis tool. To determine the causal-effect relationship between different variables, CHI Square test was used. Samples were collected from consumers and buyers of online shopping in which the total sample size of respondents are 106

DATA ANALYSIS & FINDINGS

Since data collected is nonparametric in nature therefore data analysis is done using CHI SQUARE test at 5% significance level (α=0.05). Null hypothesis is rejected where $\alpha \leq 0.05$ & it is accepted when α>0.05. SPSS software is used to analyze data. Distribution of study samples according to three strata of the research is as follows

Hypothesis 1: Age group between 25-30 years surf internet most. In order to prove above hypothesis CHI SQUARE test is conducted. H0: There is no significant difference between age & internet surfing or there is no relation between age of the respondents & internet surfing. HA: There is significant difference between age of the respondents & internet surfing, in other words there is relation between age of the respondents & internet surfing. Test statistics showed that Chi-Square calculated at 12 degree of freedom is 111.373 at 0.00% significance level. Hence null hypothesis is rejected at α=0.00 & alternate hypothesis (i.e. there is strong relation between age of the respondents & internet surfing) is accepted. However Test statistics shows that there is significant relationship between occupation and internet surfing frequency of the respondents.

Hypothesis 2: Gender does not play any role in internet surfing. Chi-square test between gender of the respondent & internet surfing data is done. Test statistics shows that Chi-square calculated at 72.9% significance level is 31.093. It means that null hypothesis is accepted i.e. there is no relation between gender and internet surfing of the respondents.

Hypothesis 3: There is high degree correlation between income of the respondents and their purchase decision. Chi-square test is conducted between income of the respondents & their purchase decision affected by e-marketing. Chi-square statistic shows that at 0.0% of significance level calculated value comes out to be 57.653 at 15 degree of freedom. Hence it can be concluded that null hypothesis is rejected & alternate hypothesis is accepted that there is significant difference of a strong relation between income of the respondents & purchase decision. Also a significant difference is found between age, occupation of the respondents and their purchase decision.

Hypothesis 4: Respondents find e-shopping more convenient because it is time saving, availability of alternatives to choose from & possibly less expensive products and services. In study liker scale (five point agreement-disagreement scale) was used to determine respondents' response. It was found that 65% were highly agreed on easy accessibility of online products. Further, most of the respondents found online shopping more convenient & time saving than brick & mortar system.

Hypothesis 5: Most of the respondents are hesitant to purchase items over internet because of security concerns. In order to prove the above hypothesis respondents were asked to rate the drawbacks of the online shopping in the rating scale of 1-5 and it was observed that 52% of the respondents claimed security concern regarding disclosure of personal information as first rank. 47% of them rated lack of physical approach on products/services offered, second. While quality & authenticity of products/services offered was rated fourth by 48% of the respondents. Chi Square test is conducted to determine that whether there is any relation between gender and security concern, lack of physical approach and quality & authenticity of the products/services offered via online trading.

Hypothesis 6: Usage of newer technology in online trading has made process more complicated, affect buying behavior of respondents. Consumers were asked if product/service of their requirements is being offered online at reasonable price, then will they prefer to buy that product/service online or purchase the same from brick &mortar system. It is found that 68% of the respondents claimed product/service of their requirement through traditional shop, while 32% favoured online purchases. To prove the hypothesis statistically Chi-Square test is conducted between age & preference of the respondents in purchasing product/service. Test statistics showed that null hypothesis is rejected at significance level 0.00%

Mode of Payment: Respondents were asked to select the mode of payments while shopping online. 52% preferred credit card payment, while 28% opted for debit card payments, 12% preferred payments through cheques and 8% preferred for demand draft or pay order services. Chi square test is conducted to determine whether the relationship between mode of payment & income group of the respondents exist or not. Test statistics showed that null hypothesis is rejected at α=0.00% and alternate hypothesis is accepted i.e. there is significant difference between the two variables or mode of payment is depended upon income of the respondents. People having monthly income Rs 10,000 or more prefer credit card payments.

CONCLUSION

E-marketing is rapidly changing the way people do business all over the world. In the business-to-consumer segment, sales through the web have been increasing dramatically over the last few years. Customers, not only those from well-developed countries but also those from developing countries, are getting used to the new shopping channel. Understanding the factors that affect intention, adoption and repurchase are important for researchers and practitioners alike. E-marketing is gaining popularity among people specially the younger generation but in today scenario to become equally popular among all age groups e-marketing will have to cover a longer distance. People have hesitations in using e-services due to security concerns, lack of physical approach towards product offered, delays in product delivery along with price & quality concerns. More-over people are more resistant to change & not easily adaptable to newer technology. **68%** of respondent found shopping from shop easier, convenient & preferable over online purchasing. Above finding clearly supports our conclusion that people are tradition bound & have doubt in mindset as far as issue of online shopping/purchase of product is concerned.

People have dubious attitude towards e-marketing of product & services mainly due to security concern related to privacy of personal information. Personal information privacy should be given preference by the companies involved in online marketing of product & services. The other major concern among people includes authenticity of product & services offered online. Companies involved in online trading should focus on building their brand awareness among people so that trust-worthy relationship can be developed between producers & consumers. On-time delivery of products purchased through online shopping will prove to be quite beneficial in a long run. Significant price-cuts should be offered to customers as there are relatively no/lesser intermediaries involved as far as e-marketing is concerned. Currency fluctuation should be dealt with great care & steps should be taken both by government & companies so as to reduce currency fluctuation to its minimal. Promotional schemes should be launched to promote e-marketing business. Advertising of web-products & services is one of the major issues where companies fail to attract potential consumer's attention. Companies should focus on offering informative advertisements which would contain product information along with additional products & services offering which best suits needs of people. Such advertisements frequency should be high so as to position the products & brands in consumer mindset. In a nut shell we can conclude that remarketing has a potential to grow, only proper boosting needs to be done both at producer and consumer level apart from government efforts.

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RESEARCH ARTICLE

Pharmacological Potential of Anti-oxidant and Anti-inflammatory Activity of Ethyl Acetate Fraction of *Moringa oleifera* Flowers (Murugai)

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ABSTRACT

Moringa oleifera, belongs to family Moringaceae, is commonly known as *Moringai* in Tamil. Several literature report suggest it to be anti-diabetic, antihypertensive, anti-cancer, immune modulators, antibacterial and anti-hyperlipidaemic activity. Fresh flowers of *Moringa oleifera* were extracted and evaluated for antioxidant activities by 2,2-Diphenyl 1-picryl hydrazyl solution (DPPH), 2,2'-azino-bis(3-ethylbenzthiazoline-6-sul- phonic acid (ABTS) assay and anti inflammatory activites by human blood cell (HRBC) membrane stabilization method and Inhibition of albumin denaturation method. The results obtained showed that the ethyl acetate fractions of *Moringa oleifera* flowers can be considered as good sources of anti-oxidants, anti-inflammatory and anti-diabetic, it can be incorporated into the drug formulations. This study justifies the anti-oxidants, anti-inflammatory and anti-diabetic activity of isolated ethyl acetate fractions of *Moringa oleifera* flowers. Further detailed analysis of this sample is required to identify the presence of bioactive compounds responsible for anti-oxidants, anti-inflammatory and anti-diabetic activity. Studies are highly needed for future drug development.

Keywords: *Moringa oleifera*, Anti-oxidant activity, Anti-inflammatory activity, anti-diabetic activity, Albumin denaturation, DPPH(2,2-Diphenyl 1-picryl hydrazyl), ABTS(2,2'-azino-bis(3-ethylbenzthiazoline-6-sul- phonic acid)assay, antioxidant activity etc.,

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INTRODUCTION

Moringa oleifera Lam. (Moringaceae) is a highly valued plant, distributed in many countries of the tropics and subtropics. It has an notable range of medicinal uses with high nutritional value. Different parts of this plant contain a profile of important minerals and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics [1]. The leaves and youthful buds of the plant are used as vegetable and can be rubbed on the temples for relieving headache while root and root bark are regarded as anti scorbutic and can be used externally as counterirritant [2]. The eye disease is treated with the juice of the leaves with honey [3]. The plant is also known to possess high nutritional value and is used in a legends medicine to treat various ailments related to pain and inflammation [4]. Dried seeds of *Moringa oleifera* are used in ophthalmic preparation, venereal infection anti-inflammatory, and purgative and as tonic. The alcoholic extract of the leaves of *Moringa oleifera* are reported to have analgesic activity [5] and the aqueous extract of *Moringa oleifera* roots also shows antifertility profile [6]. The plant is reported to have wide range of pharmacological effects that include antitumor [7], antipyretic [1], antispasmodic, diuretic [8], antiulcer [9], hypotensive [10], hypolipidemic [11], Hepatoprotective [12], anti-fungal [13] and anti-bacterial activities [14].

The purpose of this evaluation is to summarize the pharmacological properties of different parts of the plant, *Moringa oleifera*. The anti-oxidant property of *Moringa* may be due to the presence of phenolic compounds that was confirmed by phytochemical showing of the hydro-ethanolic extract. In this respect, *Moringa* pods contain important bioactive compounds including glucosinolates, isothiocyanates, thiocarbamates, and flavonoids [15]. These compounds satisfy ROS, chelate metal ions and regenerate membrane-bound antioxidants [16]. The aqueous extract of *Moringa oleifera* exhibited strong scavenging effect on 2, 2-diphenyl-2-picryl hydrazyl (DPPH) free radical, superoxide, nitric oxide radical and inhibition of lipid per oxidation. The free radical scavenging effect of *Moringa oleifera* leaf extract was as good as with that of the reference antioxidants. The extracts of *Moringa oleifera* both full-grown and tender leaves have potent anti-oxidant activity against free radicals, stop oxidative damage to major biomolecules and afford significant protection against oxidative break [17].

The anti-inflammatory achievement of an aqueous extract of root in rats with weight between 120 and 160 g was investigated [18]. The anti-inflammatory activity of inaccessible compounds was investigated with the lipopolysaccharide (LPS)-induced murine macrophage RAW 264.7 cell line. These isolated compounds 1, 2, 4 and 5. *M. oleifera* may also possess some beneficial properties that act against chemically stimulated immune-mediated inflammatory responses that are characteristic of asthma in the rat [20].

The analysis showed that *Moringa oleifera* is rich in bioactive agents that enhanced its use as medicinal plant. The result also showed a major decrease in blood glucose level of the induced rats at 3, 9 and 14 days for aqueous and ethanolic extracts of *M. oleifera* respectively. However, the aqueous extract showed more hypoglycemic effect than the ethanolic extract which suggests it to be a better solvent for the extraction of the bioactive agents for this extraction. After 14 days, there was 45.2% and 33.7% reduction of blood glucose for aqueous and ethanolic extracts respectively, while the location drug (insulin) had 58.7% reduction. The statistical analysis showed no significant difference in blood glucose drop with insulin and the *M. oliefera* extracts (p-value > 0.05). However, a significant difference exists between the glucose level after alloxan initiation and 14 days treatments (p-value< 0.05). The results from this study deep-rooted the extensive use of *M. oleifera* Lam. plant in the treatment of diabetes in ethno-medicinal practice and propose its use in drug formulation. *Moringa oleifera* Flowers contain of natural source of polyphenol that potential to have anti-oxidants, anti-inflammatory and anti-diabetic activity. The purpose of this paper is to evaluate the anti-oxidants, anti-inflammatory and anti-diabetic activities of ethyl acetate fraction of *Moringa oleifera* Flowers.

MATERIALS AND METHODS

Collection of Plant Materials
Fresh flowers of *Moringa oleifera* Flowers were collected from O.Koothur Village, Ariyalur district, Tamil Nadu, India, during the month of November and identified by Head, PG & Research Department of Botany, Periyar E.V.R. College, Trichy. Tamil Nadu.

Flower Extraction
2 kg of fresh flowers were soaked with 90% ethanol at room temperature (25°-30°C). After 72 hrs the ethanolic extract was filtered. This extract was separated from different fractions like Benzene, ether, and Ethyl acetate. Finally the ethyl acetate fraction concentrated in vacuum and the dry powder obtained was dissolved in DMSO (Dimethyl sulfoxide) to get required concentrations and were used for screening anti-oxidant and anti-inflammatory and anti-diabetic activities.

Anti-Oxidant Studies

DPPH Scavenging Assay
The ability to scavenging the stable free radical, DPPH was measured as a decrease in absorbance at 517 nm by the method.

Reagents
2,2-Diphenyl-1-picryl hydrazyl (DPPH) – 90.25mM in methanol in a dark room.

Procedure
To a methanolic solution of DPPH (90.25 mM), an equal volume of ethanolic Rhizome of Cyperus rotundus L (250-1500 μg) was added and made up to 1.0 mL with methanolic DPPH. An equal amount of methanol was added to the control. After 20 min, the absorbance was recorded at 517 nm in a Systronics UV-visible Spectrophotometer. Ascorbic acid was used as standard for comparison. The inhibition of free radicals by DPPH in percentage terms (%) was calculated by using the following equation.

% Scavenging = (OD of Control- OD of Sample/ OD of Control) X 100.

Where A control is the absorbance of the control reaction (containing all reagents except the test compound), and A sample is the absorbance of the test compound.

Anti- Inflammatory Activity
Inhibition of Albumen Denaturation
The reaction mixture was consisting of test extracts and 1% solution of bovine albumin fraction, pH of the reaction mixture was adjusted using small amount at 37°C HCl. The sample extracts were incubated at 37°C for 20 minutes and then heated to 51°C for 20 minutes after cooling the samples the turbidity was measured spectrophotometrically at 660 nm. Diclofenac sodium was taken as a standard drug. The experiment was performed in triplicates. Percent inhibition of protein denaturation was calculated as follows:

Percent inhibition (%) = (OD of Control- OD of Sample/ OD of Control) X 100.

Anti Diabetic Activity
Inhibition of Alpha-Amylase Enzyme
Starch solution (0.1% w/v) was prepared by stirring 0.1 g of potato starch in 100 ml of 16 mM of sodium acetate buffer. The enzyme solution was prepared by mixing 27.5 mg of α-amylase in 100 ml of distilled water. The colorimetric reagent is prepared by mixing sodium potassium tartarate solution and 3,5-di nitro salicylic acid

solution 96 mM. The starch solution is added to the both control and plants extract tubes and left to react with α-amylase solution, under alkaline conditions at 25ºC. The reaction was allowed for 3 min. The generation of maltose was quantified by the reduction of 3,5-dinitro salicylic acid to 3-amino-5-nitro salicylic acid. This reaction is detectable at 540 nm.

% Inhibition = (OD of Control- OD of Sample/ OD of Control) X 100.

RESULT AND DISCUSSION

Anti – Oxidant activity
DPPH Scavenging Assay Method

There are a number of methods available to assess the antioxidant activity of compounds. DPPH free radical scavenging assay is an easy, rapid, and sensitive method for the antioxidant screening of plant flower extracts. In the presence of an antioxidant, DPPH radical obtains one more electron and the absorbance decreases. In the present study, the DPPH assay activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera* flowers have high DPPH scavenging capacity, which increased with increasing concentration [Table 2 and Figure 2]. It is evident from the data presented in Table, that the sample possesses DPPH assay activity. For the ethyl acetate fractions of *Moringa oleifera* flowers, the result shows the percentage of cytotoxicity for 250 mg/ml as 13.09%, 500 mg/ml as 36% and 750 mg/ml as 47.5% and 1000 mg/ml as 51.02%. For the ethyl acetate fractions of *Moringa oleifera* leaf, the result shows the percentage of cytotoxicity for 250 mg/ml as 14.25%, 500 mg/ml as 28.5% and 750 mg/ml as 45.5% and 1000 mg/ml as 53.1%. These inhibition values are compared with standard drug of Ascorbic acid for 250 mg/ml as 23.63%, 500 mg/ml as 29.00% and 750 mg/ml as 45.25% and 1000 mg/ml as 52.05%. The DPPH assay was carried out at different concentrations of ethyl acetate fractions of *Moringa oleifera* flowers and ethyl acetate fractions of *Moringa oleifera* leaf extracts. DPPH assay did not show any significant difference at 250 mg/ml Concentrations in *Moringa Oleifera*, however, it was significant for 500 mg/ml, 750 mg/ml and 1000 mg/ml for the ethyl acetate fractions of *Moringa Oleifera*, all the values are compared with standard drug of Ascorbic acid. Hence, this assay provided information on the reactivity of test samples with a stable free radical. As a part of the investigation on the mechanism of the anti-oxidant activity, ability of extract to inhibit DPPH scavenging assay was studied. The in-vitro study of anti-oxidant activity indicates that the inhibition percentage of DPPH scavenging assay by *Moringa Oleifera. Moringa oleifera* flower extracts activity is higher than of *Moringa oleifera* leaf extracts.

Anti- Inflammatory Activity
Inhibition of Albumen Denaturation Method

There are certain problems in using animals in experimental pharmacological research, such as ethical issues and the lack of rationale for their use when other suitable methods are available. Hence, in the present study, the protein denaturation bioassay was selected for in vitro assessment of the anti-inflammatory property of albumin denaturation activity of the compound isolated from ethyl acetate fractions Moringa *Oleifera*. The Albumen Denaturation is a well-documented cause of inflammation. Most biological proteins lose their biological functions when denatured. Production of autoantigen in certain arthritic disease is due to denaturation of protein. The mechanism of denaturation involves an alteration in electrostatic hydrogen, hydrophobic, and disulfide bonding. In the present study, denaturation of proteins is the main cause of inflammation. As part of the investigation on the mechanism of the anti-inflammatory activity, ability of the extract to inhibit protein denaturation was studied. Selected extracts were effective in inhibiting heat-induced albumin denaturation. Aspirin was used as a standard anti-inflammation drug as shown in Figure [Table 2 and Figure 3]. The albumin denaturation method was carried out for *Moringa oleifera* extracts of flower and leaf at different concentrations such as 100µg/ml 200µg/ml, 300µg/ml, 400µg/ml and 500 µg/ml. For the ethyl acetate fractions of *Moringa oleifera* flowers, the result shows the percentage of cytotoxicity for 100 mg/ml as 44%, 200 mg/ml as 54%, 300 mg/ml as 57.3%, 400 mg/ml as 62.3% and 500 mg/ml as 70.3%. For the ethyl acetate fractions of *Moringa oleifera* leaf, the result shows the percentage of cytotoxicity for 100 mg/ml as 49%, 200 mg/ml as 52.02%, 300 mg/ml as 66%, 400 mg/ml as 67% and 500 mg/ml as 77%. These inhibition

values are compared with standard drug of Aspirin for 100 mg/ml as 45%, 200 mg/ml as 56.25%, 300 mg/ml as 66.20%, 400 mg/ml as 72.02% and 500 mg/ml as 82%. Albumen denaturation show significant change when the concentrations are 100mg/ml, 200 mg/ml, 300 mg/ml,400 mg/ml and 500 mg/ml for the compound isolated from ethyl acetate fractions of *Moringa oleifera* and all the values are compared with standard drug of Aspirin. As a part of the investigation on the mechanism of the anti-oxidant activity, ability of extract to inhibit Inhibition of Albumen denaturation was studied. The in-vitro study of Anti- inflammatory activity indicates that the inhibition percentage of Albumen denaturation by *Moringa oleifera*. Extracts of *Moringa oleifera* leaf extracts activity is higher than of *Moringa oleifera* flower extracts.

Anti Diabetic Activity
Inhibition of Alpha-Amylase Enzyme
Diabetes mellitus is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period. A therapeutic approach to decrease the hyperglycaemia is to inhibit the carbohydrate digesting enzymes (α-glucosidase and α-amylase), thereby preventing the breakdown of carbohydrates into monosaccharides which is a main cause of increasing blood glucose level. Therefore, developing compounds having inhibitory activities towards carbohydrate hydrolysing enzymes may be a useful way to manage diabetes. As shown in Figure 4 and Table 3, α-amylase and α-glucosidase were significantly inhibited in a dose-dependent manner by the compound isolated from ethyl acetate fractions Moringa *Oleifera*. The results suggest that with the increased samples concentration, the activity levels of enzyme were remarkably reduced, Hence, the biomolecules likely enhanced the antidiabetic potential of the synthesized NPs. α-Amylase inhibitory actions were observed in increasing order, as Acarbose (Figure 4). Comparable results were observed. However, the foregoing results suggest that the synthesized compounds have high potential antidiabetic property and could prove its effectiveness in the diabetes care. For the ethyl acetate fractions of *Moringa oleifera* flowers, the result shows the percentage of cytotoxicity for 0.05 mg/ml as 58.03%, 0.1 mg/ml as 62.06% , 0.15 mg/ml as 63.02%, 0.2 mg/ml as 64% and 0.25 mg/ml as 68.3%. For the ethyl acetate fractions of *Moringa oleifera* leaf, the result shows the percentage of cytotoxicity for 0.05 mg/ml as 32.04%, 0.1 mg/ml as 40.08% , 0.15 mg/ml as 46.04%, 0.2 mg/ml as 56.03% and 0.25 mg/ml as 58.02%. These inhibition values are compared with standard drug of Acarbose for 0.05 mg/ml as 35%, 0.1 mg/ml as 42%, 0.15 mg/ml as 56%, 0.2 mg/ml as 61% and 0.25 mg/ml as 79%. Albumen Denaturation shows significant difference, when the concentration of phyto compounds from ethyl acetate fractionate compound are increased when the concentration increased. All the values are compared with standard drug of Acarbose (Figure 5). On comparing, it was observed that when the concentration of the sample increases the inhibition also increases showing a good sign of Anti-diabetic activity. As a part of the investigation on the mechanism of the Anti diabetic activity, ability of extract to inhibit Inhibition of Alpha-Amylase Enzyme was studied. The in-vitro study of Anti diabetic activity indicates that the inhibition percentage of Alpha-Amylase Enzyme by *Moringa oleifera*. *Moringa oleifera* flower extracts activity is higher than of *Moringa oleifera* leaf extracts

CONCLUSION

The biological methods incorporate other plant or microbial mediated methods that are cheap and easily accessible in daily life. Scientists have shifted their interest from chemical or physical methods to biological methods as it does not involve a combination of abusive or toxic chemicals to human health or any involvement of immense machines or equipment. The medicinal plant *Moringa oleifera* has been used as a traditional medicinal plant due to the presence of phytochemicals in it. The various applications of the extract have already been established till date. Now, in this study, the flower and leaf from ethyl acetate fractionate compound have been used for the biogenesis of the anti-oxidant, anti- inflammatory and anti-diabetic activity. The DPPH assay is the most acceptable, fastest and simplest method for the calculation of the free radical scavenging activity. As shown in the Table 1 and Figure 2. The ethyl acetate fraction of flower and ethyl acetate fraction of leaf shows better antioxidant property when compared with the standard ascorbic acid values. Denaturation of proteins is a well-documented cause of inflammation.

Phenylbutazones, salicylic acid, flufenamic acid (anti-inflammatory drugs), have shown dose dependent ability to thermally induced protein denaturation. As a part of the investigation on the mechanism of the anti-inflammatory activity, ability of extract to inhibit protein denaturation was studied. The in-vitro study of a anti-inflammatory activity indicates that the inhibition percentage of albumin denaturation by *Moringa oleifera* from ethyl acetate fractionate compound. It is inferred that the Anti- inflammatory activity of ethyl acetate fraction of leaf synthesized from *Moringa oleifera* indicates a good and higher inhibition percentage than ethyl acetate fractionate compound of flower from *Moringa oleifera* as presented in Table 2 and Figure 3. α- amylase is a key enzyme in carbohydrate metabolism. Inhibition of α-amylase is one of the strategies for treating diabetes. Amylase inhibitors are also known as starch blockers because they contain substances that prevent dietary starches from being absorbed by the body. Amylase inhibitor with starchy meal will reduce the usual rise in blood sugar levels. The result suggests that ethyl acetate fractionate compound of flower *exhibits* well α- amylase inhibition under *in vitro* conditions (Tables 3 and Fig 4).

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Table 1: DPPH assay activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera*

Samples	Concentration of the sample (mg/ml)			
	250	**500**	**750**	**1000**
% of inhibition of the Flowers of *Moringa Oleifera*	13.09	36	47.5	51.02
% of inhibition of the Leaf of *Moringa Oleifera*	14.25	28.5	45.5	53.1
Ascorbic acid (Standard)	23.63	29	46.25	52.05

Table 2: The inhibition of albumin denaturation activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera*.

Samples	Concentration of the sample (mg/ml)				
	100	**200**	**300**	**400**	**500**
% of inhibition of the Flowers of *Moringa Oleifera*	44	54	57.3	62.3	70.3
% of inhibition of the Leaf of *Moringa Oleifera*	49	52.02	66	67	77
Aspirin (Standard)	45	56.25	66.2	72.02	82

Table 3: The inhibition of Alpha-Amylase Enzyme activity of the compound isolated from ethyl acetate fractions of *Moringa Oleifera*.

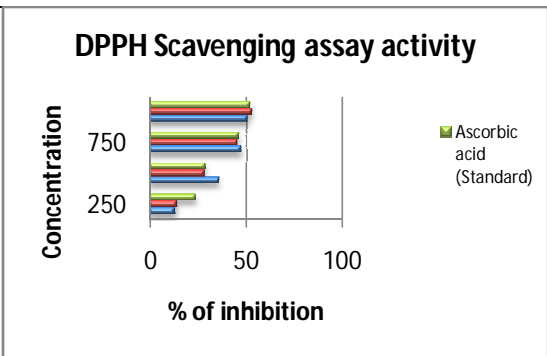| Samples	Concentration of the sample (mg/ml)				
	0.05	**0.1**	**0.15**	**0.2**	**0.25**
% of inhibition of the Flowers of *Moringa Oleifera*	58.03	62.06	63.02	64	68.3
% of inhibition of the Leaf of *Moringa Oleifera*	32.04	40.08	46.04	56.03	58.02
Acarbose (Standard)	35	42	56	61	79

Leaf **Flowers**
Fig.1. *Moringa oleifera*

Fig. 2: Graphical representation of DPPH assay activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera*.
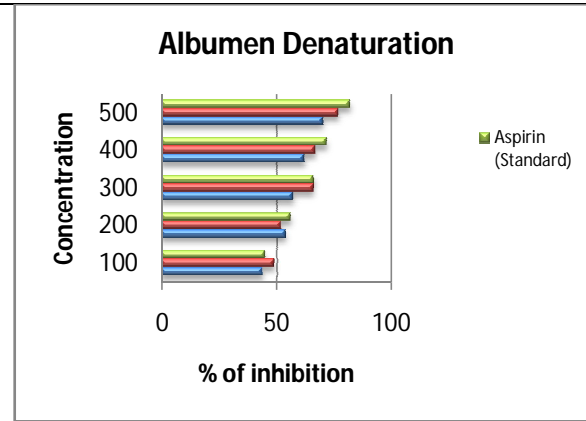
Fig.3: Graphical representation of inhibition of albumin denaturation activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera*.
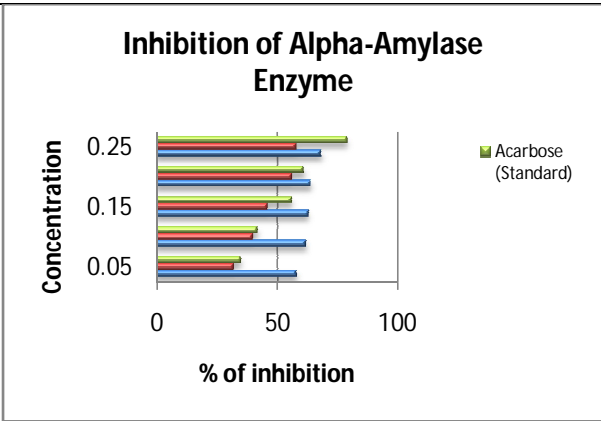
Fig.4: Graphical representation of inhibition of Alpha-Amylase Enzyme activity of the compound isolated from ethyl acetate fractions of *Moringa oleifera*.

RESEARCH ARTICLE

Preliminary Phytochemical Studies on *Solanum surratense* (Solanaceae) Stems Extract

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ABSTRACT

The present study was carried out to characterize various bioactive phytoconstituents in the methanol extract of stem extract of *Solanum surratense* (Solanaceae) using preliminary screening and antioxidant using stem extract. Examinations of restorative plants brought about the revelation of an enormous number of bioactive mixes with incredible helpful properties. *Solanum surattense*, a lasting wild-developing restorative spice, is broadly utilized in conventional medication. Comprehensive writing accessibility uncovers the presence of phytochemical mixes from various plant parts like roots, stem, leaves, natural products, and seeds answered to have a wide scope of pharmacological exercises like hepatoprotective, cardioprotective, antiasthmatic and mosquito anti-agents properties. Serious examination on phytochemical constituents brought about the confinement of alkaloid and steroidal mixes solasonoine, solamargine, campesterol, and diosgenin. Assessment of the restorative movement of confined mixes demonstrated as powerful ones concerning the norm. Current writing on the pharmacological action of *S. surattense* affirms the logical approval of legends cases and its customary use to cure various ailments.

Keywords: *Solanum surratense,* Phytochemical, Bioactive, Traditional, Therapeutic.

INTRODUCTION

Bioactive compound from plant sources have consistently been of incredible importance to create novel remedial medications. In latest things expanded consideration toward the utilization of natural medications has been noticed all through the world. India has knowledgeable information on conventional medication and its training since the old past. *Solanum surattense* (Solanaceae) is an enduring wild-developing restorative spice utilized as a customary

and fables medication utilizes current days. Antiquated Indian content *Materia medica* referenced the utilization of *S. surattense* leaves to fix a wide scope of afflictions. *Solanum surattense* normally called as Indians nightshade (English), Nelamulaka (Telugu), and Kantakari (Sanskrit). It is circulated in Ceylon, India, Polynesia, Australia, Malaysia, Malaysia, China and naturalized in South and Southeast Asia (Parmar *et al.*, 2010; 2017; Nasir, 1985). The products of *Solanum torvum* Sw. have antimicrobial (Chah *et al.*, 2000), antiviral (Arthan *et al.*, 2002), cytotoxic (Balachandran and Sivaramkrishnan, 1995), diuretic and stomach related action (Ghani, 2003). Restorative plants are critical to the soundness of people and networks by and large. The therapeutic estimation of plants lies in numerous compound substances that produce an unequivocal physiological activity on the human body. The most significant of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic mixes. Large numbers of the native restorative plants are utilized as flavors and food plants (Amin *et al.*, 2013). *S. surattense* root contains solanin, solanidine waxy substances and unsaturated fats. It likewise contains alkaloids, tannins, sugars, starch, fats, oils, proteins adhesive, lignins, and calcium oxalate. An organic product contains diosgenin, solasonine, solamargin, β-solamargin and solasodine.

Plants incorporate a huge swath of auxiliary metabolites that are significant for human existence. For remedial purposes, phytochemical examination of plants is an intriguing region of exploration, prompting the confinement of a few new mixes. Accordingly, as of late examination is more situated towards society medication, looking for new leads for the improvement of better medications against irresistible illnesses (Benkeblia, 2004) and other basic afflictions. Essentially, in the logical writing, there are not many reports on assessed pharmacological properties like antidiabetic Hemamalini and Vijusha, 2012, hepatoprotective Hemamalini et al.,2012, gastroprotective Hemamalini *et al.*, 2011, calming Niyogi *et al.*, 2012, Anti-tension, Myorelaxant Deepika *et al.*, 2013 depressants and Antidiarrheal Anurag Bhargav *et al.*, 2012. It has not been broadly utilized in customary medication possibly for its harshness which may go about as a cytotoxic specialist. In this way for *Solanum pubescens* has not been investigated for a careful quantitative and subjective phytochemical examination. There are no reports on its total phytochemical substance aside from a couple demonstrating the presence of flavonoids and alkaloids in leaf remove Krishna Kumari et al., 1985; Krishna Kumari et al., 1986. Henceforth, it is basic to take up an exhaustive phytochemical investigation to comprehend the significant gatherings of phytoconstituents present in this plant. A few plants having a place with the family solanaceae displayed solid cytotoxic and antitumor properties. *Solanum trilobatum*, *Solanum incanum*, *Solanum capsicastrum*, *Solanum indicum*, *Solanum sodomaeumand Solanum nigrum* are not many species answered to have expected antitumor exercises. Reports show that steroidal alkaloids from the Solanum species have solid cytotoxic and antitumor properties Dhar *et al.*, 1973: Vijayan *et al.*, 2004; Badami *et al.*, 2003. Solamargin from *Solanum nigram*, incanumine from *Solanum sodamaeum,* solasoinine from *Solanum crinitum* and a few other steroidal alkaloidal glycosides are known to have these properties Sumalatha *et al.*, 2013. Henceforth, in light of these reports the current examination was embraced to screen the dynamic constituents of *Solanum pubescens.* Keeping this in view, the present study was aimed to systematically screen the phytochemicals of this plant. It is believed that this study may pave the way for further high throughput analysis of the plant in terms of chemical characterization followed by the evaluation of important pharmacological properties. Activities such as antibacterial and antifungal, antinociceptive (Rahman *et al.*, 2003), antioxidant (Siddharthan *et al.*, 2007), hypoglycaemic (Kar *et al.*, 2006) and larvicidal (Sharma and Srivastava, 2007). Some of the common alkaloids are Cocaine, Atropine, Quinine, Vincristine and Nicotine. The bioactive mixes from plant sources have consistently been of extraordinary importance to create novel remedial medications. Lately, expanded consideration toward the utilization of natural medications has been noticed all through the world. India has knowledgeable information on customary medication and its training since the antiquated past. *Solanum surattense* (Solanaceae) is an enduring wild-developing therapeutic spice utilized as a conventional and legends medication. Antiquated Indian content *Materia medica* referenced the utilization of *S. surattense* leaves to fix a wide scope of illnesses. The resurgence in the use of natural medication quickens the examination of pharmacological exercises of plants utilized in conventional medication. *Solanum torvum* Sw. The products of *Solanum torvum* Sw. have antimicrobial (Chah *et al.,* 2000), antiviral (Arthan *et al.,* 2002), cytotoxic (Balachandran and Sivaramkrishnan, 1995), diuretic and stomach related movement (Ghani, 2003). Therapeutic plants are critical to the wellbeing of people and networks as a rule.

The restorative estimation of plants lies in some synthetic substances that produce a distinct physiological activity on the human body. The most significant of these bioactive constituents of plants are alkaloids, tannins, flavonoids and phenolic mixes. A significant number of the native restorative plants are utilized as flavors and food plants (Amin *et al.*, 2013). Under regular conditions alkaloid yield of plants is pitiful, these being available in little amounts, (0.612% to 0.498%). *S. surattense* root contains solanin, solanidine waxy substances and unsaturated fats. It additionally contains alkaloids, tannins, sugars, starch, fats, oils, proteins adhesive, lignins, and calcium oxalate. A natural product contains diosgenin*,* solasonine*,* solamargin*,* β-solamargin and solasodine, Petals contains apigenin and stamens contain quercetin, diglycoside and sitosterol. Plants integrate a huge range of auxiliary metabolites that are significant for human existence. For restorative purposes, the phytochemical examination of plants is an intriguing zone of exploration, prompting the detachment of a few new mixes. Accordingly, as of late examination is more situated towards society medication, looking for new leads for the advancement of better medications against irresistible infections (Benkeblia, 2004) and other regular afflictions. Essentially, in logical writing, there are not many reports on assessed pharmacological properties. It has not been broadly utilized in customary medication perhaps for its harshness which may go about as a cytotoxic specialist. In this manner, *Solanum pubescens* has not been investigated exhaustive quantitative and subjective phytochemical examination. There are no reports on its total phytochemical substance aside from a couple showing the presence of flavonoids and alkaloids in leaf remove Krishna Kumari *et al.*, 1985; Krishna Kumari *et al.*, 1986; Consequently, it is basic to take up a careful phytochemical investigation to comprehend the significant gatherings of phytoconstituents present in this plant. A few plants having a place with the family solanaceae showed solid cytotoxic and antitumor properties. *Solanum trilobatum, Solanum incanum, Solanum capsicastrum, Solanum indicum, Solanum sodomaeumand, Solanum nigrum* are not many species answered to have likely antitumor exercises. Reports demonstrate that steroidal alkaloids from the Solanum species have solid cytotoxic and antitumor properties Dhar *et al.*, 1973: Vijayan *et al.*, 2004; Badami *et al.*, 2003. Solamargin from *Solanum nigram*, incanumine from *Solanum sodamaeum*, solasoinine from *Solanum crinitum* and a few other steroidal alkaloidal glycosides are known to have these properties Sumalatha *et al.*, 2013. Thus, in view of these reports, the current investigation was attempted to screen the dynamic constituents of *Solanum pubescens.* Keeping this in view, the current investigation was intended to efficiently screen the phytochemicals of this plant. It is accepted that this examination may prepare for additional high throughput investigation of the plant regarding substance portrayal followed by the assessment of significant pharmacological properties. Exercises, for example, antibacterial and antifungal, antinociceptive (Rahman *et al.*, 2003), cell reinforcement (Siddharthan *et al.,* 2007), hypoglycaemic (Kar *et al.,* 2006) and larvicidal (Sharma and Srivastava, 2007). A portion of the regular alkaloids is Cocaine, Atropine, Quinine, Vinaistine, and Nicotine.

MATERIALS AND METHOD

Plant Collection and extract preparation
The plant *Solanum surratense* were collected from the Kalvarayan hills. The confirmation regarding the identification of plants was once again checked by local traditional siddha practitioners and online mobile application Google lens. The stem of this plant have full of throne here and there, therefore, too much of care was taken during collection of stem from plants. These stem were washed with distilled water to eliminate the adhering dust particles. They were dried in the shaded place. The dried leaf were powdered, weighed and stored in clean containers (Tamizhazhagan, *et al.,* 2017; Tamizhazhagan and Pugazhendy 2017).

Soxhlet extraction
5g of dried plant stem powder was extracted for 4-5 hrs of (150ml) polar, nonpolar and dipolar solvent (ethanol, methanol, acetone, ether, chloroform etc.) By hot continuous per location method in a Soxhlet apparatus. After the effective extraction, the solvent was concentrated using a rotary flash evaporator and water was removed by evaporated to dryness on a hot water bath to yield a soxhlet crude extract.

Photochemical analysis

The phytochemical analysis of the plant was carried out by standard methods provided by (Odebiyi and Ramstard, 1978; Waterman 1993 and Tamizhazhagan and Pugazhendy 2017).

Antioxidant assay

The Antioxidant assay of the plant stem extricates was tried utilizing two techniques: ferric thiocyanate (FTC) and thiobarbituric corrosive (TBA) strategies. The FTC strategy was utilized to gauge the measure of peroxide toward the start of the lipid peroxidation, in which peroxide responds with ferrous chloride and structure ferric particle. The ferric particle at that point consolidates with ammonium thiocyanate and produce ferric thiocyanate. The substance is red in shading. The thicker the tone was the higher the absorbance. Though the TBA techniques measure free revolutionaries present after peroxide oxidation.

DPPH radical scavenging activity

The free radical scavenging activity action by various plant removes was finished by the strategy announced by (Gyamfi et al., 2002) 3cv. Fifty microliters of the plant stem removes in methanol, yielding $100\mu g/ml$ individually in every response was blended in with 1ml of 0.1mM DPPH in methanol arrangement and $450\ \mu l$ of 50 mM Tris HCl cushion (pH 7.4). Methanol ($50\mu l$) just was utilized as control of examination. After 30 min of brooding at room temperature, the decrease of the DPPH free extremist was estimated perusing the absorbance at 517 nm. L-Ascorbic corrosive and BHT are utilized as controls. The percent restraint was determined from the accompanying condition:
% Inhibition = [Absorbance of control – Absorbance of test/Absorbance of control] 100.

In vitro nitric oxide radical (NO) Scavenging assay

Nitric Oxide created from sodium nitroprusside (SNP) was estimated by the strategy for (Marcocci *et al.*, 1994). Momentarily, the response combination (5.0 ml) containing SNP (5 mM) in phosphate buffered saline (pH 7.3), with or without the plant stem separates at various fixations, was brooded at 25°C for 180 min before an obvious polychromatic light source (25W tungsten light). The nitric oxide revolutionary in this manner created associated with oxygen to deliver the nitrite particle (Nitric Oxide) which was measured at 30 min stretches by blending 1.0 ml of the hatching combination with an equivalent measure of Griess reagent (1% sulfanilamide in 5% phosphoric corrosive and 0.1% naphthylethylenediamine dihydrochloride. The absorbance of the chromophore (purple azo color) framed during the diazotisation of nitrite particles with sulphanilamide and resulting coupling with naphthylethylenediamine dihydrochloride was estimated at 546 nm. The nitrite produced in the presence or nonappearance of the plant stem removes was assessed utilizing a standard bend dependent on sodium nitrite arrangements of known focuses. Each test was done at any rate multiple times and the information introduced as a normal of three free judgments.

RESULTS AND DISCUSSION

The phytochemical assessment is the best technique for subjective examination of auxiliary metabolites present in the *Solanum surratense* (Solanaceae). The optional metabolites different dissolvable frameworks are utilizing essential screening strategies different dissolvable to be specific polar, nonpolar and dipolar were utilized for primer screening (Table 1). The auxiliary metabolites structure terpenoids follow sum present in the metabolic concentrate and furthermore phenol compound all dissolvable present in the plant extricates it might direct the fashioning substance. Alkaloid present in the ethyl acetic acid derivation extricates, flavonoid are available in the metabolic concentrate and ethyl acetic acid derivation solvents and furthermore demonstrate benzene dissolvable presence in a more modest sums. The recognizing of auxiliary metabolites has different assessments much capability of methanolic separates contrasted with others. Henceforth this endeavor has plainly checked different test methanolic removes great dynamic compound them. The all out cancer prevention agent level was exceptionally introduced in the plant's concentrates contrasted and other standard arrangements divisions were dissected three have great movement

against standard BHT (Figure 1). The DPPH free extreme compound has been broadly used to test the free revolutionary rummaging capacity of different food tests; the cell reinforcement present kills the DPPH by the exchange of an electron or hydrogen particle. Berries of *Solanum surattense* contain high centralization of an alkaloid solosodine, which is indicated the variety in its substance in the scope of 1.1%–4.6% (Singh and Singh, 2010). Berries gathered in fall yielded the alkaloids solasonine and solamorgine. Nonetheless, the organic products gathered in summer yielded another alkaloid solasonine (Parmar *et al.*, 2010). Numerous investigations have been done with the point of featuring the limits of plant concentrates to forestall the development of bacterial life forms. Cancer prevention agents are fit for harming the responsive oxygen species (ROS) that cause oxidative harm. Free extremists respond with the biomolecules like DNA, proteins lipids and produce harmful impacts. Up until this point, countless plants announced having hostile to oxidant potential because of rich phytochemical constituents like phenols and Flavonoids. Natural product extricates likewise detailed having an obvious measure of revolutionary searching movement (about 80%) at the most minimal test concentration (250 μg/ml). The decrease limit of DPPH could be controlled by shading changes from purple to yellow by perusing at 517 nm (Figure 1). At first, the *S. surattense* organic products were explored by the Saiyed and Kanga (1936), and they were utilized for the segregation of bioactive mixes, for example, glycoalkaloid (solanosine), steroidal compound (carpesterol) and steroidal alkoloids (caffeic corrosive, coumarins, and triterpinoids).

The products of *S. surattense* have steroidal alkaloids like solanocarpine, solamorgine, and solanocarpidine (Jayakumar and Murugan, 2016). The other powerful bioactive mixes which incorporate polyphenol (caffeic corrosive), coumarins (esculentin and aesculin), steroids (carpesterol, campesterol, daucosterol, stigmasterol, cycloortanol and cholesterol), triterpinins and sapogenin (lupeol and diosgenine) have additionally been accounted for (Khanam and Sultana, 2012; Singh and Singh, 2010).

The methanolic concentrate of *Solanum surratense* showed H-giver action in our examination. The DPPH revolutionary rummaging movement of extricated material was distinguished and contrasted and standard cell reinforcement - nutrient C. The concentrate of *Solanum surratense* tried against DPPH stable extremists spectrophotometrically which uncovers that the revolutionary rummaging action of *Solanum surratense* methanol separate had magnificent cell reinforcement limit by expanded with the expanding grouping of the concentrate. At a convergence of 100 μg/ml of methanol remove, the level of hindrance was discovered to be 78%. Nonetheless, the searching action of ascorbic corrosive at a similar focus was 85.02%. The methanol concentrate of *Solanum surratense* was found at a convergence of 50 μg/ml. The methanolic concentrate of *Solanum surratense* viably decreased the age of nitric oxide from sodium nitroprusside.

Solanum surratense methanol remove indicated nitric oxide rummaging action at the convergence of 10 μg/ml while the standard nutrient C was demonstrated 50 μg/ml. Rummaging of Nitric Oxide extremist depends on the age of nitric oxide from sodium nitroprusside in cushioned saline, which responds with oxygen to deliver nitrite particles that can be estimated by utilizing Griess reagent. The absorbance of the chromophore was estimated at 546 nm within the sight of the concentrate. *Solanum surratense* extricate demonstrated to diminish in the measure of nitrite created from the decay of sodium nitroprusside in vitro. Methanol remove recorded a most extreme level of NO action of 84.11% at the grouping of 50 μg/ml. Poongothai *et al.* (2014) detailed that *S. surattense* leaf separates upgraded the degree of hostile to oxidant chemicals catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH) peroxidase in alloxan-prompted creature models. Plant removes expanded the counter oxidants to a typical level and the productivity was like standard medication glibenclamide. The astounding enemy of oxidant probability of *S. surattense* leaf concentrates may be credited to the presence of phenolic and flavonoids mixes in high amount (Poongothai *et al.*, 2014). Every one of these discoveries affirm *S. surattense* to be an ideal decision to seek after the further investigation in building up the successful normal enemies of oxidants. Compare to other plant solvents and other plant parts stem have quality bioactive and antioxidant present and to applicable medicinal purpose.

CONCLUSION

From all these logical information, it can uncover that the preliminary screening chosen methanol separate stem of *Solanum surratence*. So we can presume that as numerous restorative properties of the stem of *S. surratense* may gives therapeutic qualities in light of the fact that, from the consequences of the phytochemical examination, apparently the stem contains numerous naturally significant phytochemical mixes, and furthermore our outcomes firmly alluded plant has great cancer prevention agent movement. Notwithstanding, further pharmacological investigations would be followed for the viability and individual movement of segregated phytochemical mixes.

ACKNOWLEDGMENT

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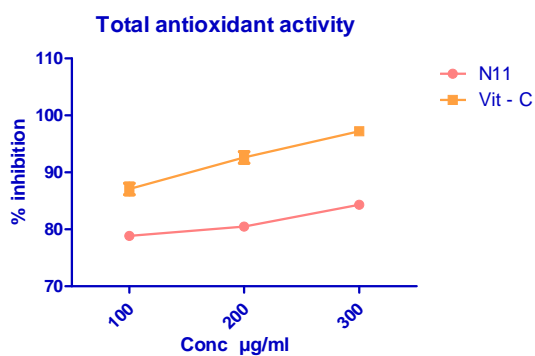36. Sheeba E. Antibacterial activity of *Solanum surattense* burm. f. Kathmandu Univ J Sci Eng Technol, 2010; 6:1–4.

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Table 1 Preliminary phytochemical screening

Phytoconstituents	Aqueous extract	Methanol extract	Pet .ether extract
Phenol	+	+	-
Flavanoid	+	+	+
Saponin	+	+	-
Steroid	-	+	+
Alkaloid	-	-	-
Tannin	-	-	-
Protein	-	+	-
Glycosides	-	-	-
Carbohydrates	+	+	-

Figure 1: Antioxidant activity of isolate

RESEARCH ARTICLE

Habits of News Reading on Mobile Apps among the Younger Generation: A Case Study

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ABSTRACT

In 1970 Tele text first digital journalism was invented in United Kingdom. In the year 2008 'Application software' we popularly knew as APPS. This application had exploded the demand for smart phone, tabs, laptop, computer and other electronics devices. Digital (online) journalism has become easily accessible as compared to print and broadcast journalism. The technology savvy younger generation primarily rely on online news websites like Dailyhunt, Feedly, Times of India, Indian Express, AajTak, Hindustan and others for news as well as information. The study is conducted under Ex-Post-Facto conditions among the students of Journalism and Mass Communication. The questionnaire was administered through a Google form and distributed among all 296 students. A total of 194 respondents were responded to the study appropriately and absolutely. Among the respondent's majority of 51 per cent are female and 49 per cent of the respondents are male. The exhibits that most of the participates are young students below the age of twenty, 21 per cent of students belong to between 20 to 25 age group mostly belongs to first year students. 34.08% of the respondents are III Semester students. 32.00% of the respondents are IV Semester students. About 46% female respondents online as source of news as compare to 37% male respondents. To a question on news apps language 50% female are in favour of English and 5% to Hindi. Among male 35% prefer English and 10% Hindi. 48% of the female respondents like to read news on mobile and 36% male opt mobile apps to read news as compare to laptop or desktop. The respondents said mobile news apps are 'easy to access' and constantly updated, save both time and money, with variety of choice for them to read, like or dislike on the subjects. The study concluded that the use of mobile news

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application has easy to access, informed and updated on their area of interest, the younger generation suppose that it saves their time and money. As well News app technology is user friendly for knowing the information in this fast-paced scenario.

Keywords: Media, Online, News, Mobile Apps, Technology, Information.

INTRODUCTION

The use of News applications is increasing day by day and has become an important phenomenon in the field media. However, it is also evident that with the boom of technology and cut throw competition among media industries, the use of online news applications is also on the rise in India especially among the youth. It is true that there is sharp evidence of decline in there a dership of newspapers in western society but the future of print media in developing countries like India doesn't seem that worse. The technology savvy younger generation primarily rely on online news websites like Dailyhunt, Feedly, Times of India, Indian Express, AajTak, Hindustan etc. for information or entertainment purposes. In the summer of 1983, Steve Jobs, in a conference in Aspen launched the first Macintosh. Twenty- four (24) years before the first **i**Phone and twenty-seven (27) years before the first **i**Pad. That day in Aspen, Steve Jobs forecast the evolution of a new digital distribution system. In the modern day, the reading of printed newspapers is fading away among the youth around the globe. Digital (online) journalism has become easily accessible as compared to print and broadcast journalism. In 1970 Tele text first digital journalism was invented in United Kingdom. In the year 2008 'Application software' we popularly knew as APPS. This application had exploded the demand for smartphone, tabs, laptop, computer and other electronics devices., Apps emerged from early PDAs (Personal Digital Assistant), through the addictively simple game Snake on the Nokia 6110 phone'*1]. In the month of July 2008 in, the Apple App Store when it made its debut 500 apps was with Apple. High speed data and faster mobile data services such as 3G and 4G give a strong online attendance among audience `[2].News apps benefits is to narrow down the news story from a large number of words to just 60 words Today almost everything ranging from news to basic necessity of daily life came on some or other apps. The sudden increase in quantity and variation of news apps, owing to the consumer necessities, takes cemented the way in the formation of an extensive choice of review, recommendation, blogs, etc. [3]. Daily hunt, Feedly, Times of India, Indian Express, AajTak, Hindustan (News applications) have revolutionized the way we expressed at the print media industry. News apps has further added new component to it by increasing its prospect from different length and breadth thus generating various prospects to a slightly unknown but possible space mostly associated to the print media production [4].

REVIEW OF LITERATURE

In the paper, ,online journalism in India: An exploratory study of Indian Newspaper on the net. ` Kiran Thakur finds in the paper, ,that online journalism changes the reading habits of our second generation. In the paper, he concluded that with the development of digital journalism and growth of smart phone user among the youth in India, change In today's world of technology mass depends on the formula of media power assumes that the audience connection is measuring is always posed by an alternative agenda community. The formula contrasts variance accounted for versus the correlations alone. Of course, there are many variables other than media that attract audiences. Media audience correlations of 1.00 would indicate that the media community is dominant, as there is no competition from the alternative community. The formula suggests that correlation of +.50 results in a complete balance of media agenda community and audiences much like the balancing of the earth and moon. By this reasoning agenda setting correlations would need to reach, as suggested, about +.75 to indicate that the power of the media agenda community is more attractive than the alternative community (other than media). The conventional print media has been

direction to a completely novel area of digitalization for its distribution structure since numerous challenges and competitions, the first part being the, News apps online journalism by the use of different Websites.

Objectives
1. To understand the readership of News-on-News Apps.
2. To evaluate the readership preferences of News, on the Mobile News Apps.
3. To analyse the 'ease to use 'technology of News Apps on Smartphone.

RESEARCH METHODOLOGY

The study is being conducted under Ex-Post-Facto conditions in the department of Journalism and Mass Communication, Manipal University Jaipur, Rajasthan, India. Keeping the study objectives in view an interview schedule was developed for data collection. The questionnaire was administered through a Google form was distributed among all 296 students. A random sample from the target audience group was selected for the study. A total of 194 respondents were responded to the survey correctly and completely.

Analysis and Interpretation
In this study with the help of primary data collected with the help of google form and analysis with the help of IBM SPSS software cross tabulation. The exhibits that most of the participates are young students below the age of twenty, 21 percent of students belong to between 20 to 25 age group mostly belongs to first year students. (34.08%) of the respondents are from III Semester. (32.00%) of the respondents are from IV Semester. Rests (6.00%) of the respondents are from II Semester Masters. On the other hand, a majority (30.0%) of the respondents are female, (25.0%) of the respondents are male belong to the first year. Other major participations of the respondents (22.0%) are female, (18.0%) of the respondents are male from the second year. Other respondents (5.02%) are postgraduates. In the study 194 respondents participate actively and completed survey absolutely and appropriately.

In a question, what are the sources of your news?
Out of fifty-one percent female students responded they read news on mobile apps, (17%), online (29.4%), offline (3.6%), (1.0%) others.
Out of forty-seven percent male students responded they read news on news apps (6.7%), online (30.4%), offline (6.7%), (2.6%) others.

In the next segment of question, which news apps you prefer to read?
Fifty-one percent female students responded they prefer (49.5%) English news apps, (1.5%), Hindi news apps, and (0.0%) others.
Forty-seven percent male students responded they prefer (35.1%) English news apps, (10.8%) Hindi news apps, and (0.5%) others.

In another question, where do you read news?
Fifty-one percent female students responded to this question, they used desktop (0.5%), laptop (0.5%), mobile (48.5%) and others (1.5%).
Forty-seven percent male students responded to this question, they used desktop (1%), laptop (3.1%), mobile (36.1%) and others (6.2%).

In another question, how much time do you spend on reading news on news Apps?
Out of (99) ninety-nine female respondents (80) eighty female students responded they spend less than (30) thirty minutes in reading news on news apps daily. (10) Ten respondents less than thirty minutes, (7) seven respondents more than one hour, and (2) two respondents less than hours.

Out of (90) ninety male respondents (63) sixty-three male students responded they spend less than (30) thirty minutes in reading news on news apps daily. (17) Seventeen respondents less than thirty minutes, (6) six respondents more than one hour, and (3) three respondents less than hour.

In the next segment of question, which online News Apps do you prefer to read?
Out of 194 respondents fifty-nine (59) female and forty-seven (47) male respondents says news apps of Times of India. Eight (8) female and ten (10) male responded to news apps of Indian Express. Five (5) each female and male responded for news apps of NDTV. Four (4) male responded in the favour of Daily hunt. Six (6) female responded in favour of Hindustan news apps. Four (4) male and two (2) female responded to AajTak news apps. Eighteen (18) female and (10) male responded for others news apps also.

In another question, do you think mobile News apps are easy to use?
In the responded of this question ninety-six (96%) respondents in favour of 'yes' News apps are ease to use on mobile online reading news articles.

In this question, why do you access News Apps?
Answer to this question most of the respondents made that news apps is 'easy to access' and news items on news apps constantly updated. Some of the respondent's mark news apps helps in both time and money save, with variety of choice for them to read, like or dislike and importantly their comments on the subjects.

CONCLUSION

The study reveals that use of mobile apps for reading news and results in support of upcoming youth journalist of our nation. The study finds that younger generation read most news on their Smartphone online. It's become the habit of using apps in smartphones among the journalist students for one or other information and also increasing the demand of Smartphones among the youth. They learn the tips of apps for their daily requirement for which they depend on their smartphones. The Study also reveals use of Smartphone easy to access, informed and constantly updated on the news apps, it saves their time and money and also, they understand the use of technology. News 8 apps technology is friendly in using and knowing information in their field of interest. News applications are rise steeply and the merely cheerful spot to the youth. Due to rising demand of Smartphones among the youth even News Agencies also modifying news gratified to Smartphone applications. Smartphone has delivered distinct knowledge with rich variances in the way public read and access news. The journey starts from the printing press than to news websites and presently the revolutionary news on Smart mobile applications (APPS).

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		Frequency	Percent	Valid Percent	Cumulative Percent
		6	3.1	3.1	3.1
	18 – 20	145	74.7	74.7	77.8
Valid	20 -25	42	21.6	21.6	99.5
	25 and above	1	.5	.5	100.0
	Total	194	100.0	100.0	

			What are the sources of your news?					Total
			News apps	Offline	Online	Others	
		Count	4	0	0	1	0	5
		% of Total	2.1%	0.0%	0.0%	0.5%	0.0%	2.6%
Gender	Female	Count	0	33	7	57	2	99
		% of Total	0.0%	17.0%	3.6%	29.4%	1.0%	51.0%
	Male	Count	0	13	13	59	5	90
		% of Total	0.0%	6.7%	6.7%	30.4%	2.6%	46.4%
Total		Count	4	46	20	117	7	194
		% of Total	2.1%	23.7%	10.3%	60.3%	3.6%	100.0%

			Which News Apps you prefer to read?				Total
			English	Hindi	Other		
Gender		Count	4	1	0	0	5
		% of Total	2.1%	0.5%	0.0%	0.0%	2.6%
	Female	Count	0	96	3	0	99
		% of Total	0.0%	49.5%	1.5%	0.0%	51.0%
	Male	Count	0	68	21	1	90
		% of Total	0.0%	35.1%	10.8%	0.5%	46.4%
Total		Count	4	165	24	1	194
		% of Total	2.1%	85.1%	12.4%	0.5%	100.0%

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			Where do you read News?					Total
			Desktop	**Laptop**	**Mobile**	**Others**		
Gender		Count	4	0	0	1	0	5
		% of Total	2.1%	0.0%	0.0%	0.5%	0.0%	2.6%
	Female	Count	0	1	1	94	3	99
		% of Total	0.0%	0.5%	0.5%	48.5%	1.5%	51.0%
	Male	Count	0	2	6	70	12	90
		% of Total	0.0%	1.0%	3.1%	36.1%	6.2%	46.4%
Total		Count	4	3	7	165	15	194
		% of Total	2.1%	1.5%	3.6%	85.1%	7.7%	100.0%

			How much time do you spend on reading news on news Apps?				Total
			Less than 1 hours	**Less than 30 min.**	**More than 1hours**	**More than 30 min.**	
Gender		Count	1	0	2	2	0	5
		% of Total	0.5%	0.0%	1.0%	1.0%	0.0%	2.6%
	Female	Count	0	2	80	7	10	99
		% of Total	0.0%	1.0%	41.2%	3.6%	5.2%	51.0%
	Male	Count	1	3	63	6	17	90
		% of Total	0.5%	1.5%	32.5%	3.1%	8.8%	46.4%
Total		Count	2	5	145	15	27	194
		% of Total	1.0%	2.6%	74.7%	7.7%	13.9%	100.0%

			Which online News Apps do you prefer to read?							Total
			AajTak	**Daily hunt**	**Hindustan**	**Indian Express**	**Ndtv**	**Others**	**Times of India**	
Gender		Count	2	0	0	0	0	0	0	3	5
		%of Total	1.0%	0.0%	0.0%	0.0%	0.0%	0.0%	0.0%	1.5%	2.6%
	Female	Count	1	2	0	6	8	5	18	59	99
		%of Total	0.5%	1.0%	0.0%	3.1%	4.1%	2.6%	9.3%	30.4%	51.0%
	Male	Count	10	4	4	0	10	5	10	47	90
		%of Total	5.2%	2.1%	2.1%	0.0%	5.2%	2.6%	5.2%	24.2%	46.4%
Total		Count	13	6	4	6	18	10	28	109	194
		%of Total	6.7%	3.1%	2.1%	3.1%	9.3%	5.2%	14.4%	56.2%	100.0%

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Gender			Do you think mobile News apps is easy to use?					Total
			Can't say	Do not known	No	Yes		
Gender		Count	1	0	1	0	3	5
		% of Total	0.5%	0.0%	0.5%	0.0%	1.5%	2.6%
	Female	Count	1	4	1	3	90	99
		% of Total	0.5%	2.1%	0.5%	1.5%	46.4%	51.0%
	Male	Count	0	8	2	5	75	90
		% of Total	0.0%	4.1%	1.0%	2.6%	38.7%	46.4%
Total		Count	2	12	4	8	168	194
		% of Total	1.0%	6.2%	2.1%	4.1%	86.6%	100.0%

			Why do you access News Apps?							Total
			Audio and video news	Both money and time save	Easy to access	Like and dislike option	Others	Update regularly	Variety of choice	
Gender		Count	1	1	2	1	0	0	0	0	5
		%of Total	0.5%	0.5%	1.0%	0.5%	0.0%	0.0%	0.0%	0.0%	2.6%
	Female	Count	0	3	9	44	1	1	33	8	99
		%of Total	0.0%	1.5%	4.6%	22.7%	0.5%	0.5%	17.0%	4.1%	51.0%
	Male	Count	2	5	11	33	0	9	26	4	90
		%of Total	1.0%	2.6%	5.7%	17.0%	0.0%	4.6%	13.4%	2.1%	46.4%
Total		Count	3	9	22	78	1	10	59	12	194
		%of Total	1.5%	4.6%	11.3%	40.2%	0.5%	5.2%	30.4%	6.2%	100.0%

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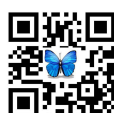
Interest of Alzheimer's Gene ApoE ε4 in the Progression of COVID to Severity: A Genetic Intervention Review

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ABSTRACT

As the nations of the world struggle to keep the newly advent SARS-CoV-2 spread under control, one of the main challenges faced by our health care professional is to prevent the progression of the disease to severity that can culminate in mortality. People with health conditions such as hypertension, diabetes, COPD, asthma, and other comorbidities have already been categorised under the high-risk group and the precautions that were taken has tremendously aided these populations. As such the identification of such potential risk factors that can cause a progression to severe COVID conditions can add to the welfare of that particular population. In a study conducted at the UK Biobank, it was found that individuals processing the ApoE gene variant ε4 were at an increased risk of contracting the SARS-CoV 2 as well as progressing to a more sever condition. The ApoE gene variants had been recognised in the past to play a major role in the metabolism of fats, atherosclerosis, cardiovascular diseases and Alzheimer's disease as well as a favourable factor for the entry of viruses and development of severe viral infections. It has also exhibited a prominent action on immunoregulation of the host that vary in an allele specific manner. The study was conducted in the UK with total participants of 322,984 who possessed the ApoE variants in the following frequency: ε4ε4-3%, ε3ε4-28%, ε3ε3-69% (most common genotype). The increased risk to susceptibility of developing the diseases well as progression to severe illness was found to be true for individuals with ApoE ε4 even without pre-existing dementia as well as other comorbidities.

Keywords: Apo E gene, Alzheimer's gene, COVID-19, susceptibility, severe condition.

INTRODUCTION

The novel corona virus disease, caused due to SARS-CoV-2 was declared as a pandemic on March 11th 2020 by the World Health Organisation as cumulative number of countries yielded to the virus. The absence of a definite therapeutic agent against the virus and increasing mortality and morbidity caused generates a complex situation for our medical community. Some of the risk factors for the progression to a severe disease have been identified and patients with comorbidities such as heart disorders, hypertension, diabetes, immunocompromised patients, cancer

patients, patients with COPD, lung disorders, ischemic heart conditions, pre-existing Alzheimer's, dementia etc have been categorized into the high risk groups and are advised to take extensive precaution. Being a newly advent pathogen researches and studies are being conducted every day and categories of risk factors is on the increase. Since nearly four decades the ApoE gene and its alleles has been extensively studied and was used for explaining the variable susceptibility of the individuals to CVD, AD, atherosclerosis etc on a genetic basis. This year however, the ApoE gene has been implicated in a different context. In a UK Bio Bank study, it was shown that the ApoE gene has shown to increase susceptibility for developing COVID as well as progression to a more sever condition, particularly for older ages in a genotype depended manner (ApoE allele $\varepsilon4\varepsilon4$ greatest risk in comparison against $\varepsilon3\varepsilon3$ allele.)

METHODOLOGY

The literature review for the following article was performed by searching the databases of Science Direct, Pub Med, Elsevier, Google scholar and research paper based journals using the keywords: ApoE gene, Alzheimer's disease, COVID-19. Suitable information was gathered from these sources and was collated for the devising of this article.

ApoE Genotype
Human ApoE gene has been mapped to chromosome 19. Till today 72 single nucleotide polymorphism of the gene has been identified and in this the two SNPs rs429358 and rs7412 determine together the three major alleles, termed $\varepsilon2$, $\varepsilon3$ and $\varepsilon4$. Two minor alleles for the gene is also present but they are only found in 0.1% of the population($\varepsilon1$ and $\varepsilon5$). The three major alleles are responsible for three homozygous ($\varepsilon2/\varepsilon2$, $\varepsilon3/\varepsilon3$, $\varepsilon4/\varepsilon4$) and three heterozygous ($\varepsilon2/\varepsilon3$, $\varepsilon2/\varepsilon4$, $\varepsilon3/\varepsilon4$) genotypes [1]. The $\varepsilon3/\varepsilon3$ is the normal functioning genotype. The product of the gene that is the ApoE protein are also of different isoforms depending on the genotype. The ApoE gene produced protein ApoE is associated with the transport of lipid in the body (LDL). ApoE is found the alveolar macrophages, hepatocytes, brain astrocytes, and arterial wall macrophages that transport the lipids.$\varepsilon2$ variant has been associated with both increased and decreased risk for atherosclerosis, $\varepsilon3$ variant has shown to play a neutral effect and $\varepsilon4$ variant has been implicated in Alzheimer's disease, atherosclerosis, faster disease progression to multiple sclerosis, HIV, ischemic brain injury and recently in a severe case of COVID-19 [2].

Geographic Allele Wise Frequency Distribution of ApoE
There exists a ethnicity wise difference in distribution of the alleles. The $\varepsilon3$ allele is usually the most prevalent, present in 50-90% of individuals, whereas $\varepsilon2$ has the lowest frequency at 0-15%, and is even absent in some native populations. The $\varepsilon4$ allelic variant occurs at a frequency of 5-30% [1]. In Europe $\varepsilon4$ allele has a higher prevalence in the northern countries and decreases toward the southern countries. The distribution of $\varepsilon4$ allele seems to increase with increase in the latitude. Globally, individuals belonging to indigenous populations, and those with a recent history of hunter gather lifestyle has exhibiter a higher frequency of $\varepsilon4$ allele and those belonging to Middle East, southern Europe, southeast Asia and central America show the lowest frequency. African American have a higher frequency of the $\varepsilon4$ allele and 37% of native Africans of south Africa possess the genotype.

ApoE and Viral Infections
The genotype ApoE E4 has been hypothesised as to allow the entry of lipophilic pathogens. In the past too ApoE e4 genotype has been implicated as a favourable factor for viral infections. It has shown to cause a faster disease progression in HIV by enabling an increase in the rate of fusion of the virus with the cell membranes [3]. ApoE4 competes less efficiently than the other isoforms for entry into neuronal cells through heparan sulphate proteoglycans, which are involved in HIV attachment and entry into cells. It has also shown a similar effect with HSV. It increases the latency of the virus and can exhibit increase oxidative damage to the CNS. In the patients carrying ApoE$\varepsilon4$ genotype, HSV infections can increase the risk of developing AD particularly in the elderly. ApoE4 protein can facilitate the entry of HSV into the brain 10 times more efficiently than ApoE3 causing a significant increase in HSV inside the spinal cord and all regions of the brain in transgenic mice as well as cause breakdown of

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the BBB by damaging the pericytes inside the blood vessels. There was a reduced risk for developing infectious HCV after knockdown of ApoE. Similarly there was about 3-5 fold reduction in the development of chronic HCV in individuals possessing ApoEε2 alleles compared to other isoforms of the gene.

Role in Immune Function Modulation

ApoE containing lipoproteins are able to modulate immune responses through inhibition or stimulation of various antigen and mitogen induced T cell activation and proliferation. Its role has been well established in the pathogenesis of multiple sclerosis and psoriasis. ApoE can regulate the function of macrophages, cause T cell proliferation, maintain integrity of BBB and BNB, maintain triglyceride homeostasis, increase NO production in platelets, increase VLDL production and lipoprotein clearance in hepatocytes, facilitate the presentation of lipid antigen by CD1 molecules to natural killer T (NKT) cells etc by interaction with cytokines that are crucial for the development of autoimmune and inflammatory responses in human [3]. The innate immune responses of the host towards infectious agents are influenced by the level of circulating lipids and triglyceride in the hosts body. Namely three cytokines: IL-6, IL-1 and TNF-α are responsible for this effect and they do so by producing elevated levels of triglycerides and VLDL , decreased levels of cholesterol, HDL and LDL or increased levels of cholesterol in the serum, depending on the nature of the infectious agents [1].ApoE deficient mice studies demonstrated that the host susceptibility to endotoxemia and klebsiella pneumonia was increased, and that of transgenic mice there was an increased proinflammatory response to an LPS challenge in isoform of ApoE E4 than E3 as substantiated by hyperthermia and cytokine release.Depending on the isoform of ApoE, there was suppression of release of TNF-α and IL-1β (E2>E3>E4). This finding was supported by observation that there was higher level of TNF-α in cultured macrophages of ApoE4 genotype than E3 genotype as well as an increase in production of PG-E2 and IL-1β by ApoE4 than other isoforms in primary adult microglia structure. ApoE3 lipoproteins have higher levels of TGF- β which is an anti-inflammatory cytokine than ApoE4. A similar genotype dependent effect was seen with the response to 17β- oestradiol through release of NO and TNF-α production from microglia activated by recombinant mouse INF-γ plus either LPS or PIC establishing that the anti-inflammatory activity of oestradiol was greatly reduced in ApoEε4 genotype than ε3 genotype, implying that the neuroprotective effect of oestradiol and HRT on brain function was also compromised in the presence of ApoE ε4 genotype [1].

The Study on Alzheimer's Gene and COVID Severity

The UK based study was conducted during the peak days of the outbreak amongst people with European ancestry with the ApoE genotypes of the following range: ε4ε4-3%, ε3ε4-28%, ε3ε3- 69% (most common genotype) out of the total participants 322,984 [4]. The study also included patients having other independent comorbidities like dementia, hypertension, coronary artery disease, diabetes which were the conditions established to cause a progression to a more severe COVID as well as patients without dementia, hypertension, coronary artery disease and diabetes. It was found that whether or not the patient had pre-existing dementia, hypertension, coronary artery disease or diabetes individuals with ε4ε4 genotype was found to be more susceptible to the development of COVID-19 as well as progression to more severe condition than individuals possessing ε3ε3 . The fact that individuals of African American decent have twice the frequency of ε4 allele than individuals of Asian and European ethnicity can provide a partial explanation for the increased incidence of severe cases of COVID-19 amongst African Americans along with other socio-economic reasons. SARS-Cov2 is a beta coronavirus having RNA as the genetic material. The symptoms and the consequent pathophysiology of ARDS caused by COVID-19 has been claimed to be because of cytokine storm, which is an uncontrolled release of pro-inflammatory cytokines and chemokines by immune effector cells in response to the virus infection[5]. Since the ApoE ε4 genotype elicits a property of worsened pathology and is a favourable factor for viruses it can be hypothesized that it must be because of the proinflammatory and other immune modulatory effects there occurs a risk for progression to severe illness. It should be taken into account that the ApoE is present in various cells of the body including the macrophages and both type 1 and 2 alveolar epithelial cells that are present in the lungs [5]. The target receptor site through which SARS-CoV2 gains entry into the cell is by means of the AT2 that is widely expressed on the type 2 alveolar epithelial cells. This detail can be used to substantiate the ApoE gene effect.

CONCLUSION

The ApoE gene, predominately studied for its role in transport of lipid and development of CVD, is now held as a risk factor for the development as well as progression to severe COVID-19 conditions. This is in direct correlation with the genotype of the gene (ε4ε4> ε3ε3.). The ApoE gene also has a role in regulation of immune functions and here also an allele dependent fashion follows. A severe COVID-19 condition is marked by the onset of ARDS, the cause of which is the cytokine storm. The ε4ε4 genotype by its ability to modify the release of inflammatory and anti-inflammatory cytokines and other immunological cells, can act as a potentiating factor for the cytokine storm in these individuals. The gene has also been held accountable for favouring the entry of viruses in to the cells of the host. As the ApoE gene is found in the alveolar cells where the AT-II receptor is present, this can provide a partial explanation to why the individuals of ApoEε4 genotype are at an increased risk of contracting the infection as well as progressing to a more severe condition.

Abbreviations

ApoE: Apolipoprotein E, **SARS-COV2**: severe acute respiratory syndrome coronavirus **ε2, ε3, ε4**: epsilon 2,3,4 **AT2**: angiotensin 2, **COPD**: chronic obstructive pulmonary disorder, **CVD**: cardio vascular diseases**, AD**: Alzheimer's disease, **HIV**: human immunodeficiency virus, **HSV**: herpes simplex virus, **HCV**: hepatitis C virus, **BBB**: blood brain barrier**, BNB**: blood nerve barrier, **NO**: nitric oxide, **TNF**: tumour necrotic factor, **IL**: interleukin**, LPS**: lipopolysaccharide, **PIC**: polyinosine- polycytidylic acid, **PG**: prostaglandin, **HRT**: hormone replacement therapy

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RESEARCH ARTICLE

Green Synthesis, Spectral Characterization and Biological Activities of Cu(II) Complex with Nitrogen Donor Ligands

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ABSTRACT

Coordination complex of Cu(II) with nitrogen donor ligands (4-Methylaminopyridine (MAP) and azide **(N_3^-)** ion) was synthesized by using microwave irradiation. The metal complex has been characterized by elemental analysis, electrical conductivity, magnetic moment, UV-Visible, FT-IR, EPR and cyclic voltammetric studies. The antibacterial and antifungal activities have been evaluated by disc diffusion method against the bacteria *viz.*, *Escherichia coli*, *Lactobacillus brevis* and *Staphylococcus* and the fungi *viz.*, *Candida albicans, Aspergillus flavus, Aspergillus niger, Aspergillus sojae* and *Aspergillus oryzae.* The formula of the complex was derived from the percentage of the metal in the complex and its molar conductivity. The electronic spectra and the magnetic moment indicate the geometry of the complexes is found to be octahedral. The FT-IR spectral data of the complex was compared with those for the two ligands (MAP and N_3^- ions) to confirm the entry of the ligands in the co-ordination sphere. The metal ligand covalancy of Cu(II) complex has been arrived at from EPR spectrum. The free radical scavenging activities of the complex and the ligand have been determined by measuring their interaction with the stable free radical DPPH. The complexes have larger antioxidant activity as compared to the ligands. DNA-binding properties have been studied by fluorescence-emissions method. The outcome suggests that the metal complexes strongly bind to DNA because of metal complexes are well-known to speed up the drug action and the ability of a therapeutic agent which can frequently be enhanced upon coordination with a metal ion.
Keywords: N-donor, 4-Methylaminopyridine, azide ion, antimicrobial.

INTRODUCTION

Some of the fast chemistry processes, mainly the microwave-assisted techniques are understood to be more eco-friendly, i.e., requiring less energy than conventional processes. Taking into account of the energy necessities for the

process and finding ways to reduce the energy are important considerations in Green chemistry or sustainable chemistry [1,2]. More recently the use of microwave radiation for heating reactions in the laboratory has extended to inorganic and materials chemistry [3,4]. Now a day, it is an established tool for accelerating the organic and inorganic reactions. It leads to high reaction selectivity and consumption of minimum amount of solvents. It is an eco-friendly technique [5,6] and gives a high yield within a short time. The transition metal complexes is becoming a spectacular area of inorganic research due to the demand for new metal based antibacterial and antifungal compounds [7,8]. The staid medical trouble [9-11] of bacterial and fungal resistance and the rate at which it develops has led to the escalating levels of resistance to conventional antibiotics. The recognition and growth of antibacterial and antifungal drugs with inspired mechanism of action have become a critical task for communicable diseases research programs [12]. Many investigations proved that binding of a drug to a metalloelement enhances its activity and in some cases the complex possesses even more therapeutic properties than the parent drug [13].

The present study aims at the microwave assisted synthesis and spectral characterization of Cu(II) complexes with 4-Methylaminopyridine and azide ion as ligands. The ligands and their complexes are also focused on the biological studies.

4-Methylaminopyridine

Sodium azide
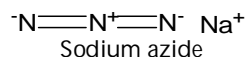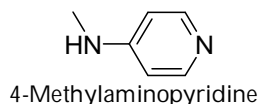
MATERIALS AND METHODS

Materials
$Cu(NO_3)_2.3H_2O$, 4-Methylaminopyridine and C_6H_5ONa were purchased from Alfa Aaser Company and used as such. The organic solvents used, *viz.,* DMSO, DMF, CH_3OH and CH_3CH_2OH were of AnalaR grade and used as such without further purification.

Synthesis of Cu(II) complex
0.83g (7.98 mmol) of 4-Methylaminopyridine in CH_3OH and 1.18g (8.44 mmol) of sodium azide in C_2H_5OH were added to the methanolic solution of $Cu(NO_3)_2.3H_2O$ 1.00g (4.14 mmol) and this was followed by microwave irradiation for few minutes after each addition by using IFB 25 BG-1S model microwave oven. The resulting precipitate was filtered off, washed with 1:1 C_2H_5OH: CH_3OH mixture and dried under vacuum. A blue colored complex was obtained with the yield of 68.5%.

Instrumentations
C,H,N elemental analyses were performed using Thermo Finnegan make, Flash EA1112 Series CHNS(O) analyzer. The electrical conductivity measurements were conducted using 10^{-3} M solutions of the metal complex in acetonitrile with Systronic Conductivity Bridge (model number-304) at 30°C. The UV-Visible spectrum of the Cu(II) complex was recorded on Varian, Cary 5000 model UV-Vis Spectrophotometer. Infrared spectra for the complex and the ligands were recorded on a Perkin Elmer, Spectrum RX-I, FT-IR Spectrometer in KBr discs at room temperature. The Far-IR Spectrum of the complex was recorded by Bruker 3000, FT- IR Spectrometer. The electron paramagnetic resonance spectra of the copper complex were recorded at room temperature using JES FA 200 EPR Spectrometer.

Antimicrobial activity
The free ligand 4-methylaminopyridine and the synthesized Cu(II) complex were tested for *in vitro* antimicrobial activity by the well diffusion method [14], using the agar nutrient as the medium. The antibacterial and the antifungal activities of the ligands and the Cu(II) complex were evaluated by the well diffusion method against the strains, cultured on potato dextrose agar as medium. In this typical procedure [15], a well was made   on the agar

medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35°C. At the end of the period, the inhibition zones formed on the medium were evaluated as millimeters (mm) diameter.

Antioxidant activity

Assessment of antioxidant activity stock solution (1 mg/ml) was diluted to final concentrations of 10–500 µg/ml. Ethanolic DPPH solution (1 ml, 0.3 mmol) was added to the sample solutions in DMSO (3 ml) at different concentrations (10–500 µg/ml) [16]. The mixture was shaken energetically and acceptable to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a UV-Vis Spectrophotometer. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ethanol was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity is designed by the following equation:

$$\text{DPPH Scavenging effect (\%)} = \frac{A_o - A_1}{A_o} \times 100$$

where A_o and A_1 are the absorbance of the control reaction and absorbance in the presence of the samples/standard.

DNA binding studies

The DNA binding experiments involving interaction of the Cu(II) complex and the ligand with calf thymus CT-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with hydrochloric acid. The CT-DNA was dissolved in Tris-HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave the ratios of UV absorbance at 260 and 280 nm above 1.8, demonstrating that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600 dm^{3}mol^{-1}cm^{-1} at 260 nm. The stock solutions were stored at 4°C and used within 4 days [17, 18]. For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EtBr) for 30 minutes. The Cu(II) complex then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm.

RESULTS AND DISCUSSION

Elemental analysis and metal estimation

The elemental analysis and metal estimation of the complex led to the formula   [Cu(MAP)$_4$(N$_3$)$_2$]. The percentages of carbon, hydrogen, nitrogen and copper in the complex were found to be 44.41(44.52), 2.88 (2.96), 5.09(5.18) and 11.01(11.70), respectively. The experimental data were in good agreement with the theoretical values.

Molar conductance

Molar conductance measurements of the complex, carried out using acetonitrile as the solvent at the concentration of 10^{-3}M, indicate non-electrolyte behaviour of the complex [19]. Thus the complex may be formulated as [Cu(MAP)$_4$(N$_3$)$_2$].

UV-Visible spectrum of [Cu(MAP)$_4$(N$_3$)$_2$]complex

The electronic spectrum of Cu(II) complex exhibits three absorbance bands at 680 nm, 343 nm and 280 nm and their corresponding transitions are $^2A_{1g} \leftarrow {}^2B_{1g}$, $^2B_{2g} \leftarrow {}^2B_{1g}$ and $^2E_g \leftarrow {}^2B_{1g}$ respectively, which indicate octahedral geometry around Cu(II) metal ion. The magnetic moment value of Cu(II) complex is 1.80 B.M, that indicates further confirming hexa-coordination around Cu(II) metal ion [20,21].

FT-IR Spectra of free ligands and their [Cu(MAP)$_4$(N$_3$)$_2$] complex

The FT-IR spectra of the free ligands and their [Cu(MAP)$_4$(N$_3$)$_2$] complex were recorded in the region of 4000-400 cm^{-1}. The free ligand 4-methylaminopyridine shows in characteristic absorption band at 3327 cm^{-1} assigned as ν(NH)

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[22,23]. A small band noticed at 3040 cm^{-1} is due to ν(CH) aryl. Aromatic ν(C= C) stretching vibration is seen as a sharp peak at 1520 cm^{-1} [24]. The azide ion shows ν(N$_3$) 645 cm^{-1}. The stretching frequency of ν(NH) in 4-methylaminopyridine and the (N$_3$) group of the azide ion underwent higher wave number at after complexation, indicating the coordination of amino nitrogen and azide nitrogen to the metal atom. The low frequency region of the spectra revealed the presence of medium intensity bands in the region of 600-300 cm^{-1} due to ν(M-N) and ν (M-N) [25] respectively in the complex which supports the involvement of N, N [26] vibrations in [Cu(MAP)$_4$(N$_3$)$_2$] complex which again supports complexation with the metal ion under investigation. Thus, the IR spectral data suggest that the 4-MAP and azide ion are bound to the metal ion through the amino nitrogen and azide nitrogen donor atoms.

Cyclic voltammetry of [Cu(MAP)$_4$(N$_3$)$_2$] complex

The cyclic voltammogram of the Cu(II) complex in DMF solution shows +1.3 to -1.3V potential range, indicating quasi-reversible one-electron process. A notable characteristic has been observed in the cyclic voltammogram of Cu(II) complex. Fig. 1 shows the cyclic voltammogram of Cu(II) complex. During the forward scan, it shows two cathodic reduction peaks, one at +0.6V and the other at -1.3V which are attributed to reduction of Cu(II)→Cu(I) and Cu(I)→Cu(0), respectively. During the reverse scan, it shows two anodic peaks, one at 0.8V and the other at -1.0V which are attributed to oxidation of Cu(0)→Cu(I) and Cu(I)→Cu(II), respectively [27].

EPR spectrum of [Cu(MAP)$_4$(N$_3$)$_2$] complex

The spectrum of DMSO solution of [Cu(MAP)$_4$(N$_3$)$_2$] complex of 4-methylaminopyridine (MAP) and azide ion measured at X-band frequency at 77 K (LNT) provide useful information which is important in studying metal ion environment. The spin Hamiltonian parameters of the complex have been calculated and are summarized in Table 1.The [Cu(MAP)$_4$(N$_3$)$_2$] complex in the frozen state at 77 K shows four well resolved peaks in the low field region and one intense peak in the high field region. The g-tensor value of the copper complex can be used to derive the ground state. In octahedral complexes, the unpaired electron lies in the dx^2-y^2 orbital [28]. For this complex, the observed g-tensor values are g$_{\parallel}$ =2.2461 > g⊥= 2.2103 > g = 2.0023 which suggest that this complex has an octahedral geometry and the ground state is ^{2}B$_1$g. The EPR parameters of the complex coincide well with the related systems which confirm that the complex has an octahedral geometry and it is axially symmetric. In the axial spectra, the g-values are related to the exchange interaction coupling constant G by the expression [29].

$$G = g_{\parallel} - 2.0023 / g_{\perp} - 2.0023$$

According to Hathaway [30] expression, if G value is larger than four, the exchange interaction is negligible because the local tetragonal axes are aligned parallel or slightly misaligned. If its value is less than four, the exchange interaction is considerable and the local tetragonal axes are misaligned. For the present Cu(II)complex, G is 0.6231, which indicates considerable exchange interaction in the solid complex.The g$_{av}$ and the covalent in-plane σ-bonding (α^2) parameters are calculated according to the following equation [31].

$$g_{av} = 1/3[g_{\parallel} + 2g_{\perp}]$$
$$\alpha^2Cu = (A_{\parallel} /0.036)   + g_{\parallel}-2.0023 +3/7(g_{\perp} -2.0023) + 0.04$$

If the α^2 value is 0.4321, it indicates a complete covalent bonding, and if the value is 1.0, it suggests a complete ionic bonding. From Table1, it is clear that the in-plane σ -bonding parameter α^2= 0.4304 [32] is less than unity and this indicates the covalent character of M-L bond [33]. These data are well in accordance with the other reported values.

Biological Activity

Antibacterial activity: The synthesized [Cu(MAP)$_4$(N$_3$)$_2$] complex and the free ligand 4-methylaminopyridine are tested against the bacteria *viz.*, *Escherichia coli*, *Lactobacillus brevis* and *Staphylococcus* by agar-well diffusion method in

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vitro conditions. The [Cu(MAP)$_4$(N$_3$)$_2$] complex has potential activity against the bacteria compared to free ligand 4-methylaminopyridine.

Antifungal activity: The antifungal activity of the free ligand 4-methylaminopyridine (MAP) and the synthesized [Cu(MAP)$_4$(N$_3$)$_2$] complex are tested against the fungi *viz., Candida albicans, Aspergillus flavus, Aspergillus niger, Aspergillus sojae* and *Aspergillus oryzae* by agar -well diffusion method. The [Cu(MAP)$_4$(N$_3$)$_2$] complex has enhanced activity against the fungi compared to free ligand 4-methylaminopyridine.

Antioxidant activity (Radical Scavenging Activity)
The antioxidant activity of the free ligand 4-methylaminopyridine (MAP) and the [Cu(MAP)$_4$(N$_3$)$_2$] complex were determined by DPPH free radical scavenging method and vitamin C as standard. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants [34]. The graph was plotted with percentage scavenging effects on the y-axis and concentration (µg/ml.) on the x-axis. The scavenging ability of the [Cu(MAP)$_4$(N$_3$)$_2$] complex were compared with Vitamin C as a standard. The metal complexes showed enhance activity as a radical scavenger compared with ascorbic acid, these results were in good agreement with previous metal complexes studies where the ligand has the antioxidant activity and it is expected that the metal moiety will increase its activity [35-37]. The scavenging activities of ligand and their complex shown in Fig.2.

DNA Binding – Emission study
The binding of free ligands and their [Cu(MAP)$_4$(N$_3$)$_2$] complex to CT-DNA can be studied by competitive binding experiments. Ethidium bromide (EtBr) is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule [38,39]. The quenching extent of fluorescence of EtBr binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with ethidium bromide causes appreciable reduction in the emission intensity, indicating the replacement of the ethidium bromide fluorophore by the complex results in a decrease of the binding constant of the ethidium to the DNA as shown in Fig. 3 & 4. According to the classical Stern-Volmer equation: Io/I = 1 + Ksvr, where Io and I are the fluorescence intensities in the absence and the presence of complex respectively. Ksv is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plots show that the quenching of ethidium bromide bound to DNA by the complex are in good agreement with the linear Stern-Volmer equation, which also indicates that the complex binds to DNA. In the plot of Io/I versus C$_{Complex}$/C$_{DNA}$, K is given by the ratio of the slope to intercept and given in Table 2.The obtained Kb values suggest that the interaction of complex with DNA is strongest, which is consistent with the above absorption spectral results. Kb values indicate that the interaction of the complex with DNA is a intercalative mode [40].

CONCLUSION

In the near study, our efforts was to synthesize and characterize a new [Cu(MAP)$_4$(N$_3$)$_2$] complex with 4-methylaminopyridine and azide ion as ligands. The new complex was synthesized using microwave irradiation. The synthesized complex was characterized by various chemical and spectral analyses. The synthesized complex was tested for antimicrobial activities. The metal complex has significant antimicrobial and antioxidant activities as compared to the free ligands. The effectiveness of the DNA binding of the complexes is being confirmed by means of change in intensity of emission in the case of emission spectral studies.

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Table 1. Spin Hamiltonian parameters of [Cu(MAP)$_4$(N$_3$)$_2$] complex at 77K

Complex	Spin Hamiltonian parameters					
[Cu(MAP)$_4$(N$_3$)$_2$]	**g$_{\parallel}$**	**g$_{\perp}$**	**g$_{av}$**	**G**	**A$_{\parallel}$10^{-4}cm^{-1}**	**α^2**
	2.2461	2.2103	0.4321	0.6231	157.62	0.4304

Table 2. DNA-binding constant (Kb) of ligand and [Cu(MAP)$_4$(N$_3$)$_2$] complex

S.No.	Ligand/Complex	Binding constant (Kb)
1	4-methylaminopyridine	1.74×10^4 M^{-1}
2	[Cu(MAP)$_4$(N$_3$)$_2$]	3.41×10^4 M^{-1}
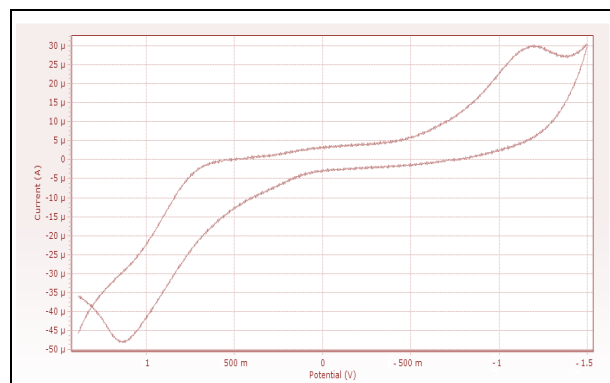
Fig. 1.Cyclic voltammogram of Cu(II) complex.
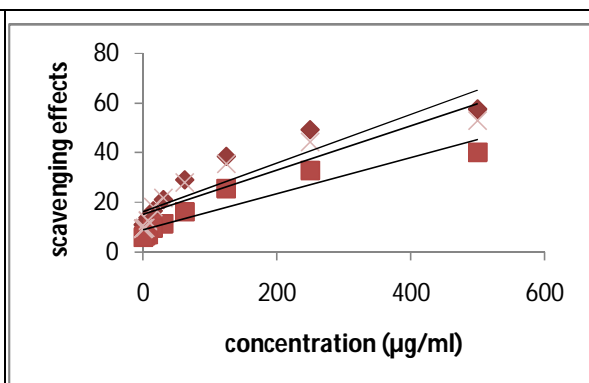
Fig.2.Antioxidant activities of free ligands and their complex.
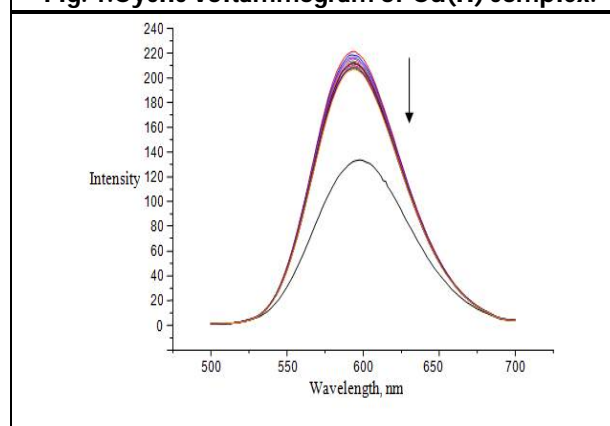
Fig.3.Emission spectrum of 4-methylaminopyridine ligand
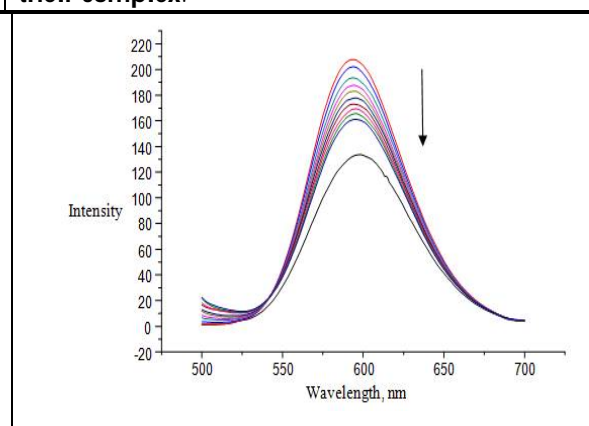
Fig.4.Emission spectrum of [Cu(MAP)$_4$(N$_3$)$_2$] complex

RESEARCH ARTICLE

Montelukast Induces Mesenchymal Properties in A549 Lung Epithelial Cells

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ABSTRACT

Background: Epithelial to mesenchymal transition (EMT) is an important event in the progression and metastasis of cancer. Montelukast is a cysteinyl leukotriene receptor antagonist, is used in asthma management, has been implicated in modifying cell behaviour, but its role in EMT is not clear. **Objective:** Our objective was to know the role of Montelukast in inducing mesenchymal properties and migration of A549 cells and what are the probable molecular alterations occurs due to this. **Methodology:** A549 cell line was used as an *In vitro* model for present studies, where wound healing assay, migration assay, invasion assay, gelatin zymography and western blotting experiments were performed subsequent to Montelukast treatment of cells. **Results:** The results show that a complete healing of wounds created by scraping the cells in monolayer by 5 and 10 μM Montelukast groups. Impact on migration and invasion measured by boyden chamber assay showed a significant increase in migration and invasion potential of cells in Montelukast treated A549 cells. Further with the help of zymography increased MMP-2 and MMP-9 activity was observed which were confirmed by western blotting where increased level of MMP-2, MMP-9 and decreased level of E-cadherin was observed. **Conclusion:** These results suggest that Montelukast increases EMT in A549 human lung epithelial cells.

Keywords: EMT, Montelukast, zymography, E-cadherin, MMP, A549

INTRODUCTION

Montelukast (ML) is a leukotriene receptor antagonist, acts as anti-inflammatory by blocking the leukotriene receptor (Tintiger *et al.,* 2010). Like ML, pranlukast and zafirlukast are also approved leukotriene receptor antagonist drugs. It

is established that 5-lipoxygenase and leukotrienes are expresses in many inflammatory diseases and also required for cancer survival, growth and metastasis (Nozaki *et al.,* 2010, Sveinbjornsson *et al.,* 2008, Poff *et al.,* 2004). ML is used for asthma management, especially in exercise- and aspirin-induced asthma. It is also used when asthma is less controlled by inhaled corticosteroid or by the combination of a long-acting β (2)-agonist (Tintinger *et al.,* 2010). Leukotriene antagonists and inhibitors of leukotriene biosynthesis are valuable as anti-allergic, anti-inflammatory and cytoprotective agents. They are also useful in treating angina, cerebral spasm, glomerular nephritis, hepatitis, endotoxemia, uveitis, and allograft rejection (Belley *et al.,* 1996). ML has been shown to have some leukotriene independent effects as well. It has been stated to possess secondary anti-inflammatory properties (Tintinger *et al.,* 2010). These activities allow ML to target eosinophils, monocytes and specially the corticosteroid-insensitive neutrophils. These effects suggest that ML may have a broader spectrum anti-inflammatory activities than originally thought.

Two type of actions regarding effect of ML are available in literature. One suggests that LO inhibition or CysLT antagonism inhibit cellular metastasis (Magi *et al.,* 2014, Nozaki *et al.,* 2010, Kaetsu *et al.,* 2007, Chen *et al.,* 1994, Tang *et al.,* 1994), while other indicates that antileukotriene agents, such as montelukast, help in cell migration and protect against injury in several inflammatory models in rats, such as ethanol-induced gastric mucosal damage, colitis, burn-induced and sepsis-induced multiple-organ damage, and renal ischemia/reperfusion injury (Celik *et al.,* 2013, Turtay *et al.,* 2010). To clarify the pricise role of ML in respiratory system, other than its role in asthama managment, we investigated the effect of ML on human lung epithelial cells, A549. Our results suggest that ML aids in the cellular migration and invasion. This effect may be useful in wound healing, on the other hand it may be harmful and may aid in metastasis. Here we are also reveling the side effect of ML for cancer patient, if he or she taking ML simultaneously for asthama, he or she may have greater chances for cancer migration.

MATERIALS AND METHODS

Chemicals
Montelukast sodium, DMSO and β-actin antibody were obtained from Sigma Aldrich, (St Louis, MO, USA). Antibodies against MMP-2 and MMP-9 were bought from Cell signalling, (Danvers, MA, US), and E-cadherin antibody was bought from BD Biosciences, (San jose, CA, USA)

Cell Culture
A549 lung cancer cell line was purchased from ATCC (Wanena, USA).Cells were grown in Roswell Park Memorial Institute (RPMI)-1640 cell culture medium with glutamine (HiMedia, India) supplemented with 10% FBS (Gibco, US) and 1% penicillin-streptomycin-amphotericin B cocktail (HiMedia, India) in a humidified atmosphere under 5% CO_2 and 37°C temperature.

Treatment of Cells
Stock solution of 10 mM was prepared by dissolving Montelukast sodium in DMSO and stored at 4°C until used. Fresh stocks were made every 1 month. DMSO at a final concentration of 0.1% (v/v) was used in all treatments, which was found non-toxic to the cells. Aliquots from the stock solution were diluted in the medium to achieve final working concentrations (2.5, 5 and 10 µM) for cell's treatments.

Wound Healing Assay
Cells were plated in 6-well plates with media supplemented with 10% FBS. At approximately 90% confluency, 2 h pretreatment of mitomycin C (0.5 µM) was given. Wounds were created with the help of 200 µl sterile tip, by scratching the confluent monolayer. Fresh media (3 ml) containing carrier or ML of specific doses (2.5, 5 and 10 µM) and mitomycin C (0.5 µM) was added to each plate and cells incubated for different time periods. Wound sizes were

recorded by bright field micro-photography at 0 h, 12 h and 36 h at three different places per plate along the length of wound after injury, using an inverted microscope equipped with digital camera

Cell Migration Assay Using Boyden Chamber

Cells grown in regular serum conditions and treated at 70% confluency with vehicle (DMSO), 2.5, 5 and 10 µM of Montelukast. After 48 h of incubation cells were harvested and approximately 30,000 live cells were seeded in upper chamber of migration transwells in serum free media. Lower chambers were filled with 500 µl of 10% FBS containing media. After incubation of 20 h, migrated cells were fixed in chilled ethanol: acetic acid solution (95:5) and stained with 0.5% crystal violet. Cells were mounted on a glass slide and counted at 200X magnification under phase contrast microscope. A minimum of 5 microscopic fields were counted in each slide; and all the treatments were done in duplicate.

Cell Invasion Assay Using Matrigel-Coated Boyden Chamber

Cells grown in regular serum conditions and treated at 70% confluency with vehicle (DMSO) or 2.5, 5 and 10 µM of Montelukast. After 48 h of incubation cells were harvested and approximately 50,000 live cells were seeded in upper chamber of migration transwells in serum free media. Lower chambers were filled with 500 µl of 10% FBS containing media. After incubation of 20 h, migrated cells were fixed in chilled ethanol: acetic acid solution (95:5) and stained with 0.5% crystal violet. Cells were mounted on a glass slide and counted at 200X magnification under phase contrast microscope. A minimum of 5 microscopic fields were counted in each slide and all the treatments were done in duplicate.

Gelatin Zymography For MMP-9 And MMP-2 Activities

Cells were treated at 70% confluency with vehicle (DMSO), 2.5, 5 and 10 µM concentrations of ML in regular media. After 48 h of incubation, the conditioned medium was collected. SDS-PAGE gels of 10% were prepared contained 0.1% gelatin also. Mixed the equal protein samples with 2X sample buffer, without β-mercaptoethanol and incubated for 10 min at RT. After running the gel was renatured with renaturing buffer to remove SDS. Gel was developed with fresh 1X zymogram developing buffer and incubated at 37°C overnight for maximum sensitivity. Destaining was done and clear areas in gel indicating protease activity were scanned with HP Scanjet G3110 scanner.

Western Blotting

Equal number of cells were seeded in 100 mm plates and treated at 70% confluency with vehicle (DMSO) or 2.5, 5 and 10 µM of ML and incubated for 48h. The cells were washed and lysed on ice with lysis buffer containing 50 Mm Tris, 1% Triton X-100, 0.1% sodium dodecyl sulfate, 150 mM NaCl, and containing protease and phosphatase inhibitors (Roche Applied Sciences, Indianapolis, USA). Equal amount of proteins were electrophoresed onto 10% SDS-PAGE gels and transferred onto nitrocellulose membrane followed by blocking with 5% non-fat milk powder for 1 h at room temperature. Membranes were probed using specific primary antibodies against MMP-2, MMP-9, E-cadherin and β-actin followed by peroxidase-conjugated appropriate secondary antibody, and visualized by the ECL detection system. Image J software was used for densitometry of the blots.

Statistical and Densitometry Analysis

Statistical analysis was done with GrapPad Prism 5 software version 5. Quantitative data are presented as mean ± SEM. Statistical significance between control and treated group was determined by one way ANOVA and/or Student's t-test and $p < 0.05$ was considered significant. To quantify zymogram gels or western blot bands, relative intensities of bands were measured by employing ImageJ analysis software tool of NIH, USA after following step by step procedure as per guidelines provided by software. Western blot bands were normalized with their β-actin.

RESULTS

Montelukast Significantly Increases the Migration of A549 Cells

For assessing the effects of ML on migration of A549, first we did the wound healing assay/scratch assay. The healing of wound was observed in ML treated groups, which was slower in control plates, during treatment duration (Figure 1A and 1B). We observed the fast healing at higher doses as compared to 2.5 µM. The complete healing of wound was observed at 5 and 10 µM ML at almost same time, but slightly faster at 5 µM ML treatment. Means ML significantly increases the migration of A549 cells.

After assessing migration of cells through wound healing assay in 2D cell culture plates, we performed the boyden chamber migration assay, which is one of the excellent *in vitro* model for assessing the migration of cells and this system is more closer to *in vivo* system. We observed that the cells which were treated with ML have more migration potential. Although, we observed that the migration promoting effect was dose-independent (Figure 2B).

Montelukast Induces the Invasion of A549 Cells

Further we did boyden chamber invasion assay. Invasion potential of cancer cells makes them able to invade distant and adjacent tissues and organs. It is an early step during metastasis. We observed a dose-independent and statistically significant increase (p<0.01 for 2.5 µM and p<0.05 for 5 and 10 µM ML) in invasion potential of A549 cells (Figure 3B). It suggests that ML increased the proteolytic enzymes production that degraded metrigel and made the cells able to invade and cross through metrigel.

Montelukast Enhances the Activity of MMP-9 and MMP-2

MMPs (Matrix metalloproteinases) are a family of enzymes which are necessary for extracellular matrix remodeling and maintenance. These have important role in embryonic development, tissue remodelling, angiogenesis, cell migration, wound healing, and tooth development (Demestrea *et al.,* 2005, Patricia *et al.,* 2005). For assessing the enzyme activity of MMPs secreted by ML treated A549 cells, we did gelatin zymography. More the enzyme activity of MMPs, more the degradation of gelatin and more the brighter white band on blue background of zymogram. We observed that the enzyme activities of MMPs were increasing dose dependently upto 1.2 fold (in MMP-9) and 1.4 fold (in MMP-2) (Figure 4A). The activity of MMPs was increased at highest (10 µM) concentration of ML. It suggested that ML treatment increases the MMP-2 and MMP-9 activity.

Effect of Montelukast on the Protein Expression of MMP-9, MMP-2 and E-Cadherin.

Next we performed western blotting for some key regulatory proteins related to cell migration, such as MMP-9, MMP-2 and E-cadherin. The western blotting analysis showed that the level of MMP-9 and MMP-2 were increasing in the cell, at higher dose of ML upto 15.8 and 1.6 fold respectively as compared to control but decreased at 10 µM ML. The level of E-cadherin was observed almost equal on Montelukast treatment, but it was decreased at 5 µM ML upto 0.2 fold in comparison to control. So the levels of MMP-9 and MMP-2 were highest at 5 µM ML and E-cadherin was lowest at same concentration of ML (Figure 4B and 4C).

DISSCUSSION

Any damage like wound or burn stimulation produces few cytokines (TNF-α, IL-1, IL-6 etc.) and growth factors like, fibroblast growth factor (FGF), platelet-derived growth factor (PDGF) and epidermal growth factor (EGF), which are released by inflammatory cells to eliminate the cause of damage. These cytokines and growth factors start regeneration trigger fibroblast proliferation on the damaged dermis (Turtay et al. 2010).Leukotrienes, prostaglandins are having inflammatory role, as these products also have the ability to cause endothelial injury and tissue damage, they may increase the damage at damaged area (Turtay et al. 2010). So, inhibition of leukotrienes and prostaglandins can also help in wound healing, which we observed in our results. We observed the healing of wound in ML treated

groups. As we discussed earlier that there are both type of literature available suggesting controversial effect of antagonism of leukotriens, here we tried to solve it. We observed the effect of ML, which is a known cysLT1 receptor antagonist, on human airway epithelial cell line (A549). Eicosanoids (leukotrienes and prostaglandins) are arachidonic acid derived potent inflammatory molecules, have been found to have important roles in the pathogenesis of various inflammatory diseases and cancer (Wisastra *et al.,* 2014, Sveinbjornsson *et al.,* 2008, Massoumi *et al.,* 2007, Claria *et al.,* 2005).

Cells adhere to each other and to ECM by adhere junctions and maintain the structure of tissue, so the loss of adhesion molecules like E-cadherin, is a characteristic of increased metastatic property. N-cadherin, which is usually expressed during organogenesis on migrating neurons and mesenchymal cells, is reported to overexpress in different invasive cancers. In our study we got very significant effect on increase in the migration of A549 cells. In wound healing assay we observed that wounds were healing in ML treated plates in lesser duration as compared to control plates. It seems that the effect was dose-independent, because although the wounds were filled at 36 h in both 5µM and 10 µM ML but it was slightly faster in 5µM ML treatment (Figure 1A and 1B).

We confirmed it with boyden chamber migration assay. This assay is well established and extensively used to examine cell migration. It is believed closer to *in vivo* conditions. Here cells migrate according to the concentration gradient of growth factors as they do *in vivo.* We found similar result that the ML helps A549 cells in migration, but the result was not as much significant as in wound healing assay (Figure 2A and 2B). Here we concluded that the trigger of wound and Montelukast worked sinergestically for migration of cells. It is reinforced by literature that the molecules (TNF-α, IL-1, IL-6, FGF, PDGF and EGF) secreted on wound trigger and blocking of leukotriens activity both helps in reepithelisation of wound.

After assessing the migration through wound healing and boyden chamber migration assay. We came to know that ML helps in migration of A549 cells. We were assuming that ML will also induce the invasive potential of A549 cells, because invasion is one of the earlier steps of migration. So, we performed boyden chamber invasion assay for assessing the effect of ML on A549 cells. Here the chambers were matrigel coated. For migration through this chamber, cells have to cross two barriers first is matrigel and second is 8 µM pore size membrane. Only the cells, which have invasion potential, can cross these barriers. Here we found that ML boosts the invasion capacity of A549 cells in dose-dependent manner (Figure 3A and 3B). The number of cells migrated through invasion chambers were significantly (p<0.01) increased at higher doses of ML. Results were more significant in boyden chamber invasion assay compared to boyden chamber migration assy. The probable reason, we thought that, it may be the expression and/or proteolytic activity of of proteolytic enzymes, like matrix metalloproteinases (MMPs). As the invasion chambers have metrigel barrier, Montelukast treatment may induces the A549 cells to express more MMPs which degrades the metrigel.

So, next we did zelatin zymography for observing the activity of MMP-2 and MMP-9. MMPs are a family of enzymes which are necessary for extracellular matrix remodeling and maintenance. They have important roles in embryonic development, tissue remodelling, angiogenesis, cell migration, wound healing, and tooth development (Demestre *et al.,* 2005, Patricia *et al.,* 2005). MMPs help the migration of cells by degrading the ECM by its proteolytic activity. Zymography is an electrophoresis technique, which is used to assess the proteins on the basis of their property to degrade their specific substrates (Patricia *et al.,* 2005). We found an increase in the activity of MMPs after ML treatment, as we expected. Thus Montelukast increases the activity of MMPs secreted in the medium. This increase was upto 1.2 fold in case of MMP-9 and upto 1.4 fold in case of MMP-2.

Further, we examined the cellular protein expression level of MMP-9, MMP-2 and E-cadherin with western blotting. E-cadherin is an important cell to cell adhesion molecule, its low expression helps the cell migration. While the higher expression of MMPs increasing the migration of cells by degrading ECM proteins. Thus both proteins have opposite role in migration. E-cadherin is also well defined in high malignant cancers (Hanahan *et al.,* 2011, Talmadge

et al., 2010). We found that both the MMPs protein expression were increased by ML treatment, however, the maximum effect was observed at 5 µM ML. For the expression level of E-cadherin, we observed almost equal expression but at 5 µM ML it was lowest.

The migration of A549 cells was increasing in wound healing assay and boyden chamber migration assay, so from the results of zymography and western blotting it can be suggested that the expression and activity of MMPs could be associated with the increase in migratory and invasive potential of A549 cells following ML treatment. However, further studies are needed to explore the dose-independent effect of ML on cytosolic levels of MMPs in A549 cells.
Our study suggests that ML do the remodeling in human lung epithelial cells that promote them to show mesenchymal properties. ML possible mechanisms that can promote the mesenchymal property of lung epithelial cells are; 1) by increasing MMPs activity and by degrading E-cadherin, 2) by activation of remodeling on trigger of ML and/or wound and 3) inhibition of leukotriene receptors is also expected to help in wound healing because it does not allow inflammation induced injury by cysLTs. Here we observed a side effect of ML for lung cancer patients who have asthama. Lung cancer patients should not prefer this drug because it may increase the risk of invasion and migration of lung cancer cells. However, further *in vivo* studies are required for evaluating the remodeling and migration promoting property of ML in lung epithelial cells and in lung cancer cells.

CONFLICT OF INTEREST

None

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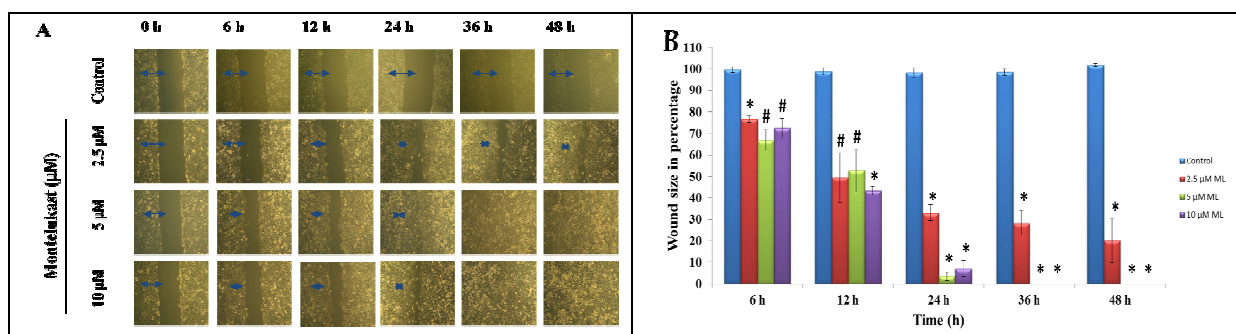
Figure 1: Effect of Montelukast (ML) on cell migration in A549 cells by wound healing assay. (A) Representative images of three independent experiments of the wound at different time periods with different doses of ML. (B) Bar diagram depicted the percent wound sizes, by considering the 0 h wound sizes as hundred percent. Results are mean±SEM (n=2). Images were taken at five independent places in each well, with digital camera fitted with inverted phase contrast microscope. Unpaired t-test was done for statistical analysis. #, $p < 0.01$; *, $p < 0.001$.
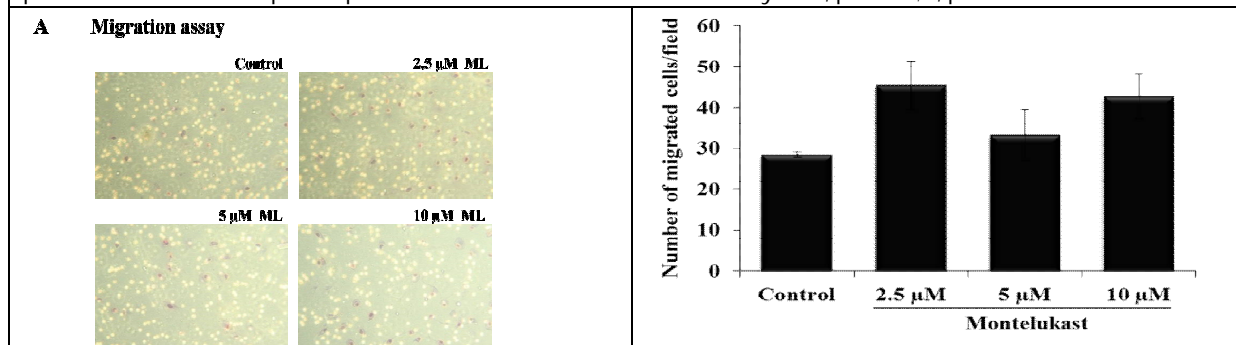
Figure 2: Effect of Montelukast (ML) on cell migration in A549 cells by boyden chamber assay. (A) Representative images of cells migrated through 8 µm pores, images were taken at 200X with digital camera fitted with inverted phase contrast microscope. (B) Bar diagram of two independent experiments. Results are in mean±SEM (n=2), showing the number of migratory cells through boyden migration chambers.

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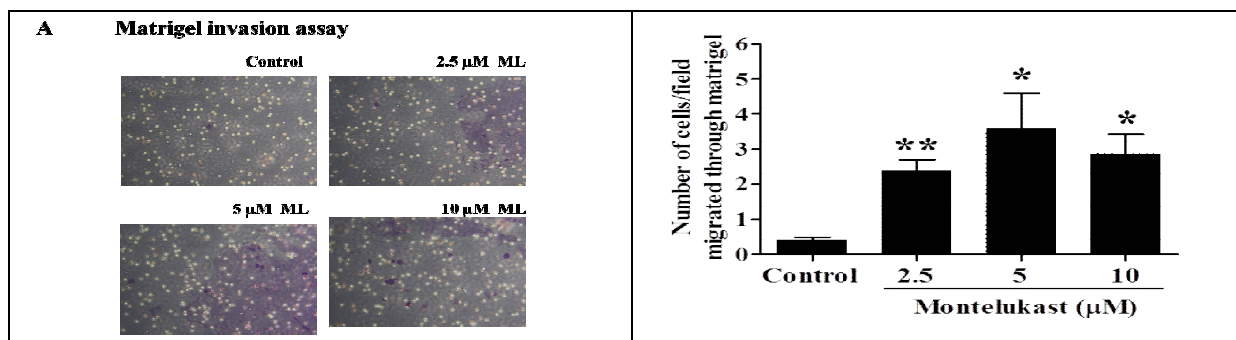
Figure 3: Effect of Montelukast on invasion capacity in human lung carcinoma A549 cells.
(A) Representative images of invaded and migrated cells through boyden chamber invasion assay. (B) Bar diagram of two independent experiments. Results are in mean±SEM (n=2), showing the number of cells which have invasion and migration potential.*, $p < 0.05$; **, $p < 0.01$.
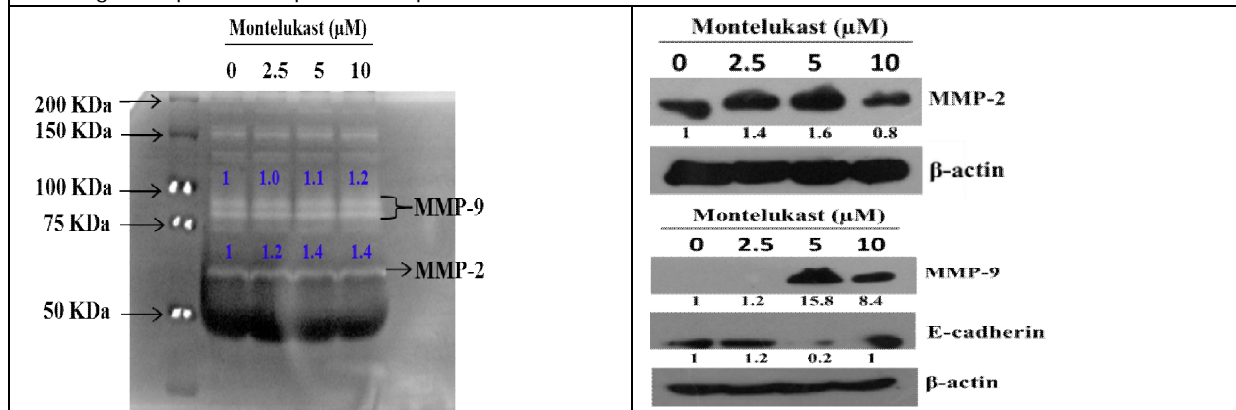
Figure 4: Effect of Montelukast (ML) on cell migration regulatory proteins in A549 cells. (A) Representative images of three independent experiments of gelatin zymography with conditioned media. White bands are showing the activity of MMP-9 and MMP-2. (B and C) Western bolt images for MMP-2, MMP-9 and E-cadherin proteins, membranes were stripped and re-probbed for loading control, β-actin. Fold change is written above each band in zymogram and below each band in western blot.

RESEARCH ARTICLE

Effect of *Dunaliella salina* Supplemented Feed Diet on Color and Growth Performance of Goldfish

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ABSTRACT

Dunaliella salina (Dunal) is a well-known microalga possible used in various fields like pharmaceuticals, industries, food additives and animal feeds. In this study, we isolated and cultivated the *D.salina* from the easterly region and cultivated it for the use of feed supplementary for goldfish. The various concentration of *D. salina* was supplemented with normal feed diet and feed to goldfish. The results showed that the supplement of *D.salina* was significantly increased body weight and feed intake ability. Moreover, *D.salina* has significantly increased the color of Fish minces. Therefore, *D.salina* could be used as an effective feed supplement for goldfish to improve color and growth performance.

Keywords: *Dunaliella salina*, microalgae, gold fish, feed supplement.

INTRODUCTION

The halophilic green micro algae *Dunaliella salina* (Dunal) is a well-known among the other algal species owing to tits highly tolerance of extreme conditions like salinity, light, temperature, and pH, as well as an abundance of integrated biologically active compounds such as carotenoids, glycerin, lipids, and others [1,2]. Substantial amounts of β-carotene can accumulate when microalgal cells are subjected to stress, so *Dunaliella* is widely used as a pro-vitamin supplement for animal husbandry, aquaculture, food additive production, food coloring and cosmetic products with antioxidant properties [3,4]. Moreover, *D. Salina* extract exhibits effective antimicrobial activity against various microbial pathogens [5].The growth rate of *D. salina* has been decreasing under stress conditions that induce the rate of carotene accumulation, so high-density cultures of *Dunaliella* cannot be obtained when it was in stress [6]. Therefore, one of the most common methods for the industrial production of β-carotene-enriched biofertilizer is the two-stage cultivation proposed by Ben- Amotz [7]. In the first "green" stage, favorable conditions are created to

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maintain a high growth rate and survival accumulates. Then, in the second "red" phase, carotenogenesisin *D. salina* cells are triggered by a variety of stress factors, namely increased salinity and light, regulation of mineral nutrients (8-12).*D. salina* is a well-known producer of β-carotene and can accumulate up to 10% of its dry cell weight. It is one of the most valuable nutritional supplements with prophylactic and therapeutic value in medicine and pharmacy. Ascarotene pigment is in high demand, it can be used as an antioxidant and food coloring (13).

D. Salina as a dietary supplement has been studied for its impact on growth, fertility, and hemorrhage parameters in different types of fish Alishahi *et al.*, (14). Found that salina has a positive effect on growth, immunity, and haematological parameters. The effect on fertility and lipid content *of Benois japonicas* Prudstox grown in ponds on *D. salina* dietary extracts was studied (15). Results have shown that salina extract may play a role in stress tolerance and increase beta carotene content. Recently, Biotechnology interests in salina extract arose, which are subject to research. Gallego-Cartagena *et al.* (16) examined the impact of stress conditions on the carotenogenic function of the Colombian mutant *D. Salina.* Recently, several reports showed that Microalgae accumulated with high concentrations of glycerol and β-carotene as an antioxidant photocatalyst as a hormonal regulatory agent (17). *D. salina* has been used as natural food. Some researchers have also proven that *D. salina* can increase the growth of several types of fish. Nutritional contents in *D. salina* such as beta carotene have also been shown to increase the immune system of fish (14). The present study aims to describe the nutritional content of *D. salina* and its effects on the growth and c0lor of goldfish *Carassius auratus*.

MATERIAL AND METHODS

Isolation and Identification of *D. salina*
Water samples were collected from a southwest part of the Parangipettai estuary region. This area is characterized by its high salinity values and the spread of halotolerant plants. Single cells of *Dunaliella salina* isolated from the collected water samples were spread on Petri plates containing Johnson′sagar dissolved in sterile seawater obtained from the marine. After incubation, cells from microcolonies on these plates were transferred aseptically to sterile test tubes containing Walne′s liquid media. The unialgal culture was incubated at 18°C under 12:12 hours dark: light cycles. Isolation and cultivation protocol was carried out according to (18,19). The isolated and purified algal culture was identified according to morphological properties (20,21).

Study and Management
Goldfishes were obtained from the CAS marine biology, Annamalai University. The obtained fishes were alienated into 4 groups of 6 fishes. Fishes were kept controlled under 2 per cycle of light and 2 per dark period. The controlled temperature and relative humidity during the experimental period were 27.0 ±3°C and 60.0 ±10%, respectively. They were fed a control diet or diets supplemented with *D. salina* (50%, 75%, and 100%) three times a day for 10 weeks. Ingredients (g/kg) of control and *Dunaliella salina* (DS) formulated feed were used during the study as shown in Table (1). The recorded parameters of water quality during the study were 7.4-8.2 mg/L of dissolved oxygen, 6.2-7.1 of pH, 27-29 °C of temperature, and <0.002 mg/L of total ammonia.

Analysis of Chemicals Composition of Feed Supplemented with *D. salina*
Ingredients of feed and fish samples were dried at 70°C in an air oven for constant weight. Both feed and fish samples were ground and analyzed for the determination of the dry matter, fat, crude protein, crude fibres, Ash, Nitrogen-free extract and energy components (22) (Table 2).

Feed Efficiency and Growth Performance
Bodyweight was recorded weekly using a digital balance (Chyo Petit Balance MK-500C-Japan). Fishes were anaesthetized using 0.1 g/L Tricaine methane sulfonates and dried before weighing. Bodyweight gain was determined by subtracting the final body weight from the initial body weight. The morphological characteristics of

fish such as body weight, total length, standard length, body depth and body thickness were determined. Tanks were cleaned before feeding using a symphony system. Diets were offered four times per day at 7.0 and 11.0 am, 2.30 pm and 6.30 pm. Feed efficiency was calculated by dividing feed intake by body weight gain.

Blood Sample Collection and Analysis
Blood samples were collected from the caudal vein at the end of the experimental period. The haematological parameters including erythrocyte counts [RBC] and packed cells volume [PCV]) were determined using a methodology of Hepler (23). Besides, glucose and total protein concentrations were determined (24-26).

Color Measurements of Fish
Color measurements of fish flesh minces were carried out using hunter according to the method of Young and Whittle (27). The color examination of L, a, and b values are used to illustrate color differences between groups.

Statistical Analysis
Statistical analysis was done according to the general linear model (GLM) of the SAS program (2008). Differences between control and *D. salina* treated groups (50%, 75% and 100%) were evaluated in growth performance, morphological, and chemical body characteristics of fish, blood profiles by one-way ANOVA. Duncan Multiple Range Test was used to test the effect of treatments (28). The data were presented as mean ± SEM.

RESULTS

Identification of *D. salina*
The isolated and purified strain was identified as *D. salina* by morphological examinations under Zeiss microscope (Figure 1) based on cell shapes, the presence of two flagella. The isolated species occurred as small green cells according to their growth stage and media conditions.

Performance Growth and Feed Efficiency
Growth performance and feed efficiency due to feeding 50,75, and 100% of *D. salina* to Gold Fish were presented in (Table 3and 4). Results showed that a significant increase in most of the growth performance parameters in *D. salina* groups compared to the control. Supplementing 50,75 and 100% of *D. salina* resulted in a significant increase in feed intake, feed efficiency compared to the control one. A feed containing 50% of *D. salina* had no significant effect in protein productive value when compared with the control versus 75 and 100% *D. salina*, which increased the parameters. The productive value of energy did not differ among control and *D. salina* treated groups.

Morphological Characterization
Morphological characteristics due to feeding 50,75, and 100% of *D. salina* to Gold Fish compared to control were presented in Table (5). The results showed that the bodyweight, total length, standard length, body depth and body thickness were significantly increased in *D. salina* groups compared to the other groups according to the concentration of *D. salina* diet used. As well as a conditional factor was found the highest in the 100% DS group.

Blood Profiles
Blood profile values (RBCs, PCV, glucose, TP) upon feeding 50,75, and 100% of *D. salina* to Gold Fish compared to control were presented in Table (6). Results showed that adding 50,75, and 100% of supplemented with *D. salina* did not show any significant effects on RBCs, PCV, glucose, TP when compared to the control.

Color Measurements of Fish
Fish scales coloration parameters upon feeding 50,75, and 100% of *D. salina* to Gold Fish compared to the control were presented in Table (7). Results showed that 100% *D. salina* caused a significant effect in terms of white flesh

compared to the control. There was a chronologically gradual white color of fish scales. The highest was in the control group followed by 50,75, and 100% groups, respectively. The feed containing 100% of *D. salina* caused a significant change in turning from whitish to yellowish flesh when compared with the control.

DISCUSSION

The need for nutritional supplements and continued dietary intake is necessary for the growth of the aquaculture sector (29) to cope with the increasing human population. Over the past decades, several studies have been carried out using feed ingredients for farmed fish and shellfish as sources of protein eg, *D. salina*. The results of the present study demonstrated the effects of supplementation of 50%, 75%, and 100% *D. salina* on the growth performance of goldfish, feed efficiency, morphological and color of flesh and blood profiles. The results indicated that the replacement of fishmeal with *D. salina* at a low level (50%) produced comparable results with the control feed regarding body weight gain, feed efficiency, morphological characteristics, and blood profiles. On the other hand, the high levels of *D. salina* (75% and 100%) were mainly increased in the parameters recorded compared to the control group, except for the fat content and the color of the meat, which have been significantly improved. The effects of *D. salina* 75% and 100% supplementations over control feed and *D. salina* 50on growth performance and feed efficiency could be attributed to palatability problems (30) as well as imbalances in food components.

Several studies (31,32) indicated an improvement in body fat due to the algae, as in 75% and 100% of the *D. salina* groups in this study. In this, survival and productive energy value did not differ between the control group and the *D. salina* group. *D. salina* algae have a high level of beta-carotene, glycerol, protein, and other fine chemicals (33-37). The chemical composition of *D. salina* algae showed 8.47% moisture, 54.17% crude protein, 0.80% fibre, 11.42% total fat, 18.47% extract. ether and 6.67% ash (26). The effect of partial replacement of fishmeal protein by microalgae on body growth, food consumption, and body composition of Atlantic cod has been studied by Walker and Berlinsky (30). They found no difference in survival and feed conversion rates, while feed consumption and growth were significantly reduced in seaweed-feed fish. Also, food intake improved in fish feed 15% algae compared to the fish feed of control.

The color of fish flesh is the first quality parameter evaluated by consumers and therefore an important quality element confirmed (38). Lipophilic pigments such as chlorophylls and carotenoids represent 3 to 5% of the dry biomass of algae (linked to market acceptance (39). In this study, a significant improvement in pulp color was obtained with the addition of 75% and 100% of *D. salina* compared to the control feed and 50% DS, which can be related to the pigment content of *D. salina*.

CONCLUSION

From the study, we infer that the supplementation of *D. salina* with feed diet to the goldfish has significantly increased the body weight, feed intake ability and other growth performances. However, it did not exhibit significant changes in blood parameters of the goldfishes which supplemented with *D. salina*. The significant encasement in color of fish minces was observed in *D. salina* supplemented fishes according to the concentrations used. Therefore, *D. salina* could be used as an effective feed supplementary for the improvement of color and body growth of goldfish.

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Table 1: Ingredients (g/kg) composition of control and *D. salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Fish powder	159.60	84.80	56.54	0.00
Soybean	280.80	290.40	299.60	332.40
Gluten	100.00	100.00	100.00	100.00
Maize	210.00	205.00	203.00	214.00
Wheat Bran	160.00	142.65	120.46	85.70
D. salina	0.00	82.15	122.40	166.90
Fish oil	64.60	70.00	73.00	76.00
Calcium diphosphate	5.00	5.00	5.00	5.00
Minerals & vitamins	5.00	5.00	5.00	5.00
Sodium chloride	10.00	10.00	10.00	10.00
Limestone	5.00	5.00	5.00	5.00
Total	1000	1000	1000	1000

Table 2: Chemical composition of feed diet supplemented with *D. salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Dry matter (%)	92.54±0.56	93.24±0.72	93.84±0.94	94.43±0.64
Crude protein (%)	30.00±0.51	32.00±0.74	33.00±0.91	34.00±0.36
Fat, %	11.16±0.08	11.32±0.86	12.08±0.64	12.78±0.51
Crude fiber (%)	3.42±0.06	3.82±0.46	4.14±0.12	4.84±0.34
Ash (%)	7.10±0.14	7.44±0.64	7.56±0.08	7.94±0.26
Nitrogen-free extract (%)	38.82±0.97	39.12±0.74	39.86±0.46	40.54±0.84

Table 3: Effect of feed supplemented with *D. salina* on goldfish growth

Parameters	Control	DS 50%	DS 75%	DS 100%
Initial body weight (g/kg)	12.35±0.10	12.86±0.17	13.32±0.21	13.92±0.19
Final body weight (g/kg)	42.51±0.20	44.46±0.11	45.52±0.19	47.87±1.07
Body weight gain (g/fish)	30.04±0.11	30.97±0.14	32.53±1.17	34.42±1.02
Body weight (%)	176.32±1.33	196.02±4.01	204.53±4.88	217.19±4.16
Daily body weight gain (g/fish)	0.36 ±0.00	0.42±0.002	0.56 ±0.01	0.64 ±0.14

Table 4: Feed efficiency of Gold Fish feed supplemented with *D. salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Average daily feed intake (g)	44.33±1.02	46.31±0.87	47.07±0.56	48.69±0.49
Average dry daily feed intake (g)	41.58±0.95	43.74±0.82	45.58±0.53	48.35±0.46
Daily feed intake (g)	2.02± 0.06	2.92 ± 0.05	3.19±0.03	4.08±0.04
Feed efficiency (%)	68.07± 1.8	72.31± 1.06	75.88±0.54	79.12± 2.67

Table 5: Morphological characteristics of Gold Fish feed supplemented with *D. salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Body weight (g)	27.06±5.65	31.70±5.58	34.39±4.29	36.08±3.22
Total length (cm)	11.75±0.79	12.56±0.77	13.37±0.67	15.11±0.51
Standard length (cm)	8.70±0.69	9.16±0.67	9.54±0.52	10.16±0.46
Body depth (cm)	2.29±0.20	2.86±0.24	3.13±0.15	3.84±0.13
Body thickness (cm)	1.28±0.15	1.76±0.19	1.92±0.13	2.18±0.10

Table 6: Blood parameters of Gold Fish feed supplemented with *Dunaliella salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Red blood cell (x10^6)	2.70±0.12	2.78±0.34	2.72±0.32	2.81±0.29
Packed cell volume (%)	28.24±2.78	28.73±2.51	29.13±3.83	29.67±3.21
Glucose (mg/100 ml)	45.33±5.13	47.33±4.05	46.67±4.14	47.33±4.51
Total protein (g/100 ml)	4.10±0.44	4.15±0.70	4.15±1.20	4.20±0.40

Table 7: Coloration of Gold Fish feed supplemented with *Dunaliella salina*

Parameters	Control	DS 50%	DS 75%	DS 100%
Lightness	52.08±2.26	55.14±2.76	57.91±2.45	58.66±2.27
Redness	6.31±1.07	8.92±1.27	9.66±1.65	10.55±1.04
Yellowness	16.25±0.21	16.76±1.23	17.09±0.57	17.87±0.47
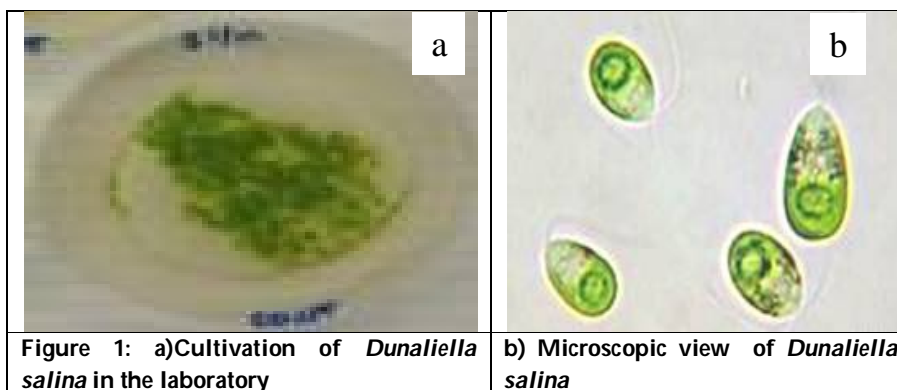
Figure 1: a)Cultivation of *Dunaliella salina* in the laboratory   b) Microscopic view of *Dunaliella salina*
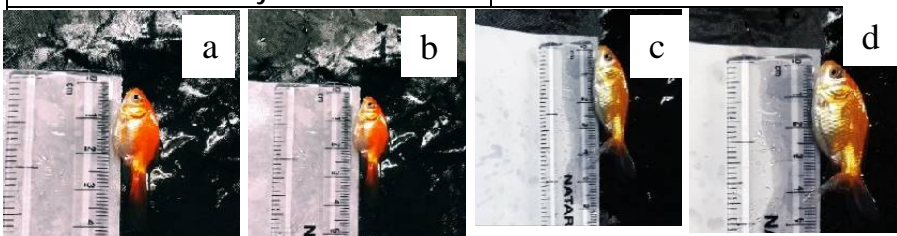
Figure 2: During the study used in Goldfish a) Normal fish feed (control) only given to the Goldfish did not change in morphology and color. b) Feed intake supplemented with *D. salina* (50%) of Goldfish showed morphology and color changes. c)supplemented with *D. salina* (75%) feed of Goldfish show significant changes to the body length and yellowish color. c)supplemented with *D. salina* (100%) intake to Goldfish increased the body length, body thickness, body depth, and yellowish color changes.

RESEARCH ARTICLE

Metal-Based Heterocyclic Compounds: Synthesis, Spectral Characterization and Pharmacological Investigation

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ABSTRACT

Cr(III) and Mn(II) metal complexes with 2-aminothiazole (ATZ) and azide ion ligands have been prepared by reacting chromium and manganese nitrates with ligand 1:1 molar ratio for complex formation. The prepared complexes have been characterized by using elemental analysis (C, H, N, M), molar conductance, IR and UV-Vis spectral analysis. Also, their CV and TGA behaviors have been studied. All the complexes have an octahedral structure. The ligands coordinated to the metal ions through the nitrogen atoms of amino group of 2-aminothiazole and azide ion. The antimicrobial activities of the ligands and their metal complexes have been studied by using different bacterial species viz., *Escherichia coli, Lactobacillus brevis, Micrococcus luteus, etc.,* and fungal species included viz., *C.albicans, Aspergillus Niger* etc., Moreover, the prepared complexes have been evaluated for antioxidant activities and DNA binding properties. The complexes have larger antioxidant activity as compared to free ligand. The DNA-binding properties have been designed by fluorescence-emissions method. The obtained results advise that the complexes effectively bind to DNA because of metal complexes are well-known to speed up the drug action and the capability of healing agent which can repeatedly be enhanced leading coordination with a metal ion.

Keywords: Cr(III), Mn(II), 2-aminothiazole, azide ion, biological activities.

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INTRODUCTION

Heterocyclic compounds containing sulphur and/or nitrogen atoms are of extensive use as building blocks in chemistry, wherever they are acknowledged as biologically active compounds with a wide range of action and textile dyes as well [1–3]. As a distinctive heterocyclic amine, 2-aminothiazole is the starting point for the synthesis of numerous compounds, together with sulfur drugs, biocides, fungicides dyes and chemical reaction accelerators and as intermediates in the synthesis of antibiotics, somewhere a huge number of 2-aminothiazoles have been substituted with different groups for pharmaceutical purposes [4–6] and are also second-hand in the syntheses of various types of dyes for synthetic fibers [7–15] next to their activity as corrosion inhibitors for mild steel protection [16,17] corrosion inhibitors for copper18 and as an ionophore in the structure of a lutetium(III)-selective membrane sensor [19]. These derivatives keep on attracting the awareness of biologists because of their prevalent use in the behavior of the biological systems.Furthermore, thiazole is classify under five-membered heterocyclic class of compounds and is establish in a lot of natural and synthetic agents. Obviously, thiazole is accessible in a large number of terrestrial and marine compounds with different pharmacological activities. Thiazole is also present in the vitamin B1 (Thiamine). In synthetic substituted thiazole derivatives, 2-aminothiazoles (20) have shown a variety of biological behavior such as anti-HIV, antitubercular, antifungal, antibacterial, anti-inflammatory, anticonvulsant, antidiabetic, antihypertensive, antiprotozoal, anticancer, dopaminergic, plasminogen activator inhibitor-1, neuro protective and antioxidant. This broad spectrum of activities makes 2-aminothiazole as an beautiful moiety in therapeutic chemistry [21].Literature search reveals that no work has been done on the mixed ligand complexes 2-aminothiazole and azide ion. In this research paper aims at the synthesis, characterization, thermal stability, antimicrobial, antioxidant and DNA- binding properties of Cr(III) and Mn(II) complexes containing 2-aminothiazole and azide ion as ligands.

MATERIALS AND METHODS

Materials
2-aminothiazole, NaN_3, $Cr(NO_3)_3.9H_2O$ and $Mn(NO_3)_4.3H_2O$ were purchased from Alfa Aaser Company and used as such. The organic solvents used were DMSO, $(\underline{CH_3})_2NC(O)H$, MeOH, EtOH which were of AnalaR grade and used as such without additional purification.
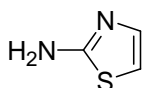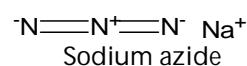
2-aminothiazole   Sodium azide

Synthesis of Metal Complexes

Synthesis of Cr(III) complex
0.75 g (7.5 mmol) of ATZ in EtOH and 0.49 g (7.53 mmol) of sodium azide in EtOH were added to the $Cr(NO_3)_3.9H_2O$ 1.00 g (2.5 mmol) in MeOH followed by microwave irradiation for few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. The obtained complex is with 82.05% yield and green colored.

Synthesis of Mn(II) complex
$Mn(NO_3)_4.3H_2O$ 1.00 g (3.64 mmol) in MeOH, 1.6 g (16 mmol) of ATZ in EtOH were added to 0.47 g (7.23 mmol) of sodium azide in EtOH the followed by microwave irradiation for few seconds after each addition by using IFB 25 BG-1S model microwave oven. The resulting impulsive was filtered off, washed with 1:1 ethanol: water mixture and dried under vacuum. The obtained complex is 80.50% yield and pale brown colored.

Instrumentations

C,H,N,S elemental analyses were performed using Thermo Finnegan make, Flash EA1112 Series CHNS(O) analyzer. The electrical conductivity measurements were conducted using 10^{-3}M solutions of the metal complex in acetonitrile with Systronic Conductivity Bridge (model number-304) at 30°C. The electronic spectra (DRS method) of Cr(III) and Mn(II) complexes were recorded on Varian, Cary 5000 model UV Spectrophotometer. Infra red spectra for the complexes and the free ligands were recorded on a Perkin Elmer, Spectrum RX-I, FT-IR spectrometer in KBr discs at room temperature. The cyclic voltammograms of the complexes were taken in acetonitrile medium using Princeton make (MC-Tech, Applied Research) equipment. Tetrabutylammoniumtetrafluoroborates was used as the supporting electrolyte. The thermogravimetric analyses of the complexes were carried out using Perkin Elmer Diamond TGA/DTA Instrument.

Biological Activities

Antimicrobial activity

The Cr(III) and Mn(II) complexes and the free ligands were tested for *in vitro* antibacterial and antifungal activity by well diffusion method using agar nutrient as medium. The antimicrobial activities of the free ligands and their complexes were evaluated against the strains cultured on potato dextrose agar as medium. The stock solution (10^{-2}M) was prepared by dissolving the compounds in DMSO and the solutions were successively diluted with different concentration. According to the typical method a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35°C. At the end of the period, inhibition zones formed on the medium were evaluated in millimeter (mm) diameter [22].

Antioxidant activity

In the assessment of antioxidant activity, the stock solution was diluted to final concentrations of 10–500 µg/ml. Ethanolic and DPPH solutions (EtOH 1 ml, DPPH 0.3 mmol) were added to sample solutions in DMSO (3 ml) at different concentrations (10–500 µg/ml). The mixture was shaken vigorously and adequate to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a ultra-violet spectrophotometer. The lower absorbance of the reaction mixture indicates superior free radical scavenging activity. EtOH was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity was designed by the following equation: DPPH Scavenging effect (%) = $A_0 – A_1 / A_0 \times 100$ where A_1 is the absorbance in the presence of the samples or standards and A_0 is the absorbance of the control reaction [23].

DNA binding properties

The DNA binding experiments involving interaction of the complexes with CT-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with HCl acid. The CT-DNA was dissolved in Tris-HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave the ratios of UV absorbance at 260 and 280 nm above 1.8, representing that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600 $dm^3mol^{-1}cm^{-1}$ at 260 nm. The stock solutions were stored at 4°C and used within 4 days [24]. For fluorescence-quenching experiments, DNA was pre-treated EtBr for 30 minutes. The ligand/ complexes were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm.

RESULTS AND DISCUSSION

Elemental analysis and metal estimation
From the C,H,N analysis and the metal estimation, the empirical formulae for the prepared complexes were determined. It is well consonance with the hypothetical values. The analytical data are given in the Table 1. The experimental values are in good agreement with the theoretical values (given in the parentheses).

Molar conductance
Molar conductance measurements of the $[Cr(N_3)_3(ATZ)_3]$ and $[Mn(N_3)_2(ATZ)_4]$ complexes carried out using acetonitrile as the solvent at the concentration of 10^{-3} M, indicates non-electrolytic nature (1:0 type) [25] and the conductivity values were found to be 67.52-78.45 $\Omega^{-1}cm^2\ mol^{-1}$. Thus the prepared complexes are non-electrolytic nature and there is no counter ion present in the out of the coordination sphere.

Magnetic moment and electronic spectra
In the diffused reflectance spectrum of $[Cr(N_3)_3(ATZ)_3]$ complex exhibits three absorption bands at 17623 cm^{-1}, 23455 cm^{-1} and 28756 cm^{-1}, which are assignable to the transitions $^4A_{2g} \rightarrow\ ^4T_{2g}$ (ν_1), $^4A_{2g} \rightarrow\ ^4T_{2g}(F)$ (ν_2) and $^4A_{2g} \rightarrow\ ^4T_{1g}(P)$ (ν_3) respectively indicates octahedral geometry around the Cr(III) metal ion [26]. The magnetic moment at room temperature is 3.35 B.M which corresponds to the expected value for octahedral geometry of Cr(III) complex and d^2sp^3 hybridisation.The electronic spectrum of $[Mn(N_3)_2(ATZ)_4]$ complex displays the weak absorbtion bands at 16712, 18345, 25947 and 36455 cm^{-1} which are characteristic of octahedral geometry around Mn(II) complex and corresponding to the transitions, $^6A_{1g} \rightarrow\ ^4T_{1g}(G)$ (ν_1), $^6A_{1g} \rightarrow\ ^4E_g(D)$ (ν_2), $^6A_{1g} \rightarrow\ ^4T_{1g}(P)$ (ν_3) and $^6A_{1g} \rightarrow\ ^4E_g(G)$ (ν_4) respectively. These bands are both Laporte and spin-forbidden. However, due to instantaneous distortion of the octahedral structure around the metal cation, weak bands sometimes appear. The magnetic moment of the Mn(II) complex is 4.45 B.M indicates octahedral structure around the metal ion [27]. The electronic spectra of Cr(III) and Mn(II) complexes shows Fig.1

FT-IR spectra
In order to determine the stretching mode of free ligands to the metal complex, the stretching frequencies of free ligands are compared with the metal complexes. The ligand 2- aminothiazole exhibited a strong band at 3332 cm^{-1} which could be assigned as $\nu(NH_2)$. The azide ion shows 645 cm^{-1} $\nu(N_3^-)$ frequency [28,29].The FT-IR spectra of the complexes were compared to those ligands 2-aminothiazole and the sodium azide. The frequency of the $\nu(NH_2)$ group was observed near 3320 cm^{-1} in complexes and they were shifted lower than those of resultant free ligand 2-aminothiazole. This shift refer to the coordination through a nitrogen atom of 2-aminothiazole and it is in good agreement with earlier examples of reported complexes. After that, the (N_3^-) group of the azide ion underwent higher frequency at after complexation, indicating the coordination of azide nitrogen to the metal atom. The low frequency region of the spectra revealed the presence of medium intensity bands in the region of 600-300 cm^{-1} due to $\nu(M-N)$ bond formation under investigation [30,31].

Cyclic voltammetry

Cr(III) Complex
The Cr(III) complex exhibits one electron transfer quasi-reversible process with a reduction peak at Epc = 0.6V with the corresponding oxidation peak at Epa= 1.3V. The peak separation (ΔEp) of this couple is 0.7V. With increasing scan rates, the ΔEp value also increases giving more evidence of quasi-reversible Cr(III)/Cr(II) couple [32]. The difference between forward and backward peak potentials can provide a rough evaluation of the degree of the reversibility.

Mn(II) complex

The Mn(II) complex is exhibit two well defined quasi-reversible one-electron cyclic responses were observed, one at Epc = 0.6V with corresponding oxidation peak at Epa = 0.9V and the other at Epc = -0.9V with the corresponding oxidation peak at Epa= -0.5V respectively. The ΔEp values for the first redox couple, 0.3V is higher than the second redox couple 0.4V. ΔEp value is higher for the complex due to the difference between the original complex and the reduced species [33].

Thermogravimetric analysis

The thermogravimetric analysis (TGA) of Cr(III) and Mn(II) complexes (with ATZ: 2-aminothiazole, azide ion ligands) show two significant weight loss with common and specific stages, termed as two stage degradation. The weight loss at the range of 190-280 ºC in TGA curves of complexes is termed the first stage of thermal degradation. In these case, the present weight loss is in the range of (19.14- 29.11%), which may be attributed to the decomposition of azide ion. The onset of second step decomposition occurs in the range of 280-340 ºC, which gives the loss of 2-aminothiazole (38.34 – 52.65%) bonding with the metal complexes. The experimental values are in full agreement with the percent weight calculated on the basis of stoichiometry proposed for the complexes [34].

Biological Activity

Antibacterial activity

The synthesized Cr(III) and Mn(II) complexes and the free ligand 2-aminothiazole are tested against the bacteria *viz.*, *Escherichia coli*, *Lactobacillus brevis*, *Micrococcus luteus*, *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Raoultella planticola*, and *Shigella flexneri* by agar-well diffusion method in *vitro* conditions as shown in Table 2. The complexes encompass prospective activity against the bacteria compared to free ligand 2-aminothiazole.

Antifungal activity

The antifungal activity of the free ligand 2-aminothiazole and the synthesized Cr(III) and Mn(II) complexes are tested against the fungi *viz.*, *C.albicans*, *Aspergillus Niger*, *Aspergillus Flavus*, *Aspergillus oryzae* and *Aspergillus sojae* by agar -well diffusion method as shown in Table 3. The complexes have enhanced activity against the fungi compared to free ligand.

Antioxidant activity (Radical Scavenging Activity)

The DPPH (2,2″-diphenyl-1-picrylhydrazyl) radical assay provides an easy and rapid way to evaluate the antiradical activities of antioxidants. Fortitude of the reaction kinetic types DPPHH is a product of the reaction between DPPH• and an antioxidant.

$$(AH): DPPH^\bullet + AH \rightarrow DPPHH + A^\bullet$$

The reversibility of the reaction is evaluated by the addition of DPPHH at the end of the reaction. If there is an increase in the percentage of remaining DPPH• at the plateau, the reaction is reversible, otherwise it is a complete reaction.The 2,2″-diphenyl-1-picrylhydrazyl radical was used as stable free radical electron accepts or hydrogen radical to become a stable diamagnetic molecule [35]. It is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis. The reduction capability of DPPH radical was determined by decrease in its absorbance at 517 nm induced by antioxidants. The graph was plotted with percentage scavenging effects on the y-axis and concentration (μg/mL.) on the x-axis. The scavenging ability of the ligand 2-aminothiazole, Cr(III) and Mn(II) complexes were compared with Vitamin C as a standard and shows enhanced activity, these results were in good agreement with previous metal complexes studies where the ligand has the antioxidant activity and it is expected that the metal moiety will increase its activity [36,37]. The Scavenging activity of free ligand and its complexes shows in Fig. 2.

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DNA Binding – Emission study

The binding of free ligand and its complexes to *CT*-DNA can be studied by competitive binding experiments. EtBr is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule [38]. The quenching extent of fluorescence of ethidium bromide binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with ethidium bromide causes appreciable reduction in the emission intensity as shown in Fig.4 & 5, indicating the replacement of the ethidium bromide fluorophore by the complex results in a decrease of the binding constant of the ethidium to the DNA.

According to the classical Stern-Volmer equation: $I_0/I = 1 + K_{sv}r$, where I_0 and I are the fluorescence intensities in the absence and the presence of complex respectively. K_{sv} is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plots illustrate that the quenching of ethidium bromide bound to DNA by the complex are in good agreement with the linear Stern-Volmer equation, which also indicates that the complex binds to DNA. In the plot of I_0/I versus $C_{Complex}/C_{DNA}$, K is given by the ratio of the slope to intercept. The data suggest that the interaction of complexes with DNA is strongest, which is consistent with the above absorption spectral results [39].

CONCLUSION

In the current study, our work were to synthesize and characterize the new Cr(III) and Mn(II) complexes (ATZ: 2-aminothiazole; N_3^-: Azide ion). The synthesized metal complexes were characterized by elemental analysis (C, H, N, M), molar conductance, IR and UV-Vis spectral analysis and also, their CV and TGA behaviors have been studied. Based on the analytical, molar conductance, spectral and magnetic moment, octahedral geometry have been suggested for the Cr(III) and Mn(II) complexes. The metal complexes have significant antimicrobial and antioxidant activities as compared to the free ligands. The effectiveness of the DNA binding of the complexes is being confirmed by means of change in intensity of emission in the case of emission spectral studies.

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Table 1.Analytical data of Cr(III) and Mn(II) Complexes

S. No.	Complex	Elements found (Calc) %				Conductance ($\Omega^{-1}cm^2\ mol^{-1}$)
		C	H	N	M	
1	$[Cr(N_3)_3(ATZ)_3]$	48.34 (51.07)	4.54 (4.90)	10.43 (11.05)	10.12 (11.32)	78.45
2	$[Mn(N_3)_2(ATZ)_4]$	51.99 (52.67)	4.48 (5.89)	11.32 (12.05)	11.22 (12.31)	67.52

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Table 2. Antibacterial activities free ligand 2-aminothiazole and its complexes

S. No.	Ligand/ Complexes	Conc. µg/ml	Zone of Inhibition in diameter (mm)						
			E.Coli	*L. brevis*	*M. luteus*	*P. vulgaris*	*P. aeruginosa*	*R. planticola*	*S. flexneri*
1	ATZ	100	06	11	07	03	05	08	05
		200	14	09	15	06	11	25	23
		400	20	13	20	12	18	20	13
2	$[Cr(N_3)_3(ATZ)_3]$	100	35	28	20	25	23	33	28
		200	44	42	32	33	28	42	38
		400	20	13	44	42	38	38	52
3	$[Mn(N_3)_2(ATZ)_4]$	100	18	18	20	20	13	20	20
		200	23	32	28	35	28	28	35
		400	28	44	38	52	35	38	52
00-15 Resistant; 16-30 Moderate; 31-46 Highly activity; 46-60 Enhanced activity									

Table 3. Antifungal activities free ligand 2-aminothiazole and its complexes

S.No.	Ligands/ Complexes	Conc. µg/ml	Zone of Inhibition in diameter (mm)				
			C. albicans	*Aspergillus Niger*	*Aspergillus Flavus*	*Aspergillus oryzae*	*Aspergillus sojae*
1	2-aminothiazole	100	08	05	07	03	05
		200	14	09	15	06	11
		400	20	13	20	12	18
2	$[Cr(N_3)_3(ATZ)_3]$	100	33	28	20	25	23
		200	42	38	32	33	28
		400	20	13	44	42	38
3	$[Mn(N_3)_2(ATZ)_4]$	100	18	18	20	20	13
		200	23	32	28	35	28
		400	28	44	38	52	35
00-15 Resistant; 16-30 Moderate; 31-46 Highly activity; 46-60 Enhanced activity							

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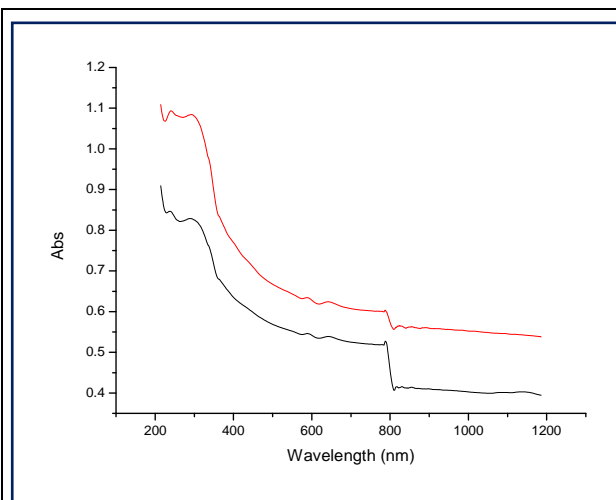
Fig.1.Electronic spectra of Cr(III) and Mn(II) Complexes
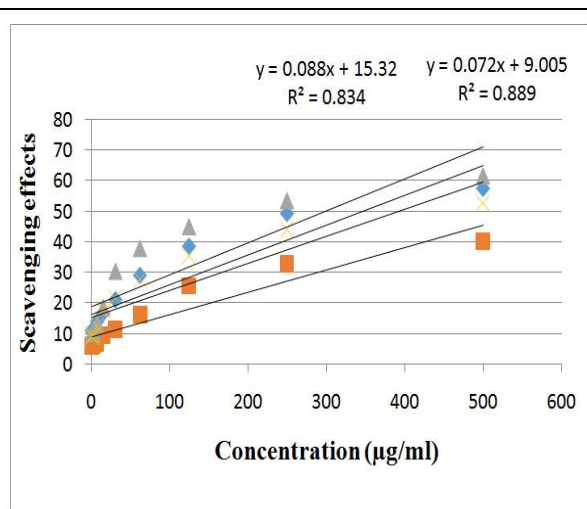
Fig.2.Scavenging activities of free ligands and their complexes
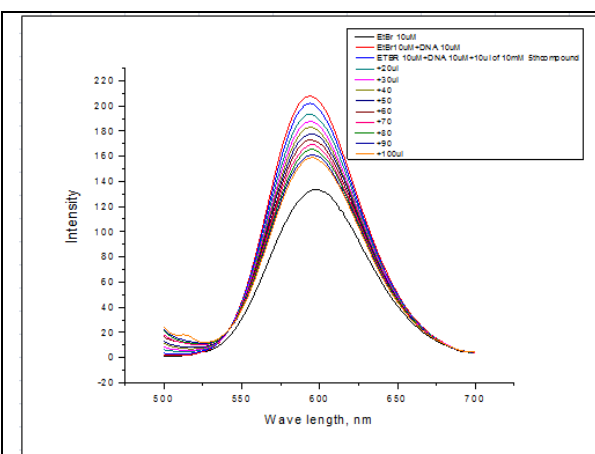
Fig.4.Emission spectrum of EtBr bound to DNA in the absence and presence of Cr(III) complex
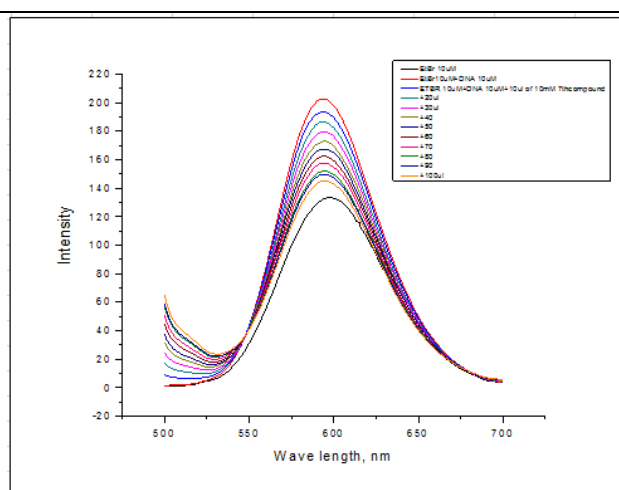
Fig.5.Emission spectrum of EtBr bound to DNA in the absence and presence of Mn(II) complex

REVIEW ARTICLE

Yogurt with the Addition of Herbs that Boost Natural Immunity - A Review

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ABSTRACT

Yogurt is a milk product found in the bacterial milk fermentation process. Yogurt is rich in riboflavin, vitamin B6, B12, calcium, protein and healthy foods rich in phosphorus. In this paper, various properties of some herbs were studied in terms of their immune-boosting properties. In this process, milk is added to the basic formulation and is mixed with sp., Bulgaricus, Lactobacillus delbrocoi and Streptococcus thermophilus. This study is done with a combination of yogurt and various immune herbal remedies. The Physico-chemical analysis and sensory responses of strong yogurt are also discussed in the paper.

Keywords: Yogurt, herbs, healthy ingredients, immune, boosting, healthy ingredients, etc.

INTRODUCTION

In human beings, corona viruses are involved in the category of viruses that cause the common cold and Acute Respiratory Syndrome (SARS). Emerging diseases such as SARS possess a major threat to public health. The virus has spread rapidly to many countries and has been declared an epidemic by the World Health Organization. By consuming good amount of water, foods rich in minerals such as zinc, magnesium, different micronutrients, herbs, vitamins C and a better lifestyle can be beneficial to promote health and help to prevent this infection. Include yogurt or yogurt in the diet of the elderly or by including them in your diet. It not only boosts their immunity but also improves their gut. Herbal treatment is very well known in Traditional Chinese Medicine (TCM). The health benefits of yogurt are well known and many yogurt-based products are consumed by people around the world (El-Shibinyet al., 2018) Herbal and spicy flavored yogurt has been developed in these products. Behrad *et al.* (2009) Adding cinnamon does not modify yogurt fermentation but allows Lactobacillus spp to increase during refrigerated storage. Cinnamon-yogurt containing probiotic bacteria inhibited the growth of Helicobacter pylori in vitro. The ability of this herb-yogurt to prevent the development of H. pylori requires further research in the highly acidic environment

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of the stomach. In addition, Bakrm and Salihin (2013) reported that the extraction of cinnamon yuvaram and allium sativum in goat, cow, and camel milk did not have a significant effect on acidification by fermentation.

Furthermore, the presence of these two herbs in milk improved the proteolytic activity of cultures, while cow's milk yogurt had the highest proteolytic activity. a. Sativam and c. Vroom promotes lactic acid bacteria (LAB) by fermentation in three types of yogurt. Therefore, A. sativum and c for the development of LAB in yogurt during fermentation. In addition, Helal and Tagliazucchi (2018) found that the addition of cinnamon powder in yogurt increased the total phenolic content and radical scavenging activity compared to natural yogurt. They show that 34.7% of the total phenolic compounds in cinnamon water extracts are formed in cinnamon-fortified yogurts, indicating that the remaining compound is actually bound to milk protein. In addition, the in-vitro digestion of yogurt is strengthened with cinnamon, resulting in gastro-intestinal stability of cinnamon polyphenols. Cinnamon-fortified yogurt can be considered an important source of nutrient bioactive polyphenols that release phenolic compounds that interact with milk protein. Recovered compounds in yogurt fortified with cinnamon are present in digested cinnamon water extracts. These results showed that the yogurt matrix improves the bioactivity and gastrointestinal stability of cinnamon polyphenols. Cinnamon yogurt can be considered an important source of nutritious biosexable polyphenols.

Background

Yogurt means I. The synthetic product obtained from a mixture of two or more products by lactic acid fermentation by the action of pasteurized, boiled, or condensed milk, pasteurized skimmed milk and/or pasteurized cream or *Delbroxy* SP. *Bulgaricus* and *S. thermophilus.* These may include *Bifidobacterium Bifidus, Lactobacillus acidophilus,* and other cultures of bacteria that produce adequate lactic acid. High-temperature pasteurization of the yogurt mixture is used to achieve a smooth and firm body. Nonfat dry milk or stabilizers can also be added to increase water holding capacity and thus improve its body. The latter is especially true for low-fat products.

Many types of yogurt are commercially available. These are plain (no extra flavor), flavorful, liquid, carbonated, and low lactose. Delicious yogurts have a sundae style in which the fruit puree is placed at the bottom of the cup and mixed with the yogurt before consumption. Another type is Swiss-style, in which plain yogurt is mixed with fruit puree before packaging. Such yogurts require high levels of solids and stabilizers to achieve the desired high viscosity. Liquid yogurt is popular in Europe, Canada, and Japan and is different from gel-type yogurt because they are homogeneous, in a miserable state. No whey should be separated during storage. There are several major stages in the preparation of yogurt: standardization, homogenization, heat treatment, cooling of the incubation temperature, vaccination with yogurt cultures, incubation, refrigeration, and packaging (Mistry, 2001).

Amirdivani and Baba (2011) have stated that herbal extracts increase the bacterial and yogurt acidity of milk yogurt. The proteolytic activity of yogurt bacteria peaked after yogurt and basil in the presence of peppermint during fermentation and refrigerated storage. These herbal yogurts have a high content of bioactive peptides and excellent antioxidant activity, offering consumers a new range of yogurts with multifunctional health properties. Hanifah *et al.* (2016) In the manufacture of goat milk-yogurt I. Acidophilus and Rosel extract are included. This yogurt is characterized by antimicrobial activity and a wide selection of Gram-positive and Gram-negative bacteria (Bacillus serius, Escherichia coli, Staphylococcus aureus, and Salmonella typhi), which can be said to have high antimicrobial activity, including antimicrobial peptides. . In addition, Liu (2018) found that Fajuan brick-tea (FZT) was compatible with yogurt and enhanced its proteolytic and b-galactosidase activity. FAST also, low synergy, improved viscosity, and the overall count of Lactobacillus acidophilus and Streptococcus thermophilus. Increased antioxidant activity of prepared yogurt and is relatively stable in refrigeration.

Kumar *et al.* (2013) prepared yogurt is replaced with crushed leaf mint at 2, 4, and 6%. They found that 2% of mint level yogurt was right for all the sensory properties. In addition, the shelf life of yogurt is 10 days 5 _ c. Mint flavored yogurt is suggested for use in burgers, sandwiches, and other bakery items. In addition, Ghalem and Zouaoui (2013)

replaced the yogurt with *Rosmarinus Officinalis* oil at a ratio of 0.14, 0.21, 0.29, and 0.36 g / L and continued for 21 days. The panelists combined the herbal yogurt with 0.14 g / l essential yogurt to give the maximum score for taste, flavor, and texture. In addition, r. Officinal's Essential Oil additionally improves the properties of yogurt by lowering pH and lactose values and dry matter, while increasing acidity, protein, ash, and fat content. In general, storage time does not affect the physicochemical properties of prepared yogurt.

Prep medicinal and culinary herbs are popular in food preparations because they are Photochemical that affect the integrity of health benefits (Exarchau et al., 2002).Deer are generally rich in antioxidant activity, which contributes to the oxidative degradation of lipids and thus improves the quality and nutritional value of foods and has significant antioxidant and antimicrobial activity. Basil and green tea extract and essential oils are on display. Basil prevents almost all types of infections from viruses, bacteria, fungi, and protozoa. Recent studies suggest that it may also help prevent the growth of HIV and cancer cells. Eugenol and sinol, essential oils of basil, cure infections. Basil helps prevent tuberculosis due to its antibiotic properties. Green tea exhibits biochemical properties and anti-oxidant, anticarcinogenic, antimicrobial heavy metal removal, and detoxification properties.

Functional Properties of Herbs
Natural antioxidants derived from plants are considered safe in nature and have been used by humans since ancient times. Antioxidants help delay the oxidation of molecules by preventing initiation, diffusion or propagation of oxidizing chain reactions by free radicals and may reduce oxidative damage to the human body. The antioxidant properties of herbs are due to presence of some vitamins, flavonoids, terpenoids, carotenoids and phytoestrogens. Some examples of spice and herbs containing antioxidants are: basil, cinnamon, clove, dill, ginger, mint, oregano, rosemary, saffron, sage, thyme, etc

There are a number of naturally occurring herbal herbs that have the potential for hypotension / antihypertensive. These herbs help control blood pressure by stimulating the bodily systems in humans. Some examples of herbs that describe fictitious properties are garlic, celery, tea, celery, ginger, lavender, drumstick, basil, kudju, radish, ravelfia, sesame, etc. Anginensin can inhibit the angiotensin-enzyme-enzyme involved in converting the enzyme (ACE).) Inhibitors, a component of the rennin-angiotensin system that regulates blood pressure, thereby lowering blood

Ashwagandha *(Vithania somnifera)*
Ashwagandha is also known as Indian Ginseng or Winter Cherry. It is one of the most revered medicinal plants used in Indian Ayurveda for centuries. It grows in the Solansi family, Africa, the Mediterranean and India. It is considered to be an adaptogen, which enhances the odor of the horse, which facilitates the ability to withstand stress and has antioxidant properties. Cytoindocytes and acylsterilglucosides are antis tress agents in equine. Horseradish contains steroid lactones, alkaloids and flavonoids. Large numbers of alkaloids- nicotine, somniferin, vithenin, vithenolides etc. are found in their source. The roots of the plant show hypotension, biocardic, antitumor, respiratory stimulatory activity and immune-stimulating effects

Cinnamon worm, synonym c. Gelatinous
Cinnamon is an herb traditionally used in many ancient cultures. They are small evergreen trees, belonging to the larcy family, native to Sri Lanka and southern India. In addition to being used as a spice and flavoring agent, cinnamon is added to some mouth-watering foods. Cinnamon has been implicated in reducing the risk of colon cancer, acting as a coagulant and preventing bleeding Cinnamon increases blood circulation in the uterus and promotes tissue regeneration. Cinnamon essential oils are antimicrobial, antifungal, antioxidant and anti-diabetic. Salmonella typhi, Salmonella paratyphi A, e. Food diseases such as coli, cinnamon. Arias shows complete antibacterial effects against Pseudomonas fluorescence and Bacillus gicenformiformes They also enforce a ban on fungal species. The minimum resistance concentration (MIC) value for cinnamon essential oil is 1.25 to 5.0%.

Cumin (Siminum)

Cumin is a small annual herbaceous plant that is a member of the aromatic plant family Ambelliferae. Cumin is cultivated in India, Morocco, Iran, Turkey, China and the United States. Plant seeds are used to add flavor to spicy dishes. They are also used to stimulate appetite and reduce stomach ailments. Dry suspension of cumin inhibits the growth of mycelium, toxin production or aflatoxin production by Aspergillus acreius, Candida versicolor and Candida flavus. Antibacterial activity has been observed in both beneficial and pathogenic Gram-positive and Gram-negative bacterial species. Mainly cuminaldehyde, carvone, limonene and linalool and to some extent limonene, eugenol, pinen etc. contribute to the antimicrobial action of cumin. The fatty oil (mainly petroselic acid) in cumin also has antimicrobial effects. The antifungal action of cumin is recorded on human pathogens including food, dermatophytes, vibrio spp., Yeast, aflatoxin and mycotoxin producers. The MIC value of cumin essential oil ranges from 6.25 to 12.5%.

Mint *(Mentha piperita)*

The mint plant is about 2-3 feet tall. The dark green, fragrant leaves grow in contrast to the white flowers. Peppermint is native to Europe and Asia, native to North America and grows wild in humid, temperate regions. Some varieties are native to South Africa, South America and Australia. The leaves and stems containing menthol (volatile oil) are used in medicine and as a flavoring in food.

Peppermint, the famous flavor of tea, helps to soothe the stomach or aid digestion. It has a calming and numbing effect and is often used to treat headaches, skin irritation, nausea, diarrhea, stru cramps, flatulence and anxiety related to depression. Menthol and methyl salicylate, the main components of peppermint, have antispasmodic and sedative effects on the gastrointestinal tract. Several studies support the use of peppermint for indigestion and irritable bowel syndrome.

Basil *(Ocimum basilicum)*

Basil is commonly known as basil. Since ancient times, this plant is known for its medicinal properties. The aqueous extract of Osimum hysterectomy showed significant reductions in blood glucose levels in normal and elexon-induced diabetic mice. Significant reduction in blood sugar, uric acid, total amino acids, total cholesterol, triglyceride and total lipids indicated the hypoglycemic and hypolipidemic effects of basil in diabetic rats. The plant has also been shown to have anti-asthma, anti-stress, anti-bacterial, anti-fungal, antiviral, anti-tumor, gastric anti-ulcer activity, antioxidant, anti-mutagenic and anti-inflammatory properties. Mint and basil (*Ocimum basilicum*) are traditionally grown in Europe and Central Asia for the production of fresh herbs, dried leaves or essential oils [42]. Parts of these plants find their applications as culinary herbs or as a minor supplement to salads and herbal teas, and as aromatic agents in the food, herbal, functional food and nutritional industries.

Aloe *(Aloe barbadensis Miller)*

Aloe vera has a long history as a topical and oral treatment. It is a parabolic, perennial plant, and belongs to the Santoraceae family. Aloe vera is a native plant of the Mediterranean region, but it is found all over the world. Aloe vera leaves can be divided into two main parts: green skin, which is rich in 1,8-dihydroxyanthroquinone derivatives and their glycosides, and is a colorless pulp rich in complex carbohydrates. Mucilageous jelly, like parenchyma, is called aloe vera paste or gel. This gel has a large content (95-99%) in water and contains carbohydrates as well as proteins, lipids, amino acids, vitamins, enzymes, inorganic compounds and small organic compounds. Asmanan (acetylated glucomannan), also known as carcinogen, is one of the most popular polysaccharides found in aloe vera gel. Aloe vera contains essential micronutrients and active phyto-chemicals such as ascorbic acid, tocopherol and phenolic compounds capable of reducing free radicals that cause CVD, carcinogenesis and oxidative reactions associated with aging. The plant has antimicrobial, laxative, radiation protection, antioxidant, anti-inflammatory, anti-tumor, anti-diabetic, antiallergic, as well as hypoglycemic, gastroprotective, immunomodulatory and wound healing effects

Herbal Processing

Herbs and spices contain volatile and volatile compounds and oils. Most spice flavors are due to volatile oil, but volatile flavor compounds can be made by heating with volatile oil. The flavor components weaken or disappear at high temperatures and the flavor components of the spices become more volatile with increasing temperature during processing. Radiation can help prolong the lifespan of herbs and reduce food-related health risks caused by pathogenic microorganisms. Currently, radiation treatment for a wide variety of foods and supplements is permitted in many countries Supercritical Fluid Extraction (SFE) has gained considerable approval these days as an alternative to conventional solvent extraction for separating organic compounds with herbs. SFE has been used successfully for the extraction of a wide variety of organic compounds from herbs and plants. SFE can be used effectively to clean up pesticides contained in herbicides.

Yogurt , Labneh (Concentrated Yogurt)

Herbal yogurt is made using cinnamon, herbs and licorice that contain probiotic bacteria. The presence of herbs did not affect the probiotic population during storage and the total permeable acidity between pH and herbal-yogurt and plain-yogurt during storage. Cinnamon-yogurt water extract has shown a very inhibitory effect on the growth of Helicobacter pylori. *L. Acidophilus* and L. Different types of herbal yoghurts were infused using Plantarum (1: 1v / v) species with pitted herbs with standard milk and incubated at 40oC for 6 hours; the herbs are basil leaf (Ocimum sanctum), *Mentha arvensis* and *Coriandrum sativum*. They reported more enzymatic activity of as-de-galactosidase in herbal yogurt than in controlling yogurt (without any herbicides). Among herbal yogurts, basil yogurt showed the highest D-D-galactosidase activity. Cinnamon-containing yogurt (6.0% cinnamon dissolved in 6.0% milk) can be successfully stored under refrigeration up to 28 days; The number of Lactobacillus species fresh in such yogurt was 19.46 x 106 cfu / ml; The number of Streptococcus thermophilus increases in cinnamon-containing yogurt for up to 7 days, when stored in the refrigerator. Labneh is a yogurt concentrate that is very popular in the Middle East. Labneh (23.0% TS) containing 0.2 ppm in each thyme, marjoram and sage essential oils extended shelf life (up to 21 days control) at 5 C. Mold from the 14th day of storage, cinnamon (23.70% TS) has a longer shelf life compared to T if it contains 0.3% cinnamon essential oil (i.e. more than 8 days) when stored at 5 C. Controls show the presence of yeast and mold from the 14th day of storage, cinnamon (23.70% TS) 0.3% cinnamon essential oil has a longer shelf life when stored at 5 ° C (i.e. more days than the control product when stored at 8 6oC

Dahi (Indian Yogurt) and Lassi (Fermented Beverage)

A functional lassi and yoghurt has been developed by replacing the herb aloe and probiotics at Karnal, NDRI, India. Animal studies of such herbal lassi have shown that lassi has better immunity than controlling. Aloe vera supplements contribute to the development of probiotic strains in fermented product. Probiotic viability during storage period of 12 days> 7 log10cfu / ml

Spread Yogurt

An herbal flavor based on yogurt spread by Kumar et al. Yogurt concentration (60% TS) was prepared to allow from whey yogurt. To concentrate this yogurt, salt and cilantro are added at the rate of 2%. To optimize mint levels, soft raw leaves were added at 2, 4 and 6% by concentrating the yogurt. The prevalence of yogurt made with 2% mint has higher sensory scores than those using the extra rate (i.e. levels 4 and 6%). The shelf life of the spread is 10 days when stored in 5 .C. Such mint spreads on flavored sandwiches, burgers, chapatis and other spicy bakery items.

Policy

MATERIALS AND METHOD

Herbs and Chemicals: Lactobacillus bulgrius and Streptococcus thermophilus and 1,1-diphenyl-2 including yoghurt, homogeneous and pasteurized milk, horseradish, other herbs and basil leaves purchased from the local market for starter culture. DPPH).

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Starter Culture Preparation: The starter culture was prepared by vaccinating the following yoghurt bacterial mixture with Lactobacillus bulgicus and Streptococcus thermophilus into pasteurized and homogeneous milk. The milk bacteria mixture is incubated overnight at 410C and the yogurt is stored at 40C. 2 weeks.

Herbal Water Extract Preparations: The herbal water extract was prepared by centrifugation (2000 rpm; 15 minutes to 40 minutes) by soaking each herb in distilled water overnight (1:10). Supernatant was used in cutting and cooling and in the preparation of herbal yogurt.

Herbal Yogurt Preparation: Homogeneous and pasteurized milk is procured from the local market. *L.Bulgaricus* and *S. athyophilus*, skim milk powder (2g), herbal water extract 1L milk dissolved starter culture (2%). Milk was added to correct the firmness (9.8%). The mixture is divided into plastic cups, plain yogurt is made without essential herbs (pH 4.5) and the incubation is done at 410C.

Yogurt Preparation

Plain and herbal (10 g) yogurts were homogenized with 2.5 ml of distilled water. The pH of the yogurt was determined and the yogurts were then acidified to pH 4.0 with HCl (0.1 M). Heat the yoghurt in a water bath (45 C) for 10 minutes, followed by centrifugation (5000 g, 10 min at 4 C). NaOH (0.1M) was added to pH 7.0 to adjust the pH of the supernatant. The supernatants were again centrifuged at (5000 g, 10 min 4 ° C) and the supernatant was harvested and stored in the -20 ° freezer until required for analysis.

Measurement of pH and Titanium Acidity (TA):

The pH of the yogurt is measured using a digital pH meter. Titratable acidity (TA) is expressed as a percentage of lactic acid.

RESULTS AND DISCUSSION

PH change in yogurt during storage: The pH for plain yogurt is similar to the pH of herbal yogurt. The amount of pH for yogurt decreased during refrigerated storage. The pH ($p < 0.05$) for all yoghurts decreased from 4.5 to 4.41 to 4.26 from the initial values by 7 days of storage. DPPH Inhibition All herbal formulations have a higher antioxidant activity (P <0.05) than plain yogurt during its storage period. Grantia yogurt (78.22 ant 1%) and basil yogurt (54.799.9%) recorded the highest antioxidant activity on day 7 during the 7-day storage period. Chowdhury, *et al.* 2008, made yogurt-based basil leaf (*Ocimum sanctum*), mint leaves (*Mentha arvensis*) and coriander (*coriander sativanam*). Basil yogurt has a maximum of one-galactosidase activity compared to other herbal yogurt. It has been suggested that the depletion of phenolic compounds and the formation of free amino acids by bacteria, even at low temperatures, alter the antioxidant activity of yogurt (Blum, 1998; McQuee and Shetty, 2005) The combination of green tea and basil enhances the antioxidant activity of herbal yogurt compared to plain yogurt. The amount of herbs increases. Affects the antioxidant action of yogurt within 7 days of refrigerated storage. Based on the presence of herbal herbal yogurt. Probiotics and herbs can provide a new range of yogurt with the multifunctional health effects that consumers want.

Sensory symptoms Yogurt treatment is classified into two groups, the highest scoring plain yogurts in the group, the second group came to boast of herbs that boast herbs. However, the presence and smell of moringa yoghurt received excellent marks similar to plain yoghurt on the first day. Meanwhile, both company totals gave a good score level. It is clear that appearance, color, and overall acceptability are affected by the brightness of the product as control yoghurts have the highest scores compared to the rest of the treatments after fresh treatment or storage. The sensory scores were the same for all parameters on the first day after 15 days of storing all the yoghurts, although there was no significant difference (p> 0.05). Different results have been reported depending on the type of culture and the concentration of green tea. The sensory results of combining bio-yogurt with green tea, horseradish and other herbs

are distilled at different concentrations (5%, 10% and 15%), indicating that there is no significant difference between the sensory nodes: Acidophilus milk has lower values High sensitivity scores have been reported from Malaysian disasters, who prefer green tea yogurt over controls. Madukwe et al In relation to Moringa and in agreement with our results. Moringa is known to prefer the control color over the drink. Combining banana with yogurt with moringa leaf powder is comparable to control, whereas alone moringa yogurt wasn't appreciated. Since prehistoric times, herbs have been used not only as a flavoring food but also for its medicinal properties. The antimicrobial and antioxidant components found in herbs make them effective as preservatives. The inclusion of herbs in dairy production improves human health and medical condition. Herbal products that promise health benefits must comply with regulatory requirements related to safety, efficacy, quality testing and marketing standardization procedures. It should be without any side effects. Requires systematic scientific studies and documentation.

Herbs and spices are natural ingredients that are widely used not only as a flavoring food but also for its health properties. The antioxidant, antimicrobial and antimicrobial components found in herbs can enhance human health and medical condition. Dairy products are always the choice of food researchers to test consumer preferences for innovation in products. The combination of herbs or spices in dairy products with gift health benefits must meet the requirements to avoid any side effects related to quality testing, safety, efficacy, pricing and marketing approval processes. However, the development of dairy products rich in herbs and spices requires a number of technical challenges, including the use of nano-form to increase their availability and efficiency. In addition, more research is needed to improve the existing methodology and to develop new approaches to improve and improve the methods of separating the active ingredients from herbs and spices.

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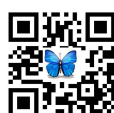
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RESEARCH ARTICLE

Phytochemical and Cytotoxicity Studies on *Andrographis paniculata*

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ABSTRACT

Antrographis paniculata, the herb that usually can grow up to a height of 30 -110 cm with its green color stem. It has a special lanced shape of leave and the leaves are hairless. Leaves size is about 8 cm long and 2.5 cm wide. The study was designed to evaluate the phytochemical compositions of *Antrographis paniculata* methanol and chloroform extracts. Also aimed to study antibacterial activity against various bacterial pathogens, antioxidant and cytotoxic activity on normal Vero cells. The results showed that the methanolic and chloroform extracts showed effective antibacterial and antioxidant activity, moreover did not exhibit significant cytotoxicity on normal Vero cells. Therefore, the use of *Antrographis paniculata* for pharmacological applications could be effective after a clinical trial.

Keywords: *Andrographis paniculata*, phytochemical screening, antibacterial, cytotoxicity.

INTRODUCTION

Medicinal plants are one of the foremost important components of the non-wood forest products sector which supplies over 80 percent of India's net forest export earnings annually. The curative properties of drugs are due to the presence of complex chemical substances of varied composition in one or more parts of these plants. These plant metabolites, according to their composition, are grouped as alkaloids, flavonoids, terpenoids, carotenoids, glycosides, corticosteroids, essential oils, and phenolic compounds. These bioactive principles are used in the treatment of various diseases like cholera, diabetes, diarrhea, fever, skin problems, aging, wound healing, and ophthalmic disorders (1).

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Different type of isolation methods has been used to get pharmacologically active compounds that can use as a drug for different diseases. The methods which include isolation from plants and other natural sources, combinatorial chemistry, synthetic chemistry, and molecular modeling (2) natural products also provide a starting point for lab syntheses with diverse structures and often with multiple stereocenters that can be challenging synthetically (3,4).Sometimes new chemical structures are very difficult to found during drug discovery from medicinal plants, in such cases known compounds with new biological activity can provide important drug directions. Molecular target playsan important role in drug discovery, since the sequencing of the human genome, a lot new molecular targets have been identified as important and useful in various diseases (5).

The developments of high-throughput screening techniques may show to the point and more selective activity directed towards these targets when using the reported compounds from medicinal plants. It has also been known that the compounds isolated from traditionally used medicinal plants are shown to act on newly validated molecular targets, one example is indirubin, which targeted and inhibit cyclin-dependent kinases (6,7).The importance of search and exploitation of natural antimicrobials and antioxidants of plant origin has greatly increased in recent years. Though in traditional medicine *A. paniculata* has been claimed to possess antimicrobial and antioxidant properties, very few studies have shown the antibacterial and antioxidant activity and to our knowledge, no study has been carried out to know its antifungal property against dermatophytes. In all the studies carried out till now on *A. paniculata*, the whole plant has been used. So, the present study aimed to investigate the antibacterial, antifungal, and antioxidant activities of *A. paniculata* leaves in various solvent systems and to determine the solvent that best extracts the compounds responsible for these activities. Aniel Kumar *et al.,* (8)*. Andrographis paniculata* Burm.F(Acanthaceae) is an efficient medicinal plant in the Indian systems of medicine. Traditionally the *A. paniculata* leaves are used as/in influenza, bronchitis, gonorrhea, cholera, fertility, and antibacterial, anticancer, antidiabetic, anti-inflammatory, and anti-snake venom, etc. This study was analyzed in phytochemicals of *A. paniculata* extract and then antibacterial activity, antioxidant activity, and Cytotoxicity activity.

MATERIALS AND METHODS

Collection of Plant Material

The whole plant of *A. paniculata* (Figure 1) was collected from the trial plots at the campus of the Faculty of Agriculture, and verified by Dr. Manivannan Professor and Head, Department of Horticulture Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu. Fresh and healthy leaf, stem, and root were used to extract bioactive fractions of *A. paniculata*. The parts of plants that were used for the extract were washed with water to remove soil and dust particles. Then they were dried under a shaded place. Dried materials were blended to form a fine powder and store in airtight bottles (9).

Preparation of Organic Solvent Extracts

Fifty grams each of the powdered material was extracted initially with 300 ml of chloroform, and methanol separately for 24 h at 23 ± 2^oC. The extract was filtered with sterile Whatman filter paper into a clean conical flask. The second extraction was carried out with the same amount of solvent for another 24 h at 23 ± 2^oC and filtered. The extracts were later pooled and transferred into the sample holder of the rotary flash evaporator for the evaporation of the solvents. The filtrate was centrifuged at 3000 rpm for 15 min and evaporated to dryness in a water bath. The extracts were stored at 4°C in the air (10).

Phytochemical Screening

Phytochemicals are secondary metabolites that are synthesized from the primary metabolites of the plant. Phytochemicals consist of several chemical classes such as terpenoids, phenols, flavonoids, saponins, and tannins that have several biological effects on microorganisms (11).

Alkaloids Test

Each of the crude extracts was dissolved in 1.0 mL of 1 % hydrochloric acid, HCl, and treated with three drops of Dragendorff's reagent. Orange-red precipitation was indicated the presence of the alkaloid compounds (12).

Phenols (Ferric chloride Test)

Each of the crude extracts was added with 1.0 mL of 5 % ferric chloride, FeCl3 solution. The blackish green color was indicated the presence of flavonoids (13).

Steroids Test

Each of the crude extracts was dissolved in 1.0 ml of chloroform. A 2.0 mL of concentrated sulphuric acid (H_2SO_4) was slowly added to form a lower layer which is in yellow with green fluorescence. A reddish-brown color on the upper layer was interpreted as a steroid ring (12).

Cardiac Glycosides Test

Each of the crude extracts was dissolved in 1.0 mL of glacial acetic acid containing one drop of 1 % FeCl3. This was followed by 2.0 mL concentrated H_2SO_4. A brown ring obtained indicates the presence of a deoxy sugar. A violet ring may appear below the ring. Meanwhile, a greenish color ring might form in the acetic acid layer and spread throughout this layer (14).

Reducing Sugars Test

Each of the crude extracts was mixed with 1.5 mL of distilled water to form an aqueous solution. Then, the extract was added to an equal volume of boiling Fehling A and B solution in separated test tubes. The presence of reducing sugars was interpreted as the formation of a brick-red precipitate (15).

Tannins Test

Each of the crude extracts was added with 2.0 mL of distilled water and boiled in a test tube and three drops of 0.1 % of FeCl3 were added. The brownish-green coloration indicates the presence of tannins (14).

Terpenoids (Salkowski Test)

Each of the crude extracts was added with 0.4 mL of chloroform. A 0.6 mL of concentrated H_2SO_4 was added, two layers will be formed. The reddish-brown coloration in the interface is indicated the presence of terpenoids (16).

Saponins Test

Each of the crude extracts was added with 1.0 mL of distilled water and boiled in a test tube for 15 min. After cooling, the mixture was shaken and a persistent froth was indicated the presence of tannin (17)

Flavonoids (Alkaline Reagent Test)

Each of the crude extracts was added with 5.0 mL of distilled water and boiled for 5 min. Three drops of 20 % NaOH solution were added. The color change from colorless to yellow. Then, 5 drops of 1 % of HCl were added to the mixture. The presence of flavonoids was interpreted by observing the decolorization of the yellow color (18).

Antibacterial Activity of *A. paniculate* Extracts

Antibacterial activity was determined by the disc diffusion method (19). For this purpose, standard bacterial strains like *Escherichia coli* (MTCC- 1258), *Klebsiella pneumoniae* (MTCC- 109), *Staphylococcus aureus* (MTCC- 6908), *Streptococcus pneumoniae* (MTCC – 5542), and *Staphylococcus epidermidis* (MTCC- 2639), were used. These bacteria were cultivated in Nutrient Broth for 24 h and were inoculated uniformly using sterile cotton swab onto Muller Hinton Agar (MHA) to test the antibacterial activities of *A. paniculata* extracts. Extracts were dissolved in DMSO and then applied to sterile disks for different concentrations (100 μg and 200 μg) then impregnated disks were placed on

the plates using sterile forceps properly spaced at equal distance. In this experiment was standard antibiotic (Chloramphenicol (20 μg)) used in control. The plates were stored for 2h to allow the extracts into the agar. Then, these plates were incubated for 24 h at 30 °C for the growth of bacterial strains. The zone of inhibition was measured and expressed in mm in diameter.

Antioxidant Activity

DPPH Free Radical- Scavenging Activity
The antioxidant activity of *A. paniculata* extracts were determined by DPPH free radical assay. The sample was reacted with the stable DPPH radical in an ethanol solution. The reaction mixture is composed of adding 0.5 mL of sample, 3 mL of absolute ethanol, and 0.3 mL of DPPH radical solution 0.5 mM in methanol. DPPH reacts with an antioxidant compound the color change will take place. The color changes were perused [Absorbance (Abs)] at 517 nm. The blend of ethanol (3.3 mL) and sample (0.5 mL) serve as blank. The control solution of Ascorbic acid was put together by mixing ethanol (3.5 mL) and DPPH radical solution. The scavenging activity percentage (AA%) was calculated according to the following formula.

$$Radical scavenging activity\ (AA\%)\ = \frac{Abs-control-Abs-sample}{Abs-control} \times 100$$

Cytotoxicity Study
Normal cells were obtained from Mahatma Gandhi Medical College and Research Institute Pondicherry, India. Vero monkey cell line was maintained at 37 °C in Dulbecco's modified Eagle's medium (DMEM) supplemented with 5% fetal calf serum, 100 U/mL penicillin, 100 mg/mL streptomycin, and 2 mM l-glutamine. Exponentially growing Vero cells were seeded at a density of 10^5 cells/mL into a 96-well plate (i.e., 250μL/well) by an 8-channel pipette. For background absorption, some wells were remained cell-free, as blank control. The cells were then treated with various concentrations (5, 10, 25, 50, 75, 100, 125, and 150μg/mL) of sample and positive control DMSO (5μg). The cells were then incubated for 24 h and cell supernatants (100 μL) were analyzed for leakage of LDH levels, using commercial spectrophotometric kits. The experiments were carried out in triplicates in each group.

Statistical Analysis
All results were presented as mean standard ± deviation (SD) after a one-way analysis of variance on the obtained data (ANOVA).

RESULTS

Phytochemical Analysis of *A. paniculate*

The plant extract was screened for the presence of major secondary metabolite classes such as Alkaloids, Flavonoids, Saponin, Terpenoid, Tannin, Glycosides, and oil and fat, according to common phytochemical methods. The tests were based on visual observation of the change in color or formation of precipitate after the addition of a specific reagent. The results of phytochemical tests carried out for *A. paniculata* with different solvents are presented in Table 1. The present study exhibited the presence and absence of phytochemical compounds in each solvent extract. It was found that Methanol showed a maximum number of phytochemicals when compared to Chloroform.

Antibacterial Activity of Solvent Extracts
The antibacterial activity of different solvent extracts against the pathogenic bacteria showed varied levels of inhibition. The methanol solvent extract was performed inantibacterial activity was shown a growth-inhibitory zone of 14.06±0.51 mm against as observed *E. coli,* which was followed by *S.aureus* (12.06±1.06 mm), and *S. epidermidis* (11.34±0.28 mm). The lowest zone of growth inhibition (09.06±0.24 mm) was observed against *K. pneumoniae.*

Notably, no growth inhibition against *S. pneumoniae* was recorded around the disc. The Chloroform solvent extract was performed in antibacterial activity was shown highest inhibition of zone of 12.04±0.23 mm against as observed*S. epidermidis,* which was followed by *E.coli* (11.09±0.84 mm). The lowest zone of growth inhibition was observed against *S. pneumoniae* (09.12±0.42 mm) and *S. aureus* (09.08±0.78 mm). No inhibition zone against *K. pneumoniae.* The standard antibiotic Chloramphenicol (20 µg) showed a zone of growth inhibition ranged from 17 to 19 mm. Among the solvent extracts tested, methanol extract had a broad spectrum of activity against pathogenic bacteria. The data showed in table 2.

Antioxidant Activity Assays

The *A. paniculata* extracts showed a concentration established anti-DPPH free radical scavenging activity. The crude secondary metabolites of *A. paniculata* exhibited the effective DPPH inhibition activity, and the maximum inhibition of 19.76±1.17 % was observed at 80 µg/mL concentration, which is considerably lesser than the inhibitory activity of 40 µg/mL of standard (Figure 2).

Cytotoxicity

The cytotoxicity of methanol and chloroformed extracts on Vero cells was studied by establishing the MTT assay. The results showed that both methanol and chloroform extracts did not exhibit significant cytotoxicity on Vero cells. The 50% inhibition of cell growth (IC50) was calculated linear regression method as 417.32±4.87 µg/mL for methanol extract and 472.31±3.25 µg/mL for chloroform extract (Figure 3).

DISCUSSION

The phytochemical screening tests have revealed that the flavonoids, tannin glycosides, and steroids are the most abundant components contained in the extract of *A. paniculata*. Further isolation and purification of the active phytochemical compounds are needed so that the specific bioactive compound can be served as the template for the production of new antibacterial, antioxidant agents, and cytotoxicity.The result from the present study indicates that the phenolic compounds and flavonoids are better extracted with ethanol than with other solvents. Phenolic acids and flavonoids are generally extracted using alcohols, water, or a mixture of water and alcohols (20).

Different plants possess different constituents and in different concentrations, which accounts for differential antimicrobial effects, as also suggested earlier (21). The secondary metabolites flavonoids, phenols and phenolic glycosides, unsaturated lactones, Sulphur compounds, saponins, cyanogenic glycosides, and glucosinolates produced by the plant have known to have antifungal activity (22).From the mass spectrum, assigned to the various molecular weight of the fragmentation possessing the different component of the bioactive molecule, the possible fragmentation patterns were obtained from the matching library Search NIST Libraries for Spectrum Parameters and named as possible (E)-4-(2-(6-hydroxy-5-(hydroxymethyl)-5,8a-dimethyl-2-methylene decahydronaphthlen-1-y1) ethylidene)-5-oxotetrahydrofuran-3-y12-hydroxypropanoate ($C_{23}H_{34}O_7$).(23)Ethanolic extract of *Andrographis paniculata* (APE) was evaluated for their in vitro cytotoxicity activity against Vero cell line and chicken embryo fibroblast (CEF) primary cell. up to 25 µg/mL (the highest concentration used in this study). The ethanolic APE also demonstrated significant ($p < 0.05$) antiproliferative activity against CEF cell lines compared to the Vero cell line. The results of the cytotoxicity evaluation were expressed as CC50 (µg/mL). A plant extract is considered safe when the CC50 value is > 20 µg/mL.

CONCLUSION

Medicinal plants are the source of the secondary metabolites i. e., alkaloids, flavonoids, terpenoids, and reducing sugars. From the study, we infer that the methanol and chloroform extracts of *Andrographis paniculata* showed significant antibacterial activity against various bacterial pathogens, and showed effective DPPH antioxidant

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activity. Moreover, it showed very little toxicity on normal Vero cells. Therefore, *Andrographis paniculata* could be used for various medicinal uses.

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Table 1: Preliminary Phytochemicals Screening of *Andrographis paniculata* extract

S. No	Components	*Andrographis paniculata*	
		Methanol	**Chloroform**
1	Alkaloids	+	+
2	Flavanoids	+	_
3	Phenols	+	+
4	Tannin	+	+
5	Saponin	+	+
6	Sugars	+	+
7	Terpenoids	+	+
8	Glycosides	+	+
9	Steroids	+	+
10	oils and fats	+	+

Presence = +; Absent= -

Table 2: Antibacterial activity of solvent extracts pathogenic bacteria

Bacteria Name	Zone of inhibition (mm in diameter)				
	Methanol		Chloroform		
	100 µg	200 µg	100 µg	200 µg	control
Escherichia coli	10.04±0.46	14.06±0.51	09.16±0.34	11.09±0.84	17.06±0.64
Klebsiella pneumoniae	_	09.06±0.24	_	_	19.08±0.12
Staphylococcus aureus	08.24±0.42	12.06±1.06	_	09.08±0.78	17.24±0.34
Streptococcus pneumoniae	_	_	_	09.12±0.42	18.32±1.06
Staphylococcus epidermidis	08.06±064	11.34±0.28	09.12±0.47	12.04±0.23	18.08±0.87

No zone of inhibition = _

Figure 1:*Andrographis paniculate*
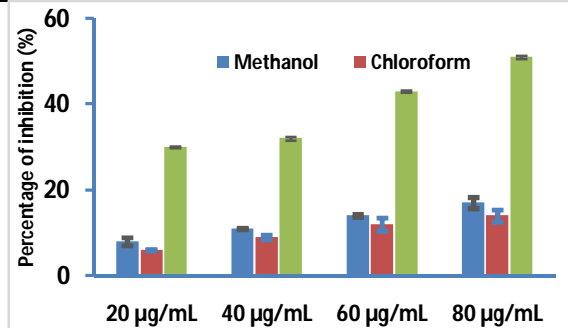
Figure 2: DPPH free radical-scavenging activity of ***Andrographis paniculata*** solvent extracts.
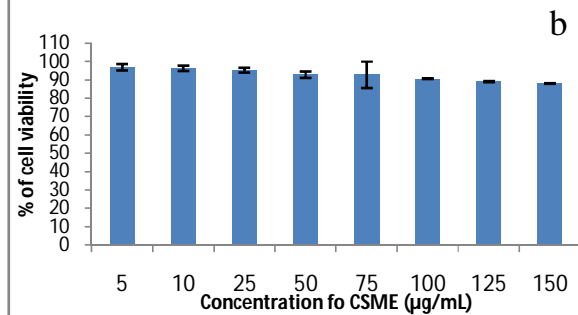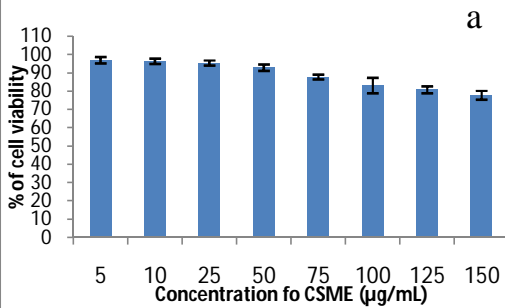
Figure 3: cytotoxicity assay of methanol (a) and Chloroform (b) extracts on normal Vero cells.

REVIEW ARTICLE

Review on *Solanum nigrum* as Traditional Medicine

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ABSTRACT

Medicinal plants are extensively used to cure various infectious diseases in human beings, Phytochemical analysis of the plant showed the presence alkaloids, terpenoids, flavonoids, saponins, steroids and phenols. Thus *Solanum nigrum* has anti-microbial activity and can be used clinically to find novel antibacterial compounds for respiratory tract pathogens. *Solanum nigrum* is well- known medicinal herb found in India. In view of the above aspects the present work provides profiles of *Solanum nigrum* leaves and fruit, with their nutritional potential, phytochemical screening and antioxidant potential activity on leaves and fruit aqueous extract *Solanum nigrum*

Keywords: *Solanum nigrum*, anti-cancer activity, carcinogenic.

INTRODUCTION

Nature has provided a complete store- house of remedies to cure all aliments of mankind (Kokate et al., 2002). This is where, nature provides us drugs in the form of herbs, plants and algae's to cure the incurable diseases without any toxic effect (Trees, Evans, 1989). The use of *Solanum nigrum Linn* as has antiseptic and ant- dysenteric properties and is given internally for cardalgia and gripe. An infusion of the plant is used as an enema in infants having abdominal upsets. Freshly prepared extracts of the plant is effective in the treatment of cirrhosis of the liver, and also serves as an antidote to opium poisoning (Acharya et al., 2006). Medicinal components from plants play an important role in conventional Western medicine. The traditional medicine all over the world is nowadays revalued by an extensive study of results of research on different plant species and their therapeutic principles (Scartezzini et al., 2000). Interest in medicinal plants has been fuelled by the rising costs of prescription drugs in the maintenance of personal health and well-being, and the bioprospecting of new plant-derived drugs (Hoareau and DaSilva 1999). Infectious diseases caused by bacteria, fungi, viruses and parasites are still a major threat to health, despite the tremendous progress in human medicine. Their impact is particularly large in developing countries due to the relative unavailability of medicines and the emergence of widespread drug resistance (Okeke et al., 2005).

Sakshi Pandey

Plant Profile
Biological Source
It consist of the dried and full grown berries of *Solanum nigrum*

Geographical Source
Medicinal Botany, National Institute of Siddha, Chennai, Tami Nadu, India.

Family
Solanaceae.

Common Name
Black nightshade, Makoi.

Macroscopy
The bark is thin and easily peeled off exposing pale yellow wood. The flowers have five petals and are generally regular in shape. They may be round and flat or star-shape, but are often bell shaped or tubular. Members of this family are often climbers or at least scrambling plants, often with hairy stem and leaves. The leaves are variable, and may be entire or dissected, without stipules, and are usually alternate. The morphological study shows the root with few branches and numerus small lateral roots, externally it is smooth pale brown. The fruit shows thin, papery epicarp, pulpy measocarp and axle placentation, seeds lie free in pulp of fruit. The fruits are berry; 6mm diameter, obtuse, usually.

Chemical Constituents
Green unripe fruits contain glycoalkaloids and their eating is a toxic to human being as well as livestock that include solamargine, solasonine, solanine, α and β-solamagrine, solasodinsolanidine (0.09-0.65%). The former two also found in leaves. Solanine is found in all parts of the plants, with the level increasing as the plant matures, though it is apparently modified by soil type and climate.

Traditional Uses
◎ Leaves are used for rheumatic and gouty joints, skin diseases, also used in the treatment of tuberculosis, nausea and nervous disorders.
◎ The decoction and juice of the berries is useful in cough, diarrohea, inflammations and skin diseases5, 6.Anti-oxidative18, anti-inflammatory and anti-pyretic effects of *Solanum nigrum* chloroform extract has also been found.
◎ The ethanol extract of dried fruits of *Solanum nigrum* showed a remarkable hepatoprotective effect against CCl4 induced oxidative damage on liver cells.
◎ The most important property of *Solanum nigrum* is its anti-cancerous property.[7]

Medicinal Uses
Some of the uses to *Solanum nigrum L.* in literature may actually apply to other species within the some species complex, and proper species identification is essential for food medicinal uses (Mohyuddint, 2010). In India, the berries are casually grown and eaten, but not cultivated for commercial use. In South India, the leaves and berries are routinely consumed as food cooking with tamarind, onion, and cumin seeds (Ignacimuthu, 2006).

The plant has a long history of medicinal usage, dating back to ancient Greece. "In the fourteenth century, we hear of the plant under the name of Petty Morel being for canker and with Horehound and wine taken with for dropsy." (Grieve, et a1 1984) It was a traditional European medicine uses as a strong sudorific, analgesic and sedative with powerful narcotic properties, but was considered a "somewhat dangerous remedy" (Schauenberg, 1977) Internal uses has fallen out of favor in Western herbalism due to its variable chemistry and toxicity, but it is used topically as

a treatment for herpes zoster.(Nohara, Ikeda, Nohara 1998,Schmelzer, 2008) *Solanum nigrum L.* is an important ingredient in traditional Indian medicines. Infusions are used in dysentery, stomach complaints, and fever. (Jain 1968) The juice of the plant is used on ulcers and skin diseases. The fruits are used as a tonic, laxative, appetite stimulant, and for treating asthma and "excessive thirst". Traditionally the plant was used to treat tuberculosis (Kaushik, et al 2009). It is known as *peddakasha pandla koora* in the Telangana region. This plant's leaves are used to treat mouth ulcer that happen during winter periods of India. It is known as *kaage soppu* in Karnataka, and apart from its use as a home remedy for mouth ulcers, is used in cooking like spinach. In North India, the boiled extract of leaves and berries are also used to alleviate liver- related ailments, including jaundice. In Assam, the juice from its roots is used against asthma and whooping cough.(Taab 2009) *Solanum nigrum L.* a widely used plant in oriental medicine where it is considered to be antitumorigenic, antioxidant, anti- inflammatory, hepatoprotective, diuretic, and antipyretic(Jain, 2011).

Nutritional Uses

The nutritional value estimation of leave and fruit of *Solanum nigrum L.* was assessed by determining proximate and phytochemical composition. The result indicates that protein content of the leave and fruit 11.16% and 13.07%, respectively other finding are carbohydrate 73.4%9 and 69.76%, crude fiber6.31% and 8.19%, Total Ash 11.85% and 11.24%, fat 2.14% and 4.25%, Moisture content 48.9% and 53 8% of the leaves and seeds respectively. Mineral analysis revealed the order Ca, Fe, K, P, Na in the leaves and seeds. Phytochemical analysis revealed high level of Ascorbic acid (Mali, Harsh, et al 2015).

The nutritional potential of the leave and fruit of *Solanum nigrum L.* was assessed by determining proximate and phytochemical composition. Results indicate protein content of the leaves and seeds as 12.9% and 15.06% respectively. Other finding is ash 10 18% and 9.05%, crude fibre 6.81% and 6.29% and carbohydrate 53.51 and 55.85% for the leaves and seeds respectively. Minerals analysis revealed the order Mg, K, Ca, Fe, Na, Mn, Zn in the leaves and seeds. Phosphorus and sulphur leaves were 75.22 and 8.55mg/100g in the leaves and 62.50 and 14.48g/100g in the seeds. vitamin content indicate the order vit-C, vit –B, Folic acid, vit- A in the both leaves and seeds (Akubugwo, Obasi, et all.,2007).

Therapeutic Uses

This herb also exhibit anti-tumor activity, anti- neoplastic activity against Sarcoma 180 in mice, anti- inflammatory and anti- convulsant activity as well as is also used in the treatment of liver diseases. Fresh juice of this herb is used for curing fever and alleviating pain (Khan, Anwar, et al., 2012).

This beneficial activity of the extract might be considered as an adjuvant drug in the treatment of liver disorders. Thus we propose that dietary intake of *Solanum nigrum L.* fruits offers protection against cellular and toxicity (Arulmozhi, etval, et al 2012).

Antidiabetic Properties

The aqueous and hypo-alcoholic extracts of different parts of *Solanum nigrum L.* plant, viz leaf, fruit and stem for hypoglycemic activity in Sprague Dawley rats. Different doses of the extract 200, 400 mg/kg body weight were employed to evaluate the oral glucose tolerance with standard Metformin. Result indicated that aqueous extracts of leaf and fruit possess significant hypoglycemic effect in dose dependent manner, followed by hydro-alcoholic extracts. The stem extract of *Solanum nigrum L.* has no profound effects (Akubugwo, et al., 2008). The effect of crude ethanolic extract of S. nigrum on blood sugar of albino rat after daily oral administration of dose at the level of 250 mg/kg body wt. for five and seven days respectively. It was noticed leads to significant decrease in blood sugar compared to control. Thus it can be concluded that *Solanum nigrum L.* has the anti- diabetic property (Ali, Singh, et al., 2010).

Immunostimulant Property

In this investigation found Immunostimulant potential plant being an alternative for preventing fish diseases. Six groups of experimental fishes were immunized with 0.2ml (4ppm) of five different extracts of Solanum nigrum through intra-peritoneal injection ad challenged with heat killed *Aphanomyces invadans.* Blood collected from immunodiffusion, antibody titration, nitro blue tetrazolium assay, determination of IgG concentration and host resistance test. In both control and exoerimental groups the peak antibody response was on day 21 after immunization and decreased towards 28th day (Leportti et al 2009). The methanol extract treated group, the antibody response was significantly enhanced on the day 14 and day 21. The highest IgG level was on day 21 and decreased towards day 28. In Chloroform extract treated group the neutrophil activity was significantly enhanced on day 6. In toluene extract treated group the neutrophil activity was significantly enhanced on day 6. The ethanol and methanol extract treated group showed less mortality rate when compared to chloroform toluene and water extract treated group. Plants extracts have great potential as Immunostimulant against micro-organisms and that they can be used in the treatment of infectious diseases caused by microorganisms (Hanifa 2011).

CONCLUSION

Solanum nigrum are nutritious food that provide sufficient amount of nutrients needed for normal body function, maintenance and reproduction. The outcome of the study demonstrated that the *Solanum nigrum* leaves can serve as a good nutritional spring in combating malnutrition as it is rich source of protein, iron and calcium in comparison to *Solanum nigrum* fruits. The presence of bioactive compounds is an assertion of the use as a leafy vegetable in the management of various ailments. Antioxidants present in *Solanum nigrum* aqueous extract shows a vital role in preventing innumerable health disorders related to oxidative stress, diabetes and CVD. Due to its strong antioxidant property which are known to diffuse the toxic free radicals, can be used in nutraceutical and biopharmaceutical industries.

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RESEARCH ARTICLE

An efficient Synthesis, Spectral Characterization and Biological Screening of Dimeric Fe(III) Complex with 2-Aminobenzonitrile and Benzoate Ion Ligands

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ABSTRACT

A novel Fe(III) complex of 2-aminobenzonitrile (2-ABN) and benzoate ion (BEN) ligands have been prepared under microwave irradiation. The structure of the compound has been investigated by using elemental analysis, molar conductance, magnetic moment, spectral (Infra Red, UV-Visible, Cyclic Voltammetry, Powder X-RD) and thermal measurements. IR spectra indicate that the ligand behaves as uninegative monodentate/ bidentate with O/N,N donor sequence towards the metal ions. Bacterial and fungal activities of the ligands and their Fe(III) complex was studied against *staphylococcus aureus, streptococcus, Escherichia coli, Klebsiella pneumonia, P. aeruginosa, salmonella typhi, Enterobacter, C.albicans, Aspergillus Niger* and *Aspergillus Flavus* by using well-diffusion method. The free radical scavenging activity of the metal complex and the ligands has been determined by measuring their interaction with the stable free radical DPPH. The complex has superior antioxidant activity as compared to the ligands. DNA-binding properties have been studied by fluorescence-emissions method. The results suggest that the complex strongly bind to DNA because of metal complexes are well known to speed up the drug action and the ability of a healing agent which can frequently be superior upon coordination with a metal ion.

INTRODUCTION

Metallo-organic multiple is far above the view interest in crystal engineering which aims to predict and control the fashion molecules assemble in the solid state [1–4]. In the designing of metal complexes with unique properties for a wide range of prospective applications including antimicrobial drugs [5,6], conductive material [7], luminous[8] and magnetic materials [9]. Among the aminobenzonitriles, 2-aminobenzonitrile (2-ABN) is second-hand for the induction of nitrilase activity in arthrobacter, radio protective agent and starting materials for the synthesis of bio-active compounds [10-13]. The 2-aminobenzonitrile is one of organic ligands in coordination chemistry which can synchronize to the metal ion through different modes *viz.,* monodentate/bidentate or bridging manner. In general, the biological activities of the metal complexes differ from those of either the ligand or the metal ion itself, and increased and/or decreased biological activities are reported for various metal complexes [13]. Alternatively, synthesis of inorganic/organic compounds using microwave irradiation has been a very rapidly developing technique in research area [14-20]. Compared with the conventional method, microwave technique is promising due to its unique effects, such as rapid volumetric heating, higher reaction rates, higher reaction selectivity, higher yields of products and energy saving. Literature search reveals that no work has been done on the mixed ligand complexes 2- aminobenzonitrile and benzoate ion. In this paper we report the synthesis, characterization, antimicrobial and DNA binding studies of Fe(III) complex containing 2-aminobenzonitrile and benzoate ion as ligands. This research mainly focused on the DNA binding properties of metal complexes. Since, in recent years, DNA binding studies of transition metal complexes have become very important in the expansion of DNA molecule probes and chemotherapeutics.

EXPERIMENTAL

Materials and Methods
2-aminobenzonitrile, C_6H_5COONa, $Fe(NO_3)_3 \cdot 9H_2O$ were purchased from Alfa Aaser Company and used as such. The organic solvents used, were DMSO, DMF, CH_3OH, EtOH which were of AnalaR grade, and used as such without further purification.

SYNTHESIS OF METAL COMPLEXES
Synthesis of Fe (III) complex
The ethanolic solution of 0.44g (3.71 mmol) of 2-ABN and the ethanolic solution of 1.23g (7.38 mmol) of sodium benzoate were added to the methanolic solution of $Fe(NO_3)_3 \cdot 9H_2O$ 1.00g (2.46 mmol) followed by microwave irradiation for a few seconds after each addition by using IFB 25 BG-1S model microwave oven. The consequential precipitate was filtered off, washed with 1:1 ethanol: water mixture and desiccated under vacuum. An orange colored complex was obtained with 85.28% yield.

Instrumentations
The elemental analyses C,H,N were performed using Thermo Finnegan make, Flash EA1112 Series CHNS(O) analyzer. The molar conductivity measurements were conducted using 10^{-3}M solutions of the metal complexes in acetonitrile with Systronic Conductivity Bridge (model number-304) at 30°C. The UV-Visible spectrum of the Fe(III) complex was recorded on Varian, Cary 5000 model UV Spectrophotometer. IR spectra for the complexes and the free ligands were recorded on a Perkin Elmer, Spectrum RX-I, FT IR spectrometer in KBr discs at room temperature. The cyclic voltammograms of the complexes were taken in acetonitrile medium using Princeton make (MC-Tech, Applied Research) equipment. Tetrabutylammonium tetrafluoroborates was used as the supporting electrolyte. The

thermogravimetric analyses of the complexes were carried out using Perkin Elmer Diamond TGA/DTA Instrument. The powder X-ray diffraction analysis of Co(II) and Ni(II) complexes was recorded on a Rigaku model X-ray Diffractometer.

BIOLOGICAL ACTIVITIES
Bacterial and fungal activity

The Fe(III) complex and the free ligands were tested against *in-vitro* bacterial and fungal activity by well diffusion method. The microbial activities of the free ligands and their complexes were evaluated against the strains cultured on potato dextrose agar as medium. The stock solution was prepared by dissolving the compounds in DMSO and the solutions were successively diluted to different concentration. According to the typical procedure a well was made on the agar medium inoculated with the microorganisms. The well was filled with the test solution using a micropipette and the plate was incubated for 24 hours for bacteria and 72 hours for fungi at 35°C. At the end of the period, zone of inhibition formed on the medium were evaluated in millimeter (mm) diameter [21, 22].

Antioxidant activity

Evaluation of antioxidant activity stock solution (1mg/ml) was diluted to final concentrations of 10–500 µg/ml. Ethanolic DPPH solution (1 ml, 0.3 mmol) was added to sample solutions in DMSO (3ml) at different concentrations (10–500 µg/ml) [23]. The mixture was shaken energetically and acceptable to stand at room temperature for 30 min. The absorbance was then measured at 517 nm in a UV-Vis Spectrophotometer. The lower absorbance of the reaction mixture indicates higher free radical scavenging activity. Ethanol was used as the solvent and ascorbic acid as the standard. The DPPH radical scavenging activity was designed by the following equation: DPPH Scavenging effect (%) = $A_0 - A_1 / A_0 \times 100$, where Ao is the absorbance of the control reaction and A_1 is the absorbance in the presence of the samples or standards.

DNA binding properties

The DNA binding experiments involving interaction of the prepared Fe(III) complex and the free ligands with calf thymus (CT)-DNA were conducted in Tris buffer containing HCl (0.01 M) adjusted to pH 7.2 with hydrochloric acid. The CT-DNA was dissolved in Tris-HCl buffer and was dialyzed against the same buffer overnight. Solutions of CT-DNA gave the ratios of UV absorbance at 260 and 280 nm above 1.8, demonstrating that the DNA was adequately free of protein. DNA concentration per nucleotide was determined by absorption spectroscopy using the molar absorption coefficient 6600dm^{3}mol^{-1}cm^{-1} at 260 nm. The stock solutions were stored at 4°C and used within 4 days [24]. For fluorescence-quenching experiments, DNA was pre-treated with ethidium bromide (EtBr) for 30 minutes. The ligand/ complex samples were then added to this mixture and their effect on the emission intensity was measured. Samples were excited at 450 nm and emission was observed between 500 nm and 800 nm.

RESULTS AND DISCUSSION

Elemental analysis

The elemental analysis data, percentages of carbon, hydrogen and nitrogen for Fe(III) complex is 56.44(55.53), 4.43(4.76), 11.72(10.73). The result was found to be good agreement with the theoretical values given in the parenthesis.

Molar conductance

Molar conductance (ΛM) value of the complexes (10^{-3}M) in acetonitrile medium was found to be 57.12 Ω^{-1}cm^{2}mol^{-1}. The low electrical conductivity value indicates that the Fe(III) complex is non-electrolytic 1:0 type [25] confirming their molecular formulae
[Fe$_2$(BEN)$_6$(2-ABN)$_3$].

UV-Visible spectra of Fe (III) complex

The electronic spectrum of Fe(III) complex gave three wavelength maximum at 680 nm (14,705cm^{-1}), 385 nm (25,974 cm^{-1}), 260 nm (38,461 cm^{-1}) which corresponding transitions are $^5E_g \rightarrow {}^5T_2g$, $^5B_{1g} \rightarrow {}^5B_{2g}$ and CT-band respectively [26, 27]. The magnetic moment of Fe(III) complex is 5.8 BM. These facts confirmed the six coordinated octahedral and dimeric geometry of the complex.

IR spectra of ligands and their Fe (III) complex

The IR spectra provide valuable information regarding the nature of functional group attached to the metal atom. In order to study the binding modes of the ligands, 2-aminobenzonitrile and benzoate ion to the complexes, the IR spectra of free ligands were compared with their Fe(III) complex as shown in Fig. 1. The 2- ABN shows in characteristic absorption bands in the 3453 cm^{-1}, 3366 cm^{-1} and 2206 cm^{-1} region, assignable to asymmetric, symmetric stretching frequencies of $\nu(NH_2)$ and ν (C≡N) respectively [28,29]. A small band noticed at 3076 cm^{-1} is due to $\nu(CH)$ aryl. Aromatic $\nu(C=C)$ stretching vibration is seen as a sharp peak at 1563 cm^{-1} [30]. The benzoate ion shows $\nu(C–O)$ at 1215 cm^{-1}. A strong band with a shoulder noticed at 1605 cm^{-1} can be attributed to $\nu(C=O)$ of the carbonyl group [31]. The band(s) are broadened at 3417-3367 cm^{-1} and the nitrile group of the ABN underwent higher frequency at 2228 cm^{-1} after complexation, indicating the coordination of amino nitrogen and cyano nitrogen (N, N) to the metal ion. In free benzoate ion the ν(C-O) stretching at 1215 cm^{-1} get shifted to the frequencies of 1235 cm^{-1} nearly in complexes, which indicates the monodentate coordination of the benzoate ion through oxygen atom.

Cyclic voltammetry of Fe (III) complex

Cyclic voltammogram of [Fe$_2$(BEN)$_6$(2-ABN)$_3$] complex was recorded in DMF solution. This shows a well redox process corresponding to the formation of Fe(III)/Fe(II) couple at Epa = -0.910V, Epc = -0.470V and the ΔEp = 0.440V.This also indicates the quasi-reversible [32] nature of one electron transfer reactions. Cyclic voltammetry of Fe(III) complex shows Fig.2.

Thermogravimetric analysis

Thermogravimetric analysis of [Fe$_2$(BEN)$_6$(2-ABN)$_3$] complex of 2-ABN and benzoate ion show two important temperature transitions of weight loss with frequent and definite stages, termed as two stages of thermal degradation. The weight loss at the range of 200-260 °C in TGA curves of complexes is termed the first stage of thermal degradation as shown in Fig.1. In this complex, the present weight loss is in the range of (22.48- 30.50%), which may be attributed to the decomposition of benzoate ion. The beginning of second step decomposition occurs in the range of 220-300 °C, which gives the loss of 2- aminobenzonitrile (45.55 – 58.33%) bonding with the metal complexes. The experimental values are in full agreement with the percent weight calculated on the basis of stoichiometry proposed for the complexes [33]. Fig.3 shows thermogram of Fe(III) complex.

Powder X-ray diffraction technique

The XRD pattern indicates that the prepared [Fe$_2$(BEN)$_6$(2-ABN)$_3$] complex have well defined crystalline patterns, with various degrees of crystallinity. In these complexes, the trend of the curves decreases from maximum to minimum intensity indicating amorphous nature of the complex [39]. The powder XRD patterns of the synthesized Fe(III) complex show sharp crystalline peaks indicating their crystalline phase. The average crystallite size (d$_{xRD}$) of the complexes was calculated using Scherer's formula [34]. The prepared complexes had an average crystallite size of 34 nm indicating its nanocrystalline in nature.

Proposed structure of the Complex

The proposed structures of complexes based on the above mentioned physico-chemical and the spectral elemental analysis, molar conductance, magnetic moment, spectral (Infra Red, UV-Visible, Cyclic Voltammetry, Powder X-RD) and thermal measurement) studies the tentative structure proposed for the complex is dimeric octahedral as shown below in Fig. 4

Biological activity

Bacterial activity

The synthesized $[Fe_2(BEN)_6(2\text{-}ABN)_3]$ complex and the free ligands 2-ABN and sodium benzoate are tested against the bacteria *viz.*, *staphylococcus aureus*, *streptococcus*, *Escherichia coli*, *Klebsiella pneumonia*, *P. aeruginosa*, *salmonella typhi* and *Enterobacter* at different concentrations by agar-well diffusion method *in-vitro* conditions. The complexes have potential activity against the bacteria compared to free ligands as shown in Table.1 and Fig.5. This is probably due to the greater lipophilic nature of the complexes. It is evident from the data that this activity significantly increases on coordination [35].

Fungal activity

The synthesized Fe(III) complexes and the free ligands 2-ABN and sodium benzoate were evaluated against the fungi, *viz., C.albicans, Aspergillus niger, Aspergillus flavus,* at different concentrations by agar-well diffusion method. The complex shows superior activity against the tested fungi. A proportional study of zone of inhibition diameter values of the ligands and their complexes indicate that the metal complexes have a better fungicidal activity than the free ligands.

Antioxidant activity (Radical Scavenging Activity)

The 2,2′′-diphenyl-1-picrylhydrazyl (DPPH) radical assay provides an easy and rapid way to evaluate the antiradical activities of antioxidants. Determination of the reaction kinetic types DPPHH is a product of the reaction between DPPH• and an antioxidant.

(AH): DPPH$^\bullet$ + AH → DPPHH + A$^\bullet$

The reversibility of the reaction is evaluated by adding DPPHH at the end of the reaction. If there is an increase in the percentage of remaining DPPH$^\bullet$ at the plateau, the reaction is reversible, otherwise it is a complete reaction. DPPH was used as stable free radical electron accepts or hydrogen radical to become a stable diamagnetic molecule [36]. DPPH is a stable free radical containing an odd electron in its structure and usually used for detection of the radical scavenging activity in chemical analysis [44]. The reduction capability of DPPH radicals was determined by decrease in its absorbance at 517 nm induced by antioxidants [37]. The graph was plotted with percentage scavenging effects on the y-axis and concentration (μg/ml) on the x-axis. The scavenging ability of the Co(II) and Ni(II) complexes were compared with Vitamin C as a standard. In Fig.5, the metal complexes showed enhance activity as a radical scavenger compared with ascorbic acid, these results were in good agreement with previous metal complexes studies where the ligand has the antioxidant activity and it is expected that the metal moiety will increase its activity [38].

DNA Binding – Emission study

The binding of free ligands and their Fe(III) complexes to *CT*-DNA can be studied by competitive binding experiments. Ethidium bromide (EB) is known to show fluorescence when bound to DNA, due to its strong intercalation between the adjacent DNA base pair. The fluorescent light is quenched by the addition of a second molecule [39,40]. The quenching extent of fluorescence of ethidium bromide binding to DNA is used to determine the extent of binding between the second molecule and DNA. The addition of the complex to DNA pretreated with ethidium bromide causes appreciable reduction in the emission intensity, indicating the replacement of the ethidium bromide fluorophore by the complex results in a decrease of the binding constant of the ethidium to the DNA as shown in Fig. 6&7. According to the classical Stern-Volmer equation: $I_0/I = 1 + K_{sv}r$, where I_0 and I are the fluorescence intensities in the absence and the presence of complex respectively. K_{sv} is a linear Stern–Volmer quenching constant, r is the ratio of the total concentration of complex to that of DNA. The quenching plots illustrate that the quenching of ethidium bromide bound to DNA by the complex are in good agreement with the linear Stern-Volmer equation, which also indicates that the complex binds to DNA. In the plot of I_0/I versus $C_{Complex}/C_{DNA}$, K is given by the ratio of the slope to intercept. The data suggest that the interaction of complexes with DNA is strongest,

which is consistent with the above absorption spectral results. K values indicate that the interaction of the complex with DNA is a intercalative mode [41].

CONCLUSION

In the present study, our efforts were to synthesize and characterize the Fe(III) complex with 2-aminobenzonitrile and benzoate ion as ligands. The new complex was prepared under microwave irradiation. The synthesized metal complex was characterized by various physico-chemical and spectral analyses. Based on the analytical, molar conductance, spectral and magnetic moment, octahedral dimeric geometry have been suggested for the Fe(III) complex. The synthesized complex was tested for antifungal activities. The metal complex has significant antifungal and antioxidant activities as compared to the free ligands. The effectiveness of the DNA binding of the complexes is being confirmed by means of change in intensity of emission in the case of emission spectral studies.

ACKNOWLEDGEMENT

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Table 1 Antibacterial activity of the free ligand and their complex - diameter of zone of inhibition (in mm)

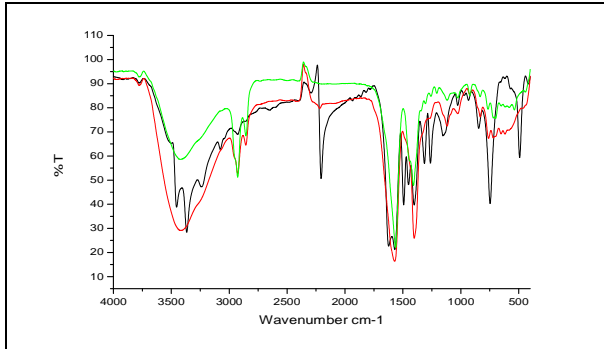| Ligand/ Complex	Conc. μg/ml	Zone of inhibition in diameter (mm)						
		S. aureus	*Strepto coccus*	*E. coli*	*Klebsiella*	*P. aeruginosa*	*S. typhi*	*Entero bacter*
2-ABN	50	04	05	06	11	14	10	11
	100	09	12	11	16	21	16	18
NaBEN	50	04	05	05	04	11	03	05
	100	08	08	07	09	17	04	09
[Fe$_2$(BEN)$_6$(2-ABN)$_3$]	50	04	05	05	04	12	07	10
	100	09	10	09	08	21	15	17

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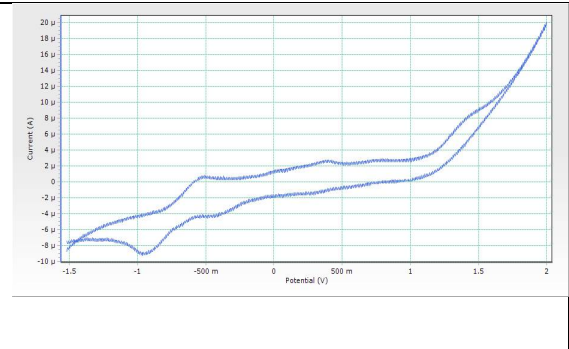**Fig.1.IR spectra of 2-aminobenzonitrile, sodium benzoate and Fe(III) complex**

Fig.2.Cyclic voltammogram of Fe(III) complex

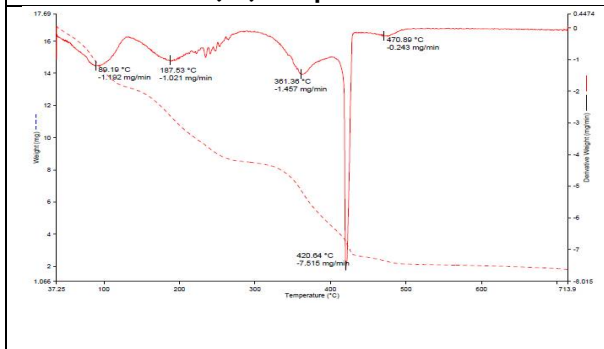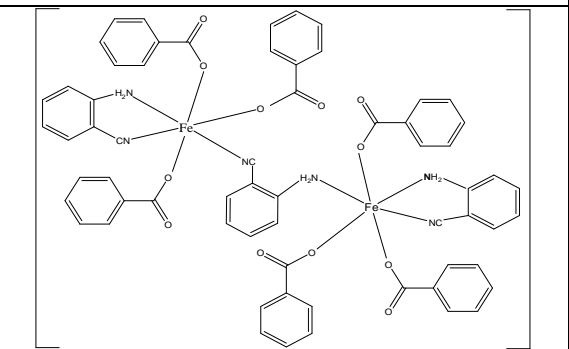**Fig. 3.Thermogram of [Fe₂(BEN)₆(2-ABN)₃]**

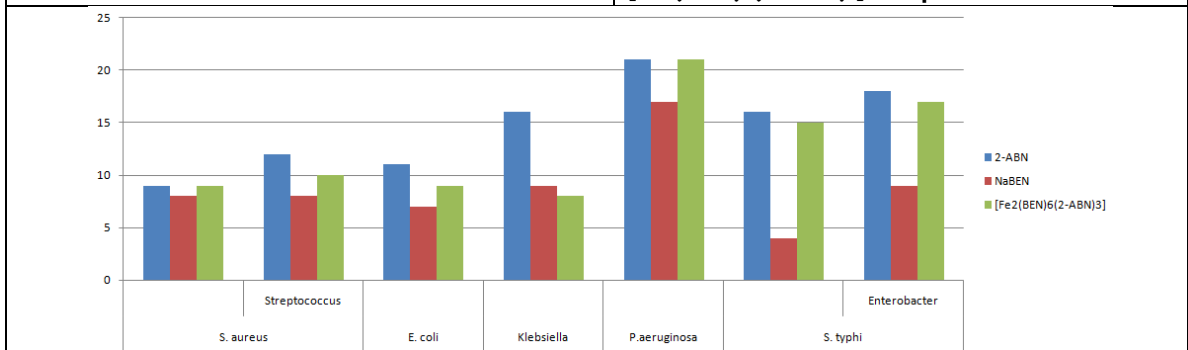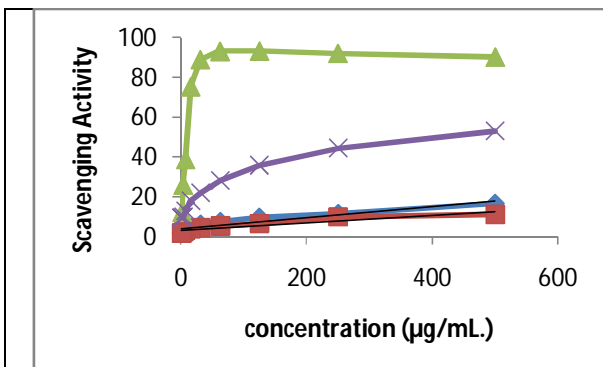**Fig.4.Dimeric octahedral structure of [Fe₂(BEN)₆(2-ABN)₃] complex**
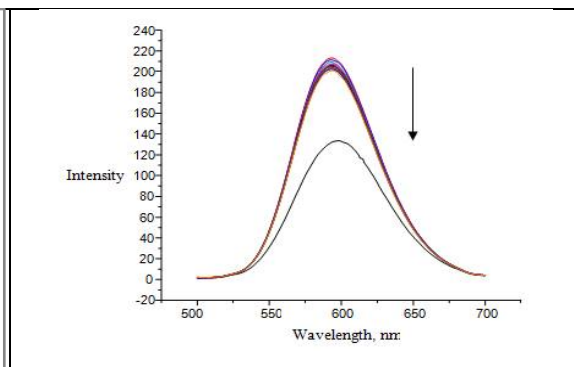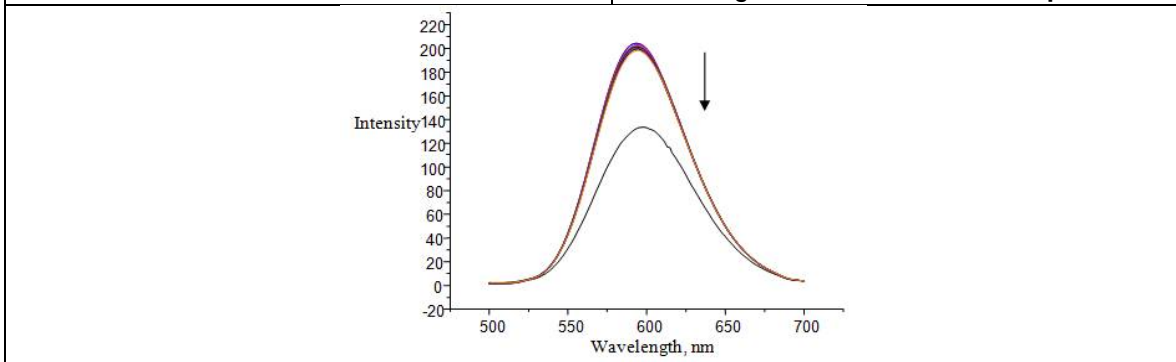

Fig. 5.Antibacterial activity-Graphical representation

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Fig.5.Antioxidant activities of free ligands and their complexes

Fig.6.Emission spectrum of EtBr bound to DNA in the absence and presence of 2-ABN. Arrows indicate the intensity changes upon increasing concentration of the complexes.

Fig.7.Emission spectrum of EtBr bound to DNA in the absence and presence of Fe(III) complex. Arrows indicate the intensity changes upon increasing concentration of the complexes.

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Women Combating against Odds: There's No Easy Path to Glory

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ABSTRACT

Independent women try really hard to rejuvenate themselves in all ways incorporating thoughts, rights, decisions, understanding etc. by leaving all the battle of the sexes. Thus, the survival of the men can only be possible because of the lifeline women have given them and made it possible to have created life. They are the ones who are fighting against "Survival for the fittest" Gender equitable societies are healthier for the society and for the growth. Women are playing great roles in different field: as workers, consumers, entrepreneurs, managers and investors. According to a report and also the survey 'Women and the Economy of the world in 1950, only one- fourth of American women of working age had a paid job. This is true in respect of every female. Gandhiji too said, "Woman is the companion of man, gifted with equal mental capacity." Without women, men would not exist. Women are actually combating in every single aspect of life as well as for every single position in the society. Despite the odds, time and again they have emerged as winners. Though it is not at all easy to be multi-tasking, and no other species is as capable as women who for centuries have managed and supervised things easily without ever revealing a sense of fatigue. This paper aims at exploring empowerment of women. They also have a sense of being systematic and organized. Moreover, they are strong in facing calamities and fighting for survival. There are many facts that can easily prove the abilities of women in every field of life. Communication is termed to be the most important a woman's strongest skill — and female leaders know how to use it! and highlighting their abilities in each and every aspect of feminism in present scenario. My endeavour is to show case that they can be the ones who can lead the world through their amazing potential and abilities. A woman has the capacity to be patient and relatively calmer in any situation. She's a wonder that is beheld by the universe and progress can only be possible with her. Today we see women playing important roles in every sphere and emerging triumphant with confidence because they sure have the

potential to emerge as the most skilful and vibrant leaders. Feminists respect individual, informed choices and believe there shouldn't be a double standard in judging a person.

Keywords: Women Empowerment, Feminism, Gender Studies, Women and Society, Role of Women, Women's Movement; Feminism; Subordination; Freedom Struggle; Non-Government

We all know that girls really nice then boys at school by the data. The annual results of Secondary and higher education have always reflected this fact and also that technically girls are performing in the best way. More women are getting higher qualification then men, and are filling most new jobs in every field. There was a time when women's education was never a priority even for the upper class and elite class. Since the last quarter of the 20th century and more of 21st century it has shown a great difference, post-1992, a growing number of women have been selected into the economic field, seeking paid work (remunerative jobs) outside the family. Women are playing bigger and bigger roles in economic field: as politician, actress, astronauts, engineers and artist. According to a report of The Economist, 'Women and the World Economy', in 1950, only one-third of American women of working age had a paid job." Reports Pooja Mondal. Gandhiji too said, "Female is just a companion of man, gifted with equal mental capacity." Thus, we see that over the ages people have recognized the worth and potential of women. "Women are leaders everywhere you look -- from the CEO who runs a Fortune 500 company to the housewife who raises her children and heads her household. As feminism challenges restrictive gender norms, improvements for health care and awareness in women, reproductive rights, and protection from violence have positive effects in the world and also it rejuvenates life expectancy and well-being, especially children. Our country was built by strong women, and we will continue to break down walls and defy stereotypes." From fighting the pandemic and also bringing stability all these American influential women—including Prime Minister Jacinda Ardern, Vice President- elect Kamala Harris and voting rights advocate Stacey Abrams They are making history in the world. Writers like Virginia Woolf have tried to empower women through their writings. "Women have served all these centuries as looking glasses possessing the magic and delicious power of reflecting the figure of a man at twice its natural size." Time and again women have tried to assert their position in the society. "I believe that it is as much a right and duty for women to do something with their lives as for men and we are not going to be satisfied with such frivolous parts as you give us." In the present times, people are realizing that the only way to create a better world and bring about a change is to empower women. "The fastest way to change society is to mobilize the women of the world."

INTRODUCTION

The stereotypes about women that have been made by the society over the ages should be defied and need to change the way that, society generally puts men on a pedestal. 2013 had been the year of change bringing about a rise in the otherwise static employment opportunities for women. We need to leave this battle of the sexes in order to emerge has the nation of morality and a vision for the future. In addition, Feminism is the belief that women should be treated as potentially equal and also with dignity and as social equals to men. A woman should have the right to vote, when an election is approaching, a woman will declare in this scenario of 21st all the selected political leaders not only represent other women but also build their opinions and rules so firm so that they could support the underprivileged women and their families. Only women can balance the professional and personal sphere and also work more than man in according to the assigned work based on the completion, if you see in today's offices and workplace about 66 percent of assigned work is completed by the girl boss support of the races and caste and other social traumas of society making it stronger than ever. These circumstances are the reason why many political parties have leaders who are women. — It's easier to approach a women leader with a personal request, or a sensitive question They not just take care of achieving heights but also avoids procrastination in comparison to the male employees. I also find women more enthusiastic in becoming mentors, and sometimes it's already such an open and responsive terms to the professional environment.

DISCUSSION AND ANALYSIS

We need to make women independent in order to rejuvenate themselves in all forms of values, thought, rights, decisions, etc. We can see the examples of this age-old battle of sexes even in "Paradise Lost" where the argument is who is more powerful, Adam or Eve? Time has come when we need to stop thinking about who is better but need to stress the fact that we as women are equally good. Women also have immense physical strength because they bring forth life on this earth besides to carry all the jobs or making home or leading life. Women have the capacity to be patient and relatively calmer in a situation. They can easily manage through stressful situations without getting hyper or panicking. Female business leaders are able to communicate with employees very profoundly Weather they are workers, copartners to execute role and responsibility. "Women lead by example, and in so many cases, women have climbed the ladder so they have experienced a variety of roles before they get to the leadership ones. Experience is key." –, Materials for the Arts

"Women are termed to be excellent leaders just because they have the tendency to do everything seamlessly and could fix every puzzle with creativity and also, they are pragmatic and resilient, there is no gender difference that lies with the position of power because the society in which we are living in create gender roles. The ideal women are one who creates history and also who is motivated to reinforce the power acquired in an equal manner Women are pragmatic, resilient and usually able to maneuver tricky situations with grace. Their perspectives are borne out of a mix of trial by fire and sheer fortitude. In an article written in 1988 the women reflect to the idea that women and men differ in power motivation is reinforced by history and culture. In the history of the west, certainly, women have had less access to most forms of power than have men. Many people believe that men are interested in power and getting power while women are not. Others hold that men and women differ in the ways that they establish, maintain and express power". Additionally, studies have shown as participation of females are increasing it also decreases corruption and unfairness as "women are less involved in bribery, and are less likely to take bribe "A survey on gender and corruption from the year 2001 also found that "cross-country data show that corruption is less severe where women hold a larger share of parliamentary seats and senior positions in the government bureaucracy, and comprise a larger share of the labor force"

It is not at all easy to be multitasking tasking, not anyone can manage and supervise things as easily as women without a sense of fatigue or burden. Have you ever seen a man, talk on the phone, feed a baby, cook dinner and manage laundry all at the same time? Women are the best in handling multiple chores at the same time and that to in the best possible way. Women also are the ones who prefer to adhere to rules, regulations and codes stipulated by governmental bodies and other agencies. Women have faced numerous odds over the centuries and thus have emerged as strong survivors who can face all kinds of situations. Maybe this is why women live longer and tend to survive alone too. We all need to admit that women too are equally good and need to give them the recognition that has been due for long in each and every field. Gender stereotype and its implications have been so harmful for the women because even if they have same qualification just like the male counterpart still because of authority and They cannot climb the corporate ladder, no matter if women work for 1000 years still, they will not be able to create the position in the society, walls have been created by the gender stereotypical society for women trying to reach positions of power which is arising the effect of glass ceiling that has the most effect on women. The women tried to work really hard in order to reach the goals and also to had the most vibrant position in the society and also in the corporate world equally to male members who are fighting for the positions in the company. They said that women cannot have CEO positions because they can't afford to be the best in the field and also that they cannot outshine the male boundaries These ignorant stereotypes had already halted the ways and the position of women; they are not the only ones affected. Many young women entering the workforce often see this domination and takes a back step in order to achieve their dreams. Gender equality is a major problem because of which many women see their mentor trapping in this black hole that is a culture begins to develop amongst female where female feel depressed and can

never attain that respect and power for self-empowerment This is a big reason why a lot of women do not run after positions of power because of lack of dignity that has been brought on by gender stereotypes and inequalities.

The glass ceiling is really effacing to days women, but with undue power on gender equality, women have to break the ceiling and effect the men and others in the business environment. Although industries are changing the roles of women and also the positions in the corporate world workplace, it is still important to discover out why women are not gaining leadership positions, even though the right of equal pay for equal wage exist. Evidence shows that the companies which good management has positive results for women. Positive management in companies has led to pay equity, more standard schedules, and equal right to great opportunity. Using innovation in the work environment and also having the leadership program has landed success in employing more women. As per the data provided by research, after extensive research, main barrier that is not letting women touching success not improving in their careers or are not being viewed as competitors for most eminent position in companies is because their interruption in the motherhood and also in the other ways to define that also even if women have full-time jobs, females are always responsible for any family problems rather than men and conditions that lies for century They have nothing but the beliefs This research indicates one top reason of dismissing females' growth in the chart in top positions in businesses is because of gender norms that have continued into the 21th century.

The paper brings a clear look at the context of the increase in feminism and women's drive and what doing gender has involved in the framework of the whole world. These three are interconnected with each other in the whole world from a very long time and also now we see the women are raising voices against crimes and being a peculiar aspect in every field and corner. One simple meaning of Feminism or Feminisms can be start from dignity and status to equal pay as per the differentiation faced by women because of their sex. The reduction in the feminism can only be achieved by different actions as well as introduction of the different organizations. Men may have accomplished a lot but it could not have been possible without coming being born through a woman. Without women, men would not exist. Thus, the survival of the men can only be possible because of the lifeline women have given them. What more is needed to be said? A woman is a wonder to behold. Scientists have actually been trying for years to create life, but have failed in every attempt. The battle between the nature and science will always prove that nature is invincible. Women are a part of that nature. One of the best rules which has been introduced by the Companies act of India states that at least one woman should be there in the board members or board of directors of any company which may lead to the strengthening of the women. This is one of the best industrial reform introduced by the government of India for empowering the women.

In India women are not seen to the part of any community or any movements which belongs to the poor families. Various sorts of restrictions are implied on them even when some of the issues requires the presence of women. One of the cases of feminism came out in Bengal during 1942-1943. The women's condition was merciless they were provided much lower quantity of food then men as a result the women started prostituting themselves as well as their daughters for food. To get relief from such kind of worse situations a group of peasant women's started kitchens which serves around 1100 people a day in 1943. This group also forced the government to lower the price of crops and also provided an idea for opening of the ration shops from which helps them in feeding more and more people per day. In MALTILDA, it is Matilda herself who actually overcomes all the troubles and problem but more importantly, she used her strange power of psycho kinesis and involved with persons in riotously smart ways. You can never judge a book by its cover and girls too are like books—full of great surprises. They have the potential to be led in every sphere. They need to be given the ultimate platform to show case their talent so that they can emerge as the spark light in the dimly lit night. They are brave warriors in the battle of life and can fight every battle if they are being given the opportunities.

"Women rule the world. It's not really worth fighting because they know what they're doing. Ask Napoleon. Ask Adam. Ask Richard Burton or Richie Sambora. Many a man have crumbled." – Jon Bon Jovi

Despite economic recession women are actually helping the economy by sharing their contribution to the economy and helping our world to be the powerful. Empowering women is the need of the society to balance everything in the right manner. Women need to have safer environment to get recognition and fame in every field, so that, the family, society or country can be proud of them. Women should be given a chance to contribute economically in order to make a country fully developed. They should be able to display the best of their abilities in each and every field and sphere--academic, political and economic. Women also have the power to either reincarnate or destroy a soul. A person can be great only when the woman as a mother nurtures and brings him or her up. To help a child flower into a worthy person, the woman sacrifices immensely.

Reading novels has always been form of escapism for me. The difference in the thinking of people on the influence of women should be resolved as is reflected in the works of women authors like Jane Austen. *Narnia* is an example of a mere girl proving her to be the most brave and confident. She's an inspiration and motivation for the girls where for men fear to trade. Even her brother refused to accept and believe her that she actually has discovered a magical land via her wardrobe but later on she was the one who was being entitled as the queen of Narnia. This proves no matter what the society feel, if a woman believes in herself, she will ultimately find a way to conquer and triumph.

Introduction to the women's study was the part of the efforts which have been done by women. Which not only educates women about their fundamental rights but also helps them in getting the prover facilities provided by the government for their growth. This study analyses the different data forms about the women which clearly states the critical lives of the women, why the views or woman's perspective kept hidden from the current academics. This helps more to make the people aware about the current position of women in the society. Lots of analysis have been done using the different data forms and tools comparing the western societies in order to fulfill the gaps between the women belongs both the societies. The women's studies made a large social reform which leads to the increase in the concern towards the issues of women in our societies which are due to poverty, unemployment, lack of education, inequality and underdevelopment of women. Women's studies also provide the different solutions for the different problems faced by women in our society by finding out the accurate cause of the particular problem.

I appreciate Hermione Granger as she gave her introduction on the Hogwarts Express. She was a girl of my age group who was confident enough to openly confess her interest for studying and reading. She did not change for anyone even for her friends who keeps on supporting her. Hermione inspires me a lot an after watching her I also confidently raise my hand in the class and ask the question from teachers or to answer the question which was asked in the class. The best of all Harry Potter series has an immense impact and aura on the mind of children and youth. The girls, who are obsessed with books, have a love for reading and writing has never been dominated by the school kids or the society. She belongs to a normal middle-class family but she has built for herself the confidence that helps her and has given her the wings to fly and reach the top. Shy and hesitant girls like me who am watching her have been inspired by her. The conference of national studies held in Bombay has a great impact on the career and also the strength of women to bring the changes to develop their mindset along with the development in their studies. They helped universities, colleges and also different institutions for the Asian studies and also the education policy for Women's Studies Womens perception need a critical and evaluated views in their own lives and the broader social reality. The gender studies and also the studies for women structures of inequality that led to marginalization, invisibility and exclusion of female from the marginalized areas, Many steps has been taken by UGC to inspire women with the best and the most appropriate education policy to facility them and come as winners.

CONCLUSION

For centuries in the past, women have every time looked upon on men, but things are changing. Yet there is a long way to go. Times when men were the only breadwinners of the family are now remaining in past. Men may be stronger in terms of physical strength, but in today's times, it's emotional stability that counts more than physical

strength and if you take us along, together we can go a long way. While the women's movement is a movement of past now the term Feminism is a modern one and has changed the perspective of world. This term seems to have been first used in 1871 in a French medical text.

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RESEARCH ARTICLE

ECG Signal Compression using Wavelet Packets

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ABSTRACT

To detect cardiac anomalies, long duration ECG of patients is sometimes required. Since this high resolution data occupies large amount of storage space it needs to be compressed for future reference. However the compressed signal has to be of good quality. Wavelet packet transform is a natural extension of Wavelet transform that leads to better frequency localization for high frequencies. Application of the Wavelet packets in the ECG signal compression is dealt with in this paper.

Keywords: Wavelet Packet transform, Electrocardiogram, compression ratio.

INTRODUCTION

Biosignal processing was carried out earlier using various time- frequency techniques like Fourier transform before the advent of Wavelet transform (WT). Due to the time frequency localization property WT has become a favorite tool for researchers especially for analyzing problematic Electrocardiogram (ECG) signals [1].Various forms of wavelet transform like the continuous Wavelet transform (CWT), the discrete Wavelet transform (DWT) and their advanced versions like stationary Wavelet transform (SWT) and Wavelet packet transform (WPT) provide different methods of analyzing the same signal to bring out the desired properties. WPT passes a signal through a series of high pass and low pass filters and their corresponding outputs are known as detail and approximation coefficients respectively. The approximations (A3, A6, A7 soon) and the details (D3, D6, D7 soon) are shown in Fig. 1. This process is called Wavelet packet decomposition.

Wavelet Packet Compression of ECG
The WPT decomposes a signal into a tree of transforms. The original signal breaks down into a low frequency component (the approximation) and a high frequency component (the detail). Each is half the length of the original signal and a tree contains many potential ways of representing the signal. Approximate and detail components are

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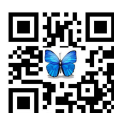
chosen in WPT in such a way that gives a potential solution for further analysis. The energy and entropy quantify how good the solution is when compared to others. The energy of a signal is the sum of squares of the signal's coordinates. The entropy measures the distribution of the energy. Low entropy means that a few components contain most of the energy, and low entropy makes for good compression [2]. The compression features of a given wavelet basis are primarily linked to the relative scarceness of the wavelet domain representation of the signal. Compression literally means that the signal can be accurately restored from a few approximation coefficients at a suitably chosen level in addition to some of the detail coefficients if necessary [3].Compression either by Wavelet packet analysis (WPA) or by wavelet analysis is exactly the same, however, the only difference is that wavelet packets result a more complex and flexible analysis wherein the details as well as approximations are split in contrast with DWT in which only approximation coefficients are decomposed.

METHODOLOGY

In the present case, the compression is illustrated by considering the lead aVL of cardiac ECG signal digitized at the rate of 500 Hz. Decomposition up to a maximum level of nine is obtained using the Daubechies 'db1' wavelet (Fig. 2). The results corresponding to approximation and detail coefficients pertaining to every level are compared with the original ECG in order to select the coefficients of a particular level which is very close to the original signal. Since appropriate diagnosis of ECG depends on visual inspection, the coefficients that generate waveform similar to the original signal can be considered for compression. The compression ratio may be improved with increased rate of sampling the signal. Figure 3.a, b, c, d and e represent the original ECG and its corresponding compressed forms from the first, second, third and fourth level approximation coefficients respectively.

RESULTS AND DISCUSSIONS

The Wavelet packet compressed ECG (Fig. 3b, c, d and e) with compression ratio (CR) of 1:2, 1:4, 1:8 and 1:15, although preserves the location and duration of components like P, QRS and T waves, needs further improvement in the compression ratio (CR). It is also observed that higher the level of approximation coefficients, the better is the CR.

CONCLUSIONS

For correct diagnosis of cardiac ailment, it may sometimes become necessary to examine an ECG taken for longer duration [2], which subsequently requires an appropriate compression technique for necessary storage of such a huge data, wherein the WPT play a very significant role.WPT based lossless compression of ECG is suggested when ECGs are recorded for a longer duration. An extended form of the Wavelet transform called Wavelet packet transform (WPT) is found to be capable of achieving lossless compression of ECG to the tune of 1:15. The CR can be further enhanced by using advanced wavelet methods for removal of baseline wandering, isoelectric line etc. [4].

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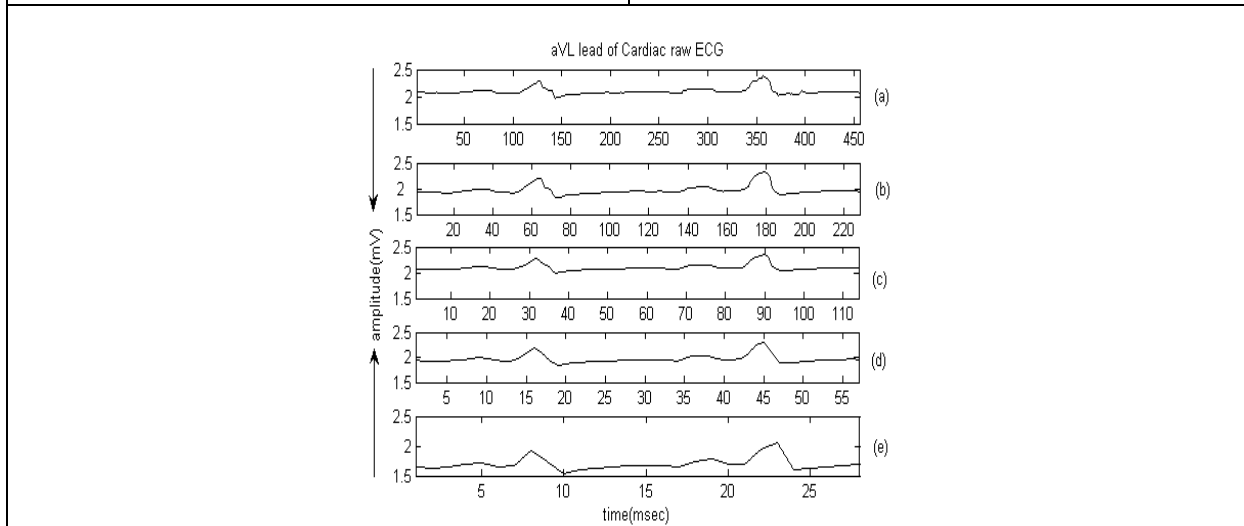
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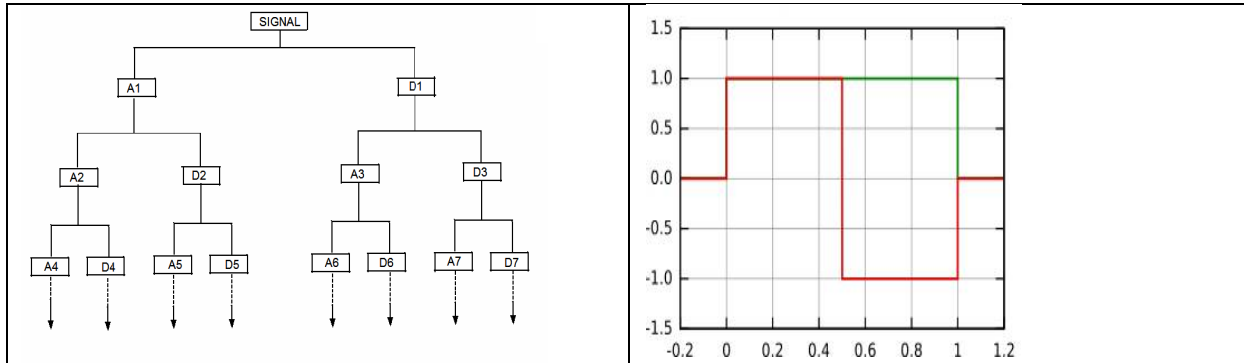
Fig. 1. Wavelet packet decomposition	Fig. 2.Daubechies wavelet 'db1' and the scaling function

Fig. 3.(a) The noisy aVL lead of Cardiac ECG (b) The first level approximate coefficients with CR =1:2 (1.76Kb: 3.51Kb) (c) The second level approximate coefficients with CR=1:4 (0.89Kb: 3.51Kb) (d) The third level approximate coefficients with CR =1:8 (0.44Kb: 3.51Kb) (e) The fourth level approximate coefficients with CR =1:15(0.23Kb: 3.51Kb)

Organic Farming in India: Problems and Prospects - Its Future

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ABSTRACT

Organic agriculture is one of the commonly used ways to prevent the adverse effects of chemical agriculture. In India, farming has become more and more unsustainable for the last three decades. Without much care for the environment and the individual's very life, the system had geared towards high production. The agricultural process that uses biofertilizers and pest control from animal or herbal waste can describe as organic farming. Organic farming was introduced to resolve the environmental harm caused by chemical and synthetic fertilizers. It means that organic agriculture is a new agriculture system that restores, maintains, and improves the ecological balance. The paper consists of secondary details. Information gathered from the publications, such as the websites, literature, historical patterns of organic farming, and its progress in India and abroad. In preparing this text, the International Federation of Organic Farming Movements, Books, Newspapers. Discussions had conducted with educated individuals, organizations, experts on agriculture, consumers, intermediaries in the industry, NGOs, and farmers. Demand is rapidly increasing for organic products. A critical factor in encouraging domestic sales is developing the demand for organic goods. The provision does not meet the demand in the country for organic products, and a lack of adequate links have been established.

Keywords: Biofertilizers, Chemical Agriculture, Demand, Ecological Balance, Environment.

INTRODUCTION

For more than a decade, sustainable development has attracted creativity and action around the world. In order to achieve the aim of sustainable growth, sustainable agriculture is essential. Sustainable Farming is the effective management of agriculture resources in order to satisfy changing human needs while maintaining or enhancing the environment's quality and preserve natural resources," the Food and Agriculture Organization (FAO) reports. Both sustainable farming concepts concentrate on sustaining a growth rate of agriculture that can satisfy every living demand without draining necessary resources. Several of the various approaches to accomplish the objectives of environmentally-friendly agriculture is organic farming. Various organic farming techniques, such as cross-cropping, mulching, and crop and livestock integration, are not new to different farming systems, including conventional farming in older nations like India. However, bio-farming depends on different laws and certification programs prohibiting the use of virtually all synthetic inputs, and the main subject of the strategy is soil sate. Unfavorable effects of modern farming practices have been recorded worldwide, not just on farming although also on the health of all living beings and the environment. Technological application, particularly in chemical fertilizers and pesticides, has convinced people all around us to believe aloud. The adverse environmental impacts are caused by soil erosion, water scarcity, salt, soil pollution, genetic erosion.

Organic agriculture is one of the most frequently used ways to prevent the adverse effects of chemical agriculture. It is the safest option. There are many organic agriculture descriptions and regarded as the most reliable and strict by the Department of Agriculture (USDA). It is characterized by using methods and substances that preserve organic agricultural products' quality until they reach the consumer. The system is intended and maintained to produce agricultural products. It was done by using compounds to satisfy any specific evolution within a scheme to preserve the long-term biological operation of the soil, ensuring effective peak management, recycling waste to remit nutrients to the soil, caring for farm animals, and manufacturing agricultural products without the use of special synthetic additives or processing in compliance with the Act In its modern past, the roots of organic farms dates back to the 1940s. The cause of organic agriculture had endorsed by Rodale in the United States, Lady Balfour in England, and Sir Albert Howard in India. In India, agriculture has become more and more unsustainable for the last three decades. Without much care for the environment and the individual's very life, the system had geared towards high production.

Meaning of Organic Farming

The agricultural process that uses biofertilizers and pest control from animal or herbal waste can describe as organic farming. Organic farming was introduced to resolve the environmental harm caused by chemical and synthetic fertilizers. It means that organic agriculture is a new agriculture system that restores, maintains, and improves the ecological balance.

Aim of Organic Farming

- To provide quality and nutritious material to the population.
- Efficient natural resource use
- Reduce/ stop all kinds of agricultural emissions
- Minimize prices for input
- To sustain long-term soil fertility.

Need For Organic Farming

The organic sector is one of America's fastest-growing agricultural sectors. Organic cultivation has such essential advantages as maintaining the organic composition of the soil.
Natural farmers use the following

- Preserve and boost soil fertility, biodiversity, and erosion.
- Minimize exposure to hazardous products to humans, animals, and the environment

- Transparent agricultural practices in order to meet local requirements of development and local markets

NIFA'S Impact
In the field of organic agriculture, NIFA programs promote work which includes:
- Promoting the development of cultivation, breeding, and processing processes for organic agriculture
- Determining possible competitive advantages for producers and processors
- Investigate the possibilities of foreign trade in organic and manufactured goods
- Assess ideal organic product characteristics
- Recognition of marketing and policy limits on organic farming expansion
- On-farm research and development that emphasizes the control, testing, and creativity of organic farms for working.
- Production of new and improved organic seed varieties
- Build educational tools for farmers to educate farmers on organic practices
- Promote the transition from traditional to organic farming.

Types of Organic Farming

Organic agriculture has two forms, namely: organic agriculture

Organic Farming Integrated: Integrated organic farming requires incorporating pesticide and nutrient management to meet the population's ecological and economic needs.

Natural Pure Agriculture: Pure organic cultivation ensures that all unnatural substances had avoided. Both fertilizer and pesticide from natural sources such as food from bone or blood had obtained in this farming process.

METHODS OF ORGANIC FARMING

Rotation of Crops: A process for the development, in sequence, by different seasons, of various types of plants in the same region

Mist Green: It is about the dead seedlings rooted and filled in the soil to be a fertilizer to improve its quality.

Mulching: It is a process that covers the earth and makes the plant growing and developing more desirable.

Organic Fertilizer: Substances which includes living microorganisms colonize the rhizosphere or else interior of the plant as it is applied to seeds, plant surfaces or soil, and support an increase in by increasing primary nutrient supply or supply to the host plant.

Compounding: Vermicompost results from the composting process using different types of worms, mostly red wigglers, white worms, and other earthworms, to produce a combination of plant or food waste, bedding, and vermicast. Vermicast is the product of this process.

Review of Literature

Ramesh et al. (2005) found that organic farming provides comparative benefits in low-precipitated areas with relatively low natural fertility and soil fertility levels. Labor has a strong return, which is relevant where there is

practically no paying labor. The costly investment in irrigation, energy, and external inputs of organic agriculture is not required, but instead, organic agricultural policies can improve local food safety, especially in marginal areas.

Rajib Roychowdhury et al. (2013) have studied the wildly divergent views of organic farming. However, there is explicit agreement that it is environmentally friendly and inherent in preserving human santé. Many studies have also shown that organic farming is efficient and sustainable. In developing countries, organic food production costs are higher as organic agriculture is labor-intensive and labor in these countries is expensive. However, organic farming is the right potential solution for chemical agriculture to human health and the environment in countries such as India, where labor is very high and fairly inexpensive. The Government of India has made efforts to support organic farming in general.

Anirudh Garg et al. (2014) analyzed new developments in agriculture due to changing demographic patterns and technological advances. These innovations must use carefully to meet the rising demands of modern farming. Upright farming and organic farming can meet changing human needs and requirements as feasible alternatives for traditional farming. Furthermore, limitations should be addressed in implementing such practices, and ties between researchers and farmers for suitable measures should be established.

Objectives of the Study

- To comprehend in the light of experience in other countries, the need for organic farming in India.
- To analyze and assess the factors that can encourage the adoption of organic agriculture in India.
- To examine India's limitations on implementing organic farming, both political and social and above all, economical.

Research Methodology

The paper consists of secondary details. Information gathered from the publications, such as the websites, literature, historical patterns of organic farming, and its progress in India and abroad. In preparing this text, the International Federation of Organic Farming Movements, Books, Newspapers. Discussions had conducted with educated individuals, organizations, experts on agriculture, consumers, intermediaries in the industry, NGOs, and farmers.

Organic Farming in India Statistics

National Program for Organic Production (NPOP)

Natural crops are grown by methods of cultivation without chemicals had fertilized. Pesticides had produced both the agricultural system and provided an environmentally and socially conscious approach to the biochemically sourced production. The agriculture method operates at the grassroots, maintaining soil's reproductive and regenerative capacities, good nutrition, and good soil management. Because of its different agro climate conditions, India has great potential to produce all forms of organic products. The long-standing past practice of organic farming in many parts of the world is a bonus. It promises that organic producers will leverage the demand in the domestic and export sectors, increasing. According to the statistics available, India ranks 8th in world organic farmland and 1st in total producers, according to 2020 (Source: FIBL & IFOAM Yearbook, 2020). APEDA introduces the national organic production program, Minister of Commerce & Industry of the State of India (NPOP). The curriculum includes accreditation of certification bodies, organic quality standards, organic agriculture, and marketing promotion. The European Commission and Switzerland have accepted the NPOP standards for processing and accreditation systems for unprocessed goods from plants equivalent to their countries' standards. The USDA also accepted something similar as analogous to NPOP's accreditation conformity evaluation processes. With such recognition, the importing countries recognize Indian organic ingredients adequately licensed by the approved Indian certification bodies. APEDA is also carrying out mutual correspondence with South Korea, Taiwan, Canada, Japan.

Zone

Being Asof 31 March 2020, 3.67 million Hectare (2019-20) had been protected by the entire organic certification field (registered under the National Organic Production Programme). It includes 2.299 a hundred thousand hectares of cultivable land and a further 1.37 million acres of wild crop collection. The country includes Madhya Pradesh, and then by Rajasthan, Maharashtra, Gujarat, Karnataka, Odhisa, Sikkim, and Uttar Pradesh, the largest field of organic certification. Sikkim has achieved a remarkable distinction during 2016 in the conversion under organic certification of its whole cultivable land (more than 75000 ha).

Production

The number of organic products that India produced was about 2.75 million tons (2019 to 20). It includes all kinds of foodstuff such as oilseeds, sugar cane, cereals and millets, cottage, pulses, aromatic and medicinal plants, tea, coffee, berries, spices, dried fruit, vegetables, food processing. The manufacture of organic cotton fabrics, foodstuffs is not restricted to the edible market. Madhya Pradesh, led by Maharashtra, Karnataka, Uttar Pradesh, and Rajasthan, is the world's largest producer. Oilseeds are the biggest single category for commodities, followed by sugar crops, cereals and millets, tea & coffee, fiber, fodder, pulses, herbal and medicinal plants, and spices & conditions.

Organic Farming Prospects

- It helps protect the health of the world by reducing emissions.
- The reduction of the residue level in the substance decreases human and animal health risks.
- It contributes to sustaining sustainable agricultural production.
- It lowers farm production costs and increases the health of soils.
- It ensures that natural resources are used optimally for the short term and conserve for future generations.
- It saves animals and machines resources and reduces the chance of crop failure.
- It improves soil's physical properties, such as granulating, right tilting, good aeration, fast root penetration, increases water retention, and decreases erosion.
- It enhances the soil's chemical properties, such as providing and preserving soil nutrients, decreasing nutrient losses in water and the atmosphere, and encouraging beneficial chemical reactions.

Organic Farming Problems

In organic farming, there are a few issues, like

- Organic manure is not readily available, and if organic inputs are purchased, it can be more costly based on plant nutrients than chemical fertilizers.
- Within the first few years, organic farm production decreases, so organic production should earn premium farmer prices.
- The organic farming, manufacturing, transport, and certification guidelines go beyond ordinary Indian farmers' knowledge.
- There is still no correctly streamlined promotion of organic goods. There are many farms in India that were never chemically controlled or cultivated or converted back to organic farming because of their convictions or because of economics. Those thousands of farmers who cultivate millions of hectares of land are not organically listed. Their goods sell them on the open market at the same price and traditional products or sell merely goodwill and trust like organics through select outlets and regular specialist markets. The costs involved and the certifiers' comprehensive documents will never allow these farmers to opt for certification.

Organic Farming Supporting Schemes in India Government

Under organic farming, the cultivable area grew by the concentrated government efforts starting at 11.83 lakh hectare in 2014 to 29.17 lakh hectare in 2020. Over the past few years, organic promotion activity has resulted in state-specific organic labels being created, increased domestic supply, and organic production exported from the north-east. The vision intended to cover an additional 20 lakh hectares of the area by 2024 and consider organic

initiatives' progress. Awareness programs, the availability of sufficient recovery facilities, marketing establishments, and bonus prices on organic produce would surely be the incentive for farmers in organic agriculture to enhance organic cover throughout the nation. The Government of India provides support through several schemes to promote organic agriculture throughout the world.

Paramparagat Krishi Vikas Yojana (PKVY)

Paramparagat Krishi Vikas Yojana supports organic farming group together with the certification of PGS (Participatory Guarantee System). The scheme facilitates Cluster recruitment, training, certification, and marketing. Rs. 50.000 per ha/3 years of assistance had given, 62% (Rs. 31,000), which would provide farmers with an opportunity to make bio-inputs.

Mission Organic Value Chain Development for North Eastern Region (MOVCDNER)

The scheme supports accredited organic niche farming by third parties in the north-east by Farmer Producer Organisations (FPOs), emphasizing exports. Applications for organic contributions, including organic manure and bio-fertilizers, are funded by Rs 25,000 per Hectare for three years. The scheme also provides funding for training FPOs, capacity building, post-harvest infrastructure to Rs 2 crore.

Capital Investment Subsidy Scheme (CISS) under Soil Health Management Scheme

Under this scheme, Government agencies for establishing mechanized fruit and vegetable market garbage, agro-waste production units up to a maximum limit of Rs 190 lakh/unit had given 100% assistance (3000 Total Per Annum TPA capacity). Likewise, up to 33 percent of Rs 63 lakh per unit of cost limit funding for individuals and private agencies is available when capital investment had paid.

National Mission on Oilseeds and Oil Palm (NMOOP)

The financial aid had given for various components, including biofertilizers, rhizobial culture, the phosphate solubilizing bacteria (PSB), and zinc solubility bacteria (ZSB), acetobacter, and vermicompost in a 50% per hectare subsidization. It involves several components, including bio-fertilizers.

National Food Security Mission (NFSM)

Financial assistance had given under the NFSM, which is 50 percent of the cost limit restricted to Rs 300 per Hectare to promote biomass fertilizers. In accordance with the conditions of certified land of 1.94 million hectares, India ranks at a ninth place according to international data on resource data from the Research Institute of Organic Agriculture and the International Federation for Organic Agricultural Movement (IFOAM) Statistics 2020. Intending to facilitate the use of biological on-farm inputs for chemical-free farming in Bharatiya, Prakritik Krishi Padhati (BPKP) of the PKVY had initiated. In order to stimulate natural agriculture under the BPKP, Andhra Pradesh and Kerala occupied one lakh hectare and 0.8 lakh hectare, each. Similarly, in 2020-21 continuous field certification and certification support for individual farmers were introduced to put conventional and organic farmers into default. Government agencies, Primary Agricultural Credit Societies (PACS), FPOs, and others could take loans for building an additional value infrastructure for organic produce under Aatmanirbhar Bharat's AGIF (1 lakh crore).

Organic Farming Techniques in India

- It promotes organic farming via agrotourism that encourages urban families to organic agriculture and provides farmers premium prices.
- Organic goods' marketing, including attractive packaging by organic waste, can also be handled better through retailing, packaging, and labeling.

- The cropped should be regarded with high-value crops with trade sustainability, industrial use, and export prospective.

India has enormous potential to capitalize on organic farming, with its vast geographical area and variety of eco-regions. However, small farmers in India are restricted by land availability, registration, a local market shortage, and other factors. Therefore, the Government and non-governmental organizations need an integrated effort to eliminate constraints that enable small farmers to take organic farming as a solution to satisfy their nutrition requests while maintaining soil, water, energy, and biological supplies.

The Organic Farming Future in India

The list of 172 countries worldwide practicing organic farming shows the showered potential of Indian farming as only 0.4% of the total agriculture is organic. The Indian organic industry's domestic and exports increased respectively by 30% and 40% in 2015. Organic agriculture has seen dramatic overall growth in virtually every crop because of increasing food safety and the environment's safety. Safe customers today will, in many ways, stimulate the development of the organic farming market. The WHO says there are currently approximately $37 billion in the overall world demand for organic foods. Of this $14 billion market, $5 trillion is projected for herbal plants and medicinal products by 2050. India has over 15,000 registered organic farms, according to a report by the International Foundation for Agriculture and Development (IFAD). Natural farms are typically more productive and environmentally sustainable, using fewer pesticides and less pesticide-intensive residues. It offers many environmental benefits and healthy food. Studies show that organic systems can produce the same results or even outweigh traditional approaches over long periods. Organizations involved in organic food must raise consumer awareness in non-metro towns. Sikkim (North-Eastern India) is an organic state with over 75,000 hectares of organically grown soil. By 2030, another north-eastern state of India, Meghalaya, is likewise looking to turn 200,000 hectares of land into an organic farm. Greater than 100,000 farmers in Kerala are engaged in organic farming.

Organic agriculture has been playing an important role worldwide because of climate changes. The Indian Government encourages organic farming across various systems under National Mission Sustainable Agriculture (NMSA) plans. In order to help support organic farming in the country, the Government implemented Paramparagat Vikas Yojna (PKVY) and Organic Value-Added Development (OCVDNER) programs under the NMSA. Under this regime, State governments will assist farmers with the funding of a maximum of one Hectare of land centered on a cluster for every 20 hectares. During the transition phase for three years, the Government has set aside about $730 per Hectare of land. The Indian Government has also announced an investment of almost $15 million to grow the organic market and about $ 44 million to invest in the Participatory Guarantee Scheme (PGS), which certifies producers involved in organic farming an organic quality assurance system. The Chandigarh State Agricultural Marketing Board's recent seminar on organic farming with KhetiVirasat Punjab's Mission to Promoting Sustainable and Environmental Farming was an effort to encourage the environmentally-friendliness of farming. In order to encourage organic farming, reduce reliance on chemicals and fertilizers on farmers, and ultimately benefit end consumers, the seminar took place among experts, farmers, consultants, and teachers. It was an effort to make organic farming take place in Chandigarh and its neighboring regions. In addition to limiting the use of fertilizer and pesticide use chemicals, the Goa State Department of Agriculture opened the State Authority regime for the first time. Approximately $150 a hectare and up to 2 hectares per beneficiary, the program offers a 50% aid in respect of organic inputs costs.

The District Administration of Bulandshahr, in the State of Uttar Pradesh, calls on farmers to start organic farming and cultivate multiple crops to reduce the adverse effects of crop combustion and global warming. In order to minimize the damage caused by unpredictable weather changes, it was intended to spread knowledge about the demerits of crop furnace use and encourage weatherproof farming. Arum Kumar Sharma has started the tradition and promotion of organic farming in the Dantiwara village of Rajasthan to ensure food safety in India. His team concluded in eight years of extensive study at Central Arid Zone Research Centre (CAZRI), queuing all the adverse

events caused by chemical fertilizers and pesticides is organic farming. He claims that the Green Revolution needs to be introduced, emphasizing organic farming rather than chemical farming, concentrating on 60% of the dryland that we have today. Around 400 Bigha farms have been subject to organic cultivation in Dantiwara and have been inspired continuously to spread the word across the region. For more than a decade, India has sufficed for itself in food staples. Bio-farming can flourish in India and contribute 1.5 billion individuals by 2030. The organic farming market in India will hit about $1.36 billion, together with growth rates ranging from 25 to 30 percent per year in 2020, according to statistics by Assocham and Tech Sci. Organic farming in India is rapidly growing, and investors accept that difficulties are facing this sector. However, the positive economic outcome will follow as soon as understanding and training on the benefits and establishing organic farming is extended to farmers.

CONCLUSION

In India, it harms agricultural development, environmental destruction, health, and health issues. In terms of unsustainability, the traditional farming method. Like an alternative to the industrial system, organic farming is gaining momentum. In many nations, 2-10% of their cultivated fields were converted into organic farming. Demand is rapidly increasing for organic products. The adoption of organic farming continues to be well behind India. The only accomplishment so far appears to have been establishing the National Organic Production Standards (NSOPs) and the recognition of four accreditation agencies (all government agencies), with their expertise restricted to just a few crops. The following are some concerns that need attention at the government policy level if the work to spread organic agriculture in the country is to be completed. In order to encourage organic agriculture, significant financial support is required from governments (Central, State, and lower levels of Government). The governments' very liberal subsidies were a significant factor behind the significant organic countries' progress. Bio farmers in India do not receive government subsidies because they target traditional farming. Given its minimal risk bearing capability, the need to make an appealing proposition at least at a preliminary level, the possible failures in productivity anticipated for several years, and the presence of organic marketing networks provided by financial aid must be adequate. A successful proposal is to start the implementation of biological farming in India's north-eastern area and dry farms. Nevertheless, it should be noted that the poorest and least developed groups in these regions are dependent on farming for livelihoods. In cash and kind, the full payoutto agricultural producers in the event of loss of production they experience until it gets organic farming level within days before adoption must be supported entirely in the program on organic farming in such areas. A fair, fast, and effective distribution mechanism for such assistance should also be in place in advance, likely holding the government bureaucracy at a distance. A critical factor in encouraging domestic sales is developing the demand for organic goods. The provision fails to meet the demand in the country for organic goods, and a lack of adequate links have been established

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RESEARCH ARTICLE

Heavy Metal (Lead) and Antibiotic Susceptibility Profiles of *Staphylococcus* sp. Isolated from Marine Fish Samples

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ABSTRACT

The present study aimed to evaluate the heavy metal resistance and antibiotic susceptibility pattern for *Staphylococcus* sp. isolated from marine fish collected from Nagapattinam. Antibiotic susceptibility pattern, heavy metal (lead) tolerance, minimum inhibitory concentration and accumulation level of lead in bone sample of fish was evaluated. The maximum resistant (100%) recorded in tetracyclin antibiotic and minimum zone of inhibition recorded in amoxylin and norfloxacin antibiotics against *Staphylococcus* sp., while no zone of inhibition in doxycycline antibiotics were determined. The minimum inhibitory concentration recorded in between 0.5 – 1.1 mg/ml. the heavy metal analyzed in bone sample the permissible value 0.45mg/l detected and compared to WHO reports. This study concludes that the persistence of lead in the fish bone samples indicates metal pollution and the lead resistant *Staphylococcus* can be used for bioremediation but antibiotic resistance and heavy metal pollution is alarming.

Keywords: Fish, antibiotics, heavy metal, bacteria.

INTRODUCTION

Heavy metals are natural components of the earth's crust. Weathering and erosion causes metals to wash from the land into the waters. They can be subdivided into essential and nonessential based on their needs for living organisms. Essential micronutrients (iron, copper, zinc, cobalt and nickel) are critical for normal development and growth of organisms (Marschner 1995, Broadeley, 2007). But excess of metals affects both biochemical and physiological processes in living organisms. Hence the persistence of heavy metals beyond the threshold level are

considered as toxic and non essential heavy metals such as cadmium (cd), Mercury (Hg) and lead (Pb) have no known essential role in living organisms exhibit extreme toxicity even at very low exposure levels and have been regarded as the main threats to all forms of life especially human health. Mercury poisoning affects the formation of microtubules and thus neuronal migration and cell division. Inhalation of lead containing dust particles, fluids or fumes causes effect on neurological development. Calcium inhibits the growth of plants, and cadmium accumulated in the plant products .The metabolism of animals and humans are affected by consuming these plant products. The load of heavy metals is increasing in the environment due to many different anthropogenic sources such as industrial, municipal and agricultural waste. As a result of increased concentration of heavy metals in the atmosphere subsequently leads to an increase in ocean metal concentration. They are a serious and persistent pollutant of terrestrial and aquatic ecosystem. Contamination of soils by heavy metals is a long term problem. By the process of bioaccumulation such metals enter into the food chain and magnified in the ecosystem and finally assimilated by human consumers. Even though microbes are sensitive to heavy metals, continuous exposure compels them to adapt the environment and they convert heavy metals into harmless forms through biosorption or enzymatic transformation. By the similar process microorganisms readily develop antibiotic resistance also. Microorganisms resistant to both metals and antibiotics have been isolated frequently from different environments and clinical samples (Henriette *et al.* 1991; Sundin and Blender 1993). *Staphylococcus aureus* have been reported to be resistant to a number of heavy metals such as lead, chromium and iron. These metal resistant organisms play an important role in bioremediation of metal contaminated areas. This study focuses on the metal resistant *Staphylococcus* which was isolated from marine fish samples.

MATERIALS AND METHODS

Sample Collection
The fish samples were collected from marine environment at Nagapattinam. The fish samples, packed in propylene bags with ice cubes, were stored at 20^oC in deep freezer in the laboratory for further analysis.

Isolation of Microorganisms
Fish samples (*Sphyraenidae sphyraena*) were separated into eyes, gills, head, skin, muscles and bones with dissecting knife. Separated organs were swabbed and directly streaked over on Nutrient agar medium, Macconkey agar and Blood agar medium. Plates were incubated at 37^oC for 24 hours.

Identification of Microorganism
Isolated colonies were identified as morphological and biochemical test, after confirmed by Determination of bacteriology 9th edition.

Antibiotic Susceptibility Test
The Antibiotic Susceptibility Test was performed using Kirby Bauer Disc Diffusion Assay with frequently used eleven antibiotics.

Lead Tolerance Capacity
Nutrient agar was prepared and poured into petriplates (thickness of medium should be 4mm.) Inoculated the isolated bacterial strains on the surface of the plate and spread by making use of L-rod. Pieces of lead metal (2gm) were placed on the centre of the medium and were incubated at 37^oC for 18-24 hours. They were examined and observed the zone of inhibition around the lead pieces.

Minimum Inhibitory Concentration
Isolated bacterial strains were exposed to lead nitrate Pb $(NO_2)_3$ salt concentration in the range of 0.5mg/ml to 1.1mg/ml for heavy metal exposure. MIC was determined by observing the turbidity.

Heavy metal (lead) analysis in bone sample

The bones were then separated from 1kg of fish oven-dried at 105°C and then ground into powder. Extraction of metal from the fish samples was done by mixed acid digestion using 10ml of 65% HNO_3, 5ml of 37% HCL and 2ml of 35% H_2O_2 at 80°C in a chamber until colorless liquid was obtained. The solution was allowed to cool, filtered through whatmann number 1 filter paper into a 50ml standard flask. The filtrate obtained was made up to 50ml mark with distilled water and used for heavy metals determination against those of the blank and calibration standards using atomic absorption spectrophotometer.

RESULT

The fish sample collected from Dr.MGR Fisheries College and Research Institute at Nagapattinam. And they indicated that fish belongs to the family *Sphyraenidae sphyraena*.

Isolation of Organism

The colonies grown in Nutrient agar, Macconkey and Blood agar were identified through preliminary test confirmed by biochemicals test. Both Gram positive and Gram negative organism such as *Staphylococcus, Streptococcus* and *E.coli* were identified. Among this organism only *Staphylococci* were isolated and maintained in pure culture for further studies (Table 1 and 2; fig. 1).

Antibiotic Susceptibility Test

The antibiotic susceptibility test performed by Kirby Bauer Disc Diffusion method and its ranges were illustrated in table 3.Tetracyclin shows 100% resistance.

Lead tolerance capacity

Lead tolerance capacity was observed on Nutrient agar plate and there was no zone of inhibition. It indicates the tolerance capacity of the organism (Fig. 3).

Minimum inhibitory concentration

The range of minimum inhibitory concentration of heavy metal lead against *Staphylococcus sp* was illustrated in fig: 4. The MIC value was 1.1 mg/ml.

Atomic Absorption Spectroscopy

The level of lead was estimated through atomic absorption spectroscopy in NIT at Trichy. The permissible limit of lead was 0.05mg /kg (WHO report) in the present study the level of lead exceeds the permissible limit 0.45mg/L (Table 4).

DISCUSSION

Seafood products are important source of proteins, polyunsaturated lipids and also of vitamins. The fishery industry including fish aqua culture, in coastal oceans has expanded rapidly around the world in the last few decades because of increasing demand for fish as a protein source. As the two sides of coin, fish industry as the concentration of heavy metals as tail side increasing equally (Belias and Dassenakis 2002, Dalsgaard and Kouse Jensen, 2006). Hence, the monitoring of metal concentration in fish is also important in considering with food safety regulations. This present study concentrated on heavy metal (lead) in marine fish samples and the antibiotic susceptibility pattern of *S. aureus* isolated from the fish samples. There were lot of reports relating the antibiotic resistance and heavy metal resistances in various environments, such as sewage, hospitals and also in marine water samples, which clearly indicates the heavy metals pollution and antibiotic abuse in the environment. Similar to the present study, Kacar and

Koujigit (2013). Characterized the heavy metal and antibiotic resistance in *Bacillus* sp. isolated from Bastern Aegran Sea coast was resistant to gentamycin followed by to bramicin where as in the present study *Staphylococcus* sp. isolated from the marine fish showed 100% resistance against tetracycline. Divya *et al.* (2014) observed the MIC to heavy metal resistance for clinical microbes. MIC to lead was higher in *Acinetobacter* species (600- 1000mg/ml) and lower in *S.aureus* (50-150 mg/ml). But in the present study the MIC of marine fish isolate *Staphylococcus* sp. was high. Quiongxia Hu and Lanming Chen (2016) revealed that 74.5% of *Vibrio* sp., isolated from crustaceans and shellfish in china, showed multidrug resistance and tolerance to the heavy metal cu^{2+}, Pb^{2+} and cd^{2+}. In the present study also *Staphylococcus* sp. was resistant to the heavy metal lead. Prevalence of heavy metal in the environment can proliferate resistance to heavy metals and antibiotic through co-resistance and cross resistance mechanism (Nguyen *et al* 2019). In coherent to this, in the present study heavy metal (lead) concentration in the bone sample of fish was 0.45 mg/kg which exceeding the WHO permissible limit. They also insisted that the total metal bio accumulation were greatest in the liver, gills and lowest in the muscle. Hence that fish were still safe for human consumption as liver and gills are not edible part for humans. Hence this study suggested that controlling of heavy metals pollution will provide healthy fish for future human consumption. In addition, heavy metals tolerant organism has significant role in the detoxification of polluted environment, which can be widely used for bioremediation, but the spread of the antibiotic resistance is alarming (Erum Hanif and Shafag Aiyaz Hassan, 2016).

CONCLUSION

Fish and seafood are good food, which contain amount of rich protein and omega-3 fatty acid and it's very healthy for human and also for its ecological importance. Contamination of marine and fresh water bodies due to release of "Toxic metal" (anthropogenic sources) lead and mercury posses serious threat to natural biota including humans. Heavy metals (or) toxic metals such as lead, cadmium, copper, zinc and chromium cannot be biodegraded and therefore persist in the environment for long period and causes serious effects. Efforts should be taken to prevent environment pollution with heavy metals and policy should be undertaken for the usage of antibiotics.

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Table1: Isolation of bacteria from different parts of fish

S.No	Different Parts of fish	CFU/ml
1	Eyes	5
2	Gills	13
3	Head	12
4	Skin	16
5	Muscles	14
6	Bone	9

Table 2: Biochemical characteristics of isolated bacteria

S.No	Biochemical test	1	2	3
1	Gram staining	+	+	-
2	Shape	Cocci (chain)	Cocci (cluster)	Rod
3	Motility	-	-	+
4	Indole	-	-	+
5	Methyl red	+	+	+
6	Voges proskauer	-	-	-
7	Citrate	+	+	-
8	Catalase	-	-	+
9	Coagulase	+	-	-
10	Hemolysis	+	+	-
11	Identified as	*Staphylococcus*	*Streptococcus*	*E.coli*

Table 3: Antibiotics resistance percentage of *Staphylococcus* sp.

S.No	Antibiotic	*Staphylococcus* sp. (% of Resistance)
1.	Ampicillin	64%
2.	Cefuroxime	32%
3.	Amoxicillin	16%
4.	Ceftaroline	40%
5.	Doripenem	36%
6.	Tetracycline	100%
7.	Doxycycline	0%
8.	Levofloxacin	20%
9.	Cefoxitin	20%
10.	Norfloxacin	16%
11.	Ofloxacin	24%

Table 4: Lead estimated by atomic absorption spectroscopy

Sample concentration (mg/l)	Stnd. Concentration (mg/l)	Blank signal	Time	Analyte signal stored
0.55	0.556	0.0773	15:46:44	No
0.413	0.413	0.0567	15:46:48	No
0.393	0.393	0.0538	15:46:51	No
0.454	0.454	0.0626	-	-
0.885	0.885	0.0128	-	-
19.498	19.498	20.48	-	-

Fig 1: Isolation of bacteria from fish sample

Fig 2: Antibiotic sensitivity test

Fig 3: Lead tolerance test

Fig 4: Minimum Inhibitory Concentration

RESEARCH ARTICLE
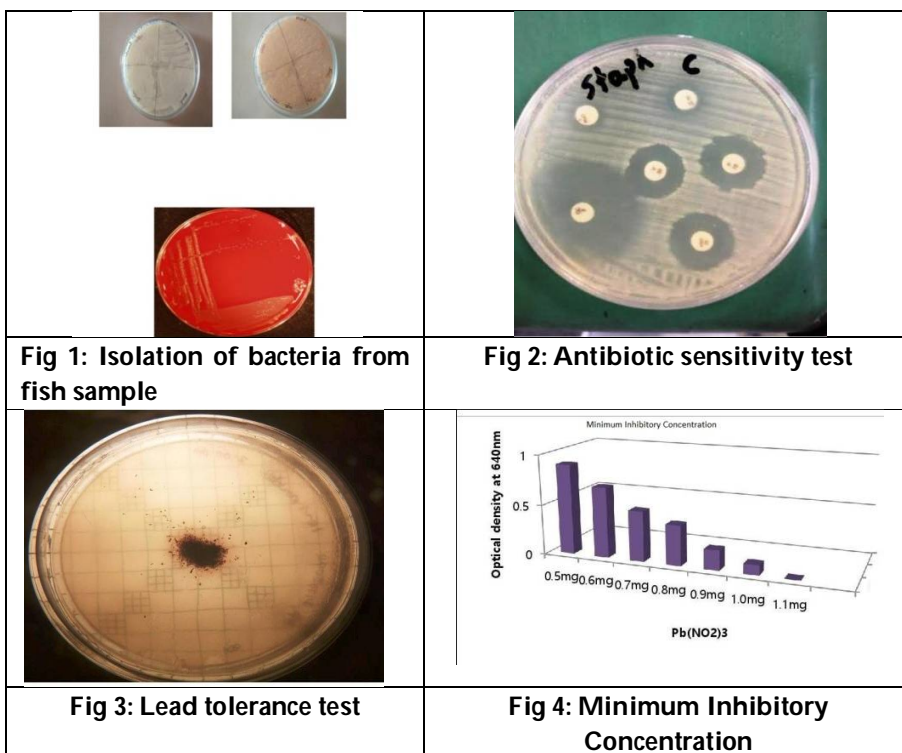
Preposition as a Cognitive Tool in Knowing about the Learning of a Poem

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ABSTRACT

Prepositions can be used as a cognitive tool to identify the path towards understanding the complexity of a metaphysical poem. Cognitive poetics is a new way of thinking which can be implemented in understanding literature. It involves the application of psychology and cognitive science. It is used to illuminate the study of literary reading. It is an emerging discipline in the field of literature. Cognitive poetics is used to analyse the text but it recognises that the content has an important role to play in understanding the theme of the poem. Metaphysical poetry is a type of complex poetry that can be understood easily by applying cognitive poetics. Strange imagery, unusual comparisons and ingenious conceits are some of the characteristics of metaphysical poetry. This paper will explain in detail about applying cognitive poetics in understanding the theme of some of the metaphysical poetry which uses prepositional phrases as their titles.

Keywords: Cognitive Poetics, Metaphysical Poetry, Preposition.

INTRODUCTION

Cognitive poetics is about reading literature. Cognitive poetics can be applied in reading the art of literature that is poetics involving the mental processes [1]. It is a school that deals with literary criticism which uses the principles of cognitive science particularly psychology to the interpretation of literary texts. It is about reading literature. It usually focuses on the reader's understanding of a poem rather than the author or the content. It is used to have a
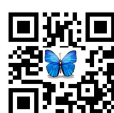
deep analysis while reading a poem. While applying cognitive poetics the context plays an important role in understanding the meaning of a poem. Cognitive poetics is "applying the principles of cognitive science to the interpretation of literary texts" [2]. It acts as a link between humanities and sciences. Cognitive poetics is also known to be "a new brand of poetics" [3] and "a new science of literature and reading" [1]. This allows the reader to come up with new ideas and helps them to think about literature and science and to link them. It helps to relate the psychological effects on the reader to the literary text. Cognitive poetics helps to grasp the subject of a poem by linking the things realized before. It assists in understanding the subject of a poem. It is important to discover the association between the title and the lines of the poem to comprehend the metaphorical significance. The preposition serves as a cognitive tool for understanding the association here. As a cognitive tool that plays a vital role in understanding the theme of a poem, a preposition can be used. To understand the relationship of a noun with another word in the sentence, a preposition is often used. It associates words in the title as well as the context when a preposition is used in the title. To connect the title and the context, it acts as a bridge. Metaphysical poetry is set apart by the utilization of expanded metaphorical dialects, unique vanities, paradoxes, and philosophical subjects. The word 'metaphysical' alludes to style, not the subject matter yet style represent an attitude towards experience. Metaphysical poetry discusses the idea of the universe. Subjects of interest regularly included love, religion, and profound quality, which the poets considered through unusual correlations, as often as possible utilizing unforeseen likenesses and representations in shows of wit. Joan Van Emden expresses that metaphysical poetry is "emotion and intellect are called to action at the same time" [4] metaphysical poetry is a school of poetry in which cognitive poetics can be applied to understand the complexity of a poem and the innermost meaning and theme can be revealed. When a preposition is used in the title of a poem it eases the work of a reader to understand the main theme if, cognitive poetics is applied as a tool.

Here are some of the metaphysical poems where prepositions were used as the titles
- 'To Time' by Thomas Randolph
- 'To Her at Her Departure' by Robert Heath
- 'On a Drop of Dew' by Andrew Marvell

Analysis of "To Time"

Thomas Randolph (1605-1635) was an English poet and dramatist. He was a humorous writer and his works were admired by Ben Jonson. From the title 'To Time' it is clearly understood that he is addressing time. The preposition 'to' plays a vital part in understanding the concept. In this poem he addresses time. He just posted a question at the beginning of the poem [5] by asking

> *"Why should we not accuse thee of a crime*
> *And justly call thee envious, time?"*

The writer starts the line by saying that time resembles a criminal endeavoured wrongdoing. Also, wish to call time jealous. The writer denounces time in the absolute first line.

> *"When in our pleasures we desire to stay*
> *With swallow's speed thou flyest away;*
> *But if a grief in our sad hearts do keep*
> *Then thou art like a snail and will but creep;"*

At the point when the poet and his mistress were appreciating the delights, they wish that the time should remain yet, with speed the time passes quickly away. He says that time passes quickly during cheerful minutes however in tragic minutes it creeps like a snail. He utilizes a comparison to contrast the time and the snail. The poet requests that the time change the speed and permit the sand to stream gradually in the hourglass when he remains with his mistress. He wishes that the time should run in reverse. He expects the time to fix the moment and not confine their happiness because his mistress was a divine gift to him. At that point, the poet understands that when their

repetitive acts of love become old and when they can move without speedy spirits and when they started to blackout and lie like a half-dead ivy twine with their branches wilted then, the time can take its wings again and can fly.

Study of "To Her at Her Departure"

Robert Heath (1620-1685) poet, was baptized at St Martin Ludgate on 22nd October 1620; he was the fourth son of sir Robert Heath and his wife, Margaret. In his poem 'To Her at Her Departure', the poet uses double preposition to make the reader understand the context very well. Preposition in the title clarifies that the poet is tending to his mistress and the subsequent preposition clarifies that she is leaving. Llewelyn Morgan classicist at Brasenose College, in his recent tweet [6] on 14th December 2020 calls this poem a perfect little poem. It was first printed in *Clarastella* (1650)[5]. The poem begins by saying

> *" They err*
> *That think we parted are:*
> *Two souls we carry,*
> *Half of which though it travel far*
> *Yet both at home do tarry."*

The poet intends to tell that the people may mistake thinking that the poet and his mistress will be separated however he says that they are conveying two spirits in one and even though the portion of it travel far when they arrive at home the two of them stay in one spot. During the afternoon under the sun, their shadow comes nearer.

> *"My soul, your shadow, when I'm gone,*
> *Waits closer through desire."*

Even when the poet has gone and was separated physically, his mistress' shadow waits closer. He is addressing his mistress as "Dear Heart" and asks her not to be sad because they are separated, as distance cannot separate them.

> *"For Though My Body Walks Apart*
> *Yet I Am With You Ever."*

He concludes the poem by saying that despite the poet leaves she is with his mistress forever. In this poem, he is directly pointing towards his mistress and that can be perceived from the title where the preposition plays a vital role. Preposition in the title clarifies two things one is, to whom is the poet addressing and the second thing is that at what circumstance the poet clarifies. When the reader reads the title cognitive mind helps the reader to look at the preposition in the title and connects the link between the title and the context. This encourages the reader to comprehend the circumstance of the poem in their mind.

Marvell's "On a Drop of Dew"

Andrew Marvell (1621-1678) composed a shifted exhibit of flawless verses that blend cavalier elegance with metaphysical wit and multifaceted nature. Leigh Hunt did a lot to promote his writings. He is of the view that Marvell is a thoughtful and elegant poet, a skilful prose-writer and controversialist, and above all a morally sound nationalist [7]. Blair Worden is of the view that like Milton, Andrew Marvell can appear to be a representative for singularity. Marvell's composition, like Milton's, makes temperance of independence [8].

Andrew Marvell in his 'On a Drop of Dew' [5] made his influence on the belief of the soul effect on a consistent hold back to be unified with his maker. Joan Van Emden is of the view that "the poem is not a mere academic exercise, but

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a lively and intricate analysis of Marvell's vision of the human soul."[4] Joan Bennett comments that it is "a devotional poem depending on nature-imagery" [9].

> *"See how the orient dew,*
> *Shed from the bosom of the morn*
> *Into the blowing roses,"*

The poet describes the dewdrop as how it drops from the skies in the morning into the blooming roses. He is addressing the reader to look into the dew which was beautifully landed on the roses.

> *"Yet careless of its mansion new,*
> *For the clear region where 'twas born*
> *Round in itself encloses:"*

It was born in a clear region. It is round and it looks back into the sky where it came from. Even though it is lying in the purple flower it is looking into the sky with the mournful light as it is longing to go back to its place.

> *"Shines with a mournful light;*
> *Like its own tear,*
> *Because so long divided from the sphere."*

It is sparkling yet in despairing and shed tears and that tear itself a dewdrop. It was separated from its place for quite a while. It is in horrendous dread as it believes that it might become unclean on account of the contact with the world. The dewdrop felt isolated from the sky thus it is in torment and the sun shows pity on the dewdrop with the goal that it can move back to where it is originated. The poet compares the dewdrop to the human soul. The soul dwells from heaven and resides in the human body. It is longing to stay away from the world's tribulations to stay pure. It is pure and attempts to communicate about paradise. It permits the paradise's light to fall upon it. It seeks a way to travel from earth to heaven openly. It is prepared to move upwards. At that point in the closing lines, the poet gives a biblical reference to manna given to the Israelites. The manna is the food given to the Israelites which was round and white which was frozen and cold; it evaporates when the sun rises. In the same way, the soul frozen by the world's tribulations will move back to the glories of the almighty sun that alludes to God.

CONCLUSION

In the poem 'To Time' the poet clearly explains time and he is straightforwardly highlighting time. In the second poem, "To Her at Her Departure", the poet explains the parting of the soul. Even though the poet and his mistress were isolated, their spirits stay as one. Furthermore, the title here clarifies that the poet is conversing with his mistress. Marvell explains about the soul in his 'On a Drop of Dew'. He attempted to clarify about the aching of a soul to arrive at heaven. He compares the soul to a dewdrop. In the event that the prepositions in the titles are taken out, it is impossible for the reader to comprehend the association between the title and the context. For instance, if it is time rather than 'To Time' and drop of dew rather than 'On a Drop of Dew' some association is missing. So preposition as a cognitive tool connects the mind of the reader and the context of the poem through the title. These three poems accompany three distinctive settings yet these three poems have preposition as their titles. While applying preposition as a cognitive tool, it is simple for the reader to interface the title with the content subsequently assists with understanding the setting quite well.

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RESEARCH ARTICLE

A Study on Effect of Antibiotic on Methanolic and Ethyl Acetate Extracts of *Aerva lanata* Linn. against Multi-Drug Resistant Microorganisms

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ABSTRACT

The objective of the project is to evaluate the effect of extracts (Methanolic and Ethyl acetate) of plant *Aerva lanata* on the Antibiotic Gentamicin. The Methanolic and Ethyl acetate extracts of the plant is obtained through sox let extraction process and the phytochemical analysis were done which shows the presence of alkaloids , carbohydrates and flavanoids in Ethyl acetate extract and alkaloid, carbohydrates, tannins, phenols, flavonoids and saponins in Methanolic extracts.Antimicrobial assay of the extracts was done against two bacteria: *Escherichia.coli* and Staphylococcus aureus, by Agar well diffusion method in the concentration of 100μg/ml, 500μg/ml and 1000μg/ml, which shows that the Ethyl acetate extract produce high antimicrobial activity against *E.coli* and *S.aureus* compared to Methanolic extract. Also, each extracts were mixed at equal ratio (1:1) with Antibiotic Gentamicin and its antimicrobial activity was observed at concentrations 250μg/ml, 500μg/ml and 1000μg/ml , which exhibited cumulative increase in the Antimicrobial activity of Gentamicin.

Keywords: *Aervalanata*, Phytochemical constituents, Antimicrobial activity

INTRODUCTION

Plant which is a source of many medicinal compounds plays an important role in the maintenance of human health since ancient times .As per WHO, plan t extract or their active constituents are used in traditional therapies, in which 80% of the world drugs are of natural origin. Plants, due to its medicinal value, are of great importance to the health of individual sand communities. The medicinal activity of the plant is used to the chemical substances present that produce a definite physiological action on the human body. The most important bioactive constituents present are

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alkaloid, tannins, flavonoid and phenolic compounds. Advantage of medicinal plant over synthetic drugs is the lack of side effects due high content of antioxidant compounds. This provides a protective action against diseases without reducing their therapeuticefficacy. Plant remains the foremost common source of antimicrobial agents.

A plants remain the most common source of antimicrobial agents. Their usage as traditional health remedy is the most popular for 80% of world population in Asia, Latin America and Africa and is reported to have minimal side effects. According to the investigations conducted recently, evidence shows that infectious diseases are one of the leading cause of death worldwide. This is mainly due to the development of antibiotic resistance. The efficacy of many antibiotics is threatened by multi-drug resistant pathogens Antibiotics are used to kill bacteria which can cause illness and disease. They have a major contribution to human health. Many diseases that once killed people can now be treated effectively with antibiotics. However, some bacteria became immune to commonly used antibiotics. Antibiotic resistant bacteria are bacteria that aren't controlled or killed by antibiotics. They are able to survive and even multiply in presence of an antibiotic. Most infection causing bacteria can become resistant to at least some antibiotics.Bacteria that are resistant to many antibiotics are known as multi- resistant organism.[MRO]. The important examples are;

- Methicillin-resistant *Staphylococcus aureus* [MRSA].
- Vancomycin-resistant *Enterococcus* [BRE].
- Multidrug resistant *Mycobacterium tuberculosis* [MDR-TB]

Infectious diseases are the leading cause of death worldwide. Antibiotic resistance has become a global concern. The clinical efficacy of many existing antibiotics is being threatened by the emergence of multidrug-resistant pathogens.The steady increase in multi-drug resistant bacteria to existing antibiotics is a serious problem to existing antibiotics that significantly cause treatment failure to infections and thus increase the mortality rates. There is an urgent need to develop new antimicrobial compounds that block the resistance mechanisms and improve the therapeutic activity to eradicate the resistant strains. Many infectious diseases are known to be treated with herbal remedies throughout the history of mankind. Natural products, either as pure compounds or as standardized plant extracts, provide unlimited opportunities for the development of new drug because of the unmatched availability of chemical using two or more antibacterial agents, is one of the most important strategies to overcome multi- drug resistant organisms. Recently, plant antimicrobials have been found as synergistic enhancers such a way that they may not have any antimicrobial properties alone, but when they are taken concurrently with standard drugs, they enhance the effect of thatdrug.

Aerva lanata
Aerva lanata (L.) commonly known as Polpala of Amaranthaceae is a perennial shrub which is seen commonly in different parts of India. They are originated in India; Africa as well as Australia .I t is familiar in the name of knotgrass. They are branching shrub. It is mainly used for urinary disorders. The cultivation no of *Aerva lanata* is done by seed propagation. Sunlight is needed for the growth of plant. These plants are cultivated during the month of September.

Chemical Constituents
The plant contains many constituents like alkaloid (11.4%). They are biological active canthin-6-onealkaloids, 10 methoxycanthin-6-one, beta D-glucopyranosyloxycanthin- 6-one, aervoside, 6-methoxy beta carbolin, Ervolanine and Propionic acid. And it also contains sugars, minerals, saponins (7.67%),oxalate (3.69%) and phytin-phosphorus (6.60%). It contain Flavanoids (3.75%) such as Kaempferol, Quercetin, Isohamnetin, Galactoside, Favanone glucoside pernisol,Persinoside A & B. Miscellaneous chemical constituents are Lupeo lacetate benzoicacid, Methylgrevullate, β-sitisterylacetate and Tannicacid(0.35%). Leaves are high in carbohydrate (26.6/100g), crude protein (22.6/100g) and ash (31.2g/100g).

Plant Description Taxonomical Classification
Kingdom: Plantae (Plants)
Sub-kingdom: Tracheobionta (Vascularplants)
Division: Magnoliophyta (Angiospermes, flowering plants)
Class: Magnoliopsida (Dicotylédones)
Subclass:Caryophyllidae
Order:Caryophyllales
Family:Amaranthaceae
Genus: Aerva
Species: *Aerva lanata* (L.) A. L. Juss. ExSchultes.

Vernacular Names:
In India, *Aerva lanata* is known by a wide variety of vernacular names, they are
English:Polpala
Hindi: Gorkhabundi, Kapurijadi
Bengali:Chaya
Rajasthani :Bhui
Punabi: Bui-kaltan
Kannada:Bili
Marathi: Kumra, Kapumadhura
Malayalam :Cherula, Peela,Cheruvula

Pharmacological Actions
It is used as diuretic and demulcent. Its diuretics action is very effective in the treatment of urethral discharges and gonorrhea. It is also used for treating headache, cough, liver congestion, jaundice, biliousness, dyspepsia, pneumonia, typhoid, urinary and gall stones, skin diseases, scorpion stings and snake bites. It is extensively studied for various pharmacological activities such as antimicrobial, anti-diabetic, antitumor, nephron-protective, hepatoprotective, immune modulatory, antiurolithiatic, antifertility, anti-metastatic and anti-HIV activity.

MATERIALS AND METHODS
Materials
Table1: Photochemical analysis
Test Organisms
- *Escherichia coli*
- *Staphylococcus auerus*

Plant Materials
The fresh plants of A.lanata were collected in the month of August. The plant was identified taxonomically by Dr. Sr Tessy Joseph, HOD, Department of Botany, Nirmala College, Muvattupuzha.

METHODS
Preparation of Nutrients Agar Media
Agar   -10g
Nutrients Broth -6.8g
Water   -500ml
Dissolved measured quantity of agar and nutrient in 500ml distilled water. Boiled for 15 min by continuous stirring. Cooled and sterilized in autoclave.

Preparation of Plant Extract

Fresh whole plant of *Aerva lanata* was rinsed thoroughly in running tap water, shade dried at room temperature(22-25°C) for a period of 21 days and 30148 September into coarse powder using a mechanical grinder. Approximately 120g of coarse powder was obtained. About 50g of powder were weighed and packed inside a filter paper and subjected to soxhlet extraction using methanol and ethyl acetate as solvent. The extraction was continued until the colour of the solvent in the column turns colourless. The solvent was removed by distillation and the resulting semisolid mass was dried and the percentage yield was calculated.

Evaluation of Antimicrobial Activity

500ml of nutrient broth was prepared by dissolving 6.8g of commercially available nutrient broth and 10g of agar in 500 ml distilled water and boiled to dissolve the medium completely. The medium was dispersed as desired and sterilized by autoclaving at 15lbs pressure (121°C) for 15 minutes.

Petri plates containing 20ml nutrient agar medium were seeded with bacterial culture of *E.coli* and *Staphylococcus aureus*. Wells of approximately 8 mm was bored using a cork borer and mixtures of concentration of plant extract such as 250µg/ml, 500µg/ml and 1000µg/ml were added. The plates were then incubated at 37° C for 24 hours. The antibacterial activity was assayed by measuring the diameter of the inhibition zone formed around the well. Streptomycin was used as a positive control.

RESULTS

Phytochemical screening and Antimicrobial activity of Ethyl acetate of *Aerva lanata* against *E.coli and S.aureus*.
This study investigated the antimicrobial activity of the phytochemical extracts (methanol and ethyl acetate) of *Aerva lanata* against gram positive *Staphylococcus aureus* and gram negative *Escherichia coli*. The results obtained are shown in the table 3 given below:

GA+ AL EA- Gentamicin + Ethyl acetate extract of *A.lanata*
GA+ AL ME- Gentamicin + Methanolic extract of *A.lanata*

From the statistical analysis, It is observed that the antimicrobial activity of Gentamicin show a significant variation when Gentamicin is combined with plantextracts.

DISCUSSION

Plants are the backbones of traditional medicines and antimicrobial activity of plant extract is due to the phytochemical constituents of plants.The analysis of various phytochemical constituents revealed the presence of constituents like tannins, alkaloids, flavonoids which are the main secondary metabolites of plants. Therefore, this explains the antimicrobial activity of plant *Aerva lanata*. Due to overuse of Antibiotics, bacteria that are resistant to antibiotics are born. This may cause major problem in the future. To avoid this dangerous crisis that may occur in the future it is necessary to take precautions .Many studies are conducted in various parts of the world to develop an alternative way to fight against antibiotic resistance and one o f the way found is use of plant extracts, with sufficient antimicrobial activity, in combination with anantibiotic.

In this study, it was observed that the Methanolic and Ethyl acetate extracts of whole plant of *A.lanata* was found to be effective against both gram positive and gram negative bacteria. Out of the two solvents used for extraction (Methanol& Ethyl acetate) of whole plant, Ethyl acetate extract has proved to have higher activity than methanolic extract on the tested microorganisms. Moreover, when an Antibiotic such as Gentamicin was used in combination with these extracts, it was observed that both the extracts exhibited a cumulative increase in the antimicrobial activity of *Aerva lanata* L.As per the statistical analysis conducted, it was observed that the Antimicrobial activity of

Gentamicin show a significant variation when it was combined with plant extracts.

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Table1: Phytochemical screening of *Aerva lanata* L.

SL.NO.	TEST	ETHYL ACETATE EXTRACT	METHANOL EXTRACT
1	Alkaloids	+	+
2	Carbohydrates	+	+
3	Glycosides	-	-
4	Tannins and phenols	-	+
5	Flavanoids	+	+
6	Saponins	-	+
7	Proteins and amino acids	-	-

Table2: Antimicrobial activity of *A.lanata*

Solvent extracts	Concentration(µg/ml)	Zone of inhibition (mm) *E.coli*	Zone of inhibition (mm)*S.aureus*
Methanol	250	4	4
	500	6	6
	1000	9	8
Ethyl acetate	250	6	6
	500	9	8
	1000	13	9

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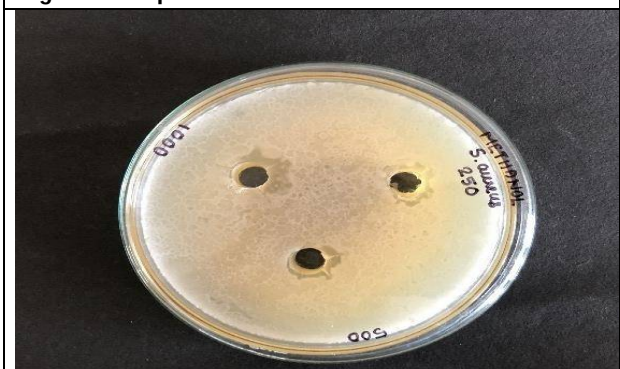**Table 3: Evaluation of effect of Antibiotic on both extracts of plant**

	E.COLI			S.AUREUS		
	GENTAMICIN	**GA+AL EA**	**GA+AL ME**	**GENTAMICIN**	**GA+AL EA**	**GA+AL ME**
CONC 250	18	25	27	20	29	30
CONC 500	20	27	28	24	30	31
CONC 1000	22	29	30	28	32	32

Fig.1: Whole plant of *Aerva lanata*

Fig.2: Leaves and flowers of *Aerva lanata*

Fig3: Methanol extract against S.aureus [Conc 250,500,1000 μg/m

Fig4: Methanol extract against E.coli [Conc. 250,500,1000 μg/ml]

Fig.5: Ethyl acetate extract against E.coli [Conc 250, 500, 1000 µg/ml]

Fig.6: Ethyl acetate extract against S.aureus [Conc 250, 500, 1000µg/ml]

Fig.7: Gentamicin against S.aureus [Conc 250µg/ml]

Fig.8: Gentamicin against S.aureus [Conc 500µg/ml
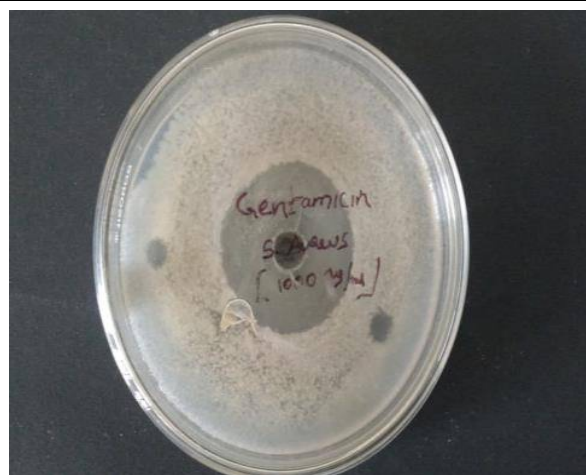
Fig.9: Gentamicin against S.aureus [Conc 1000µg/ml]
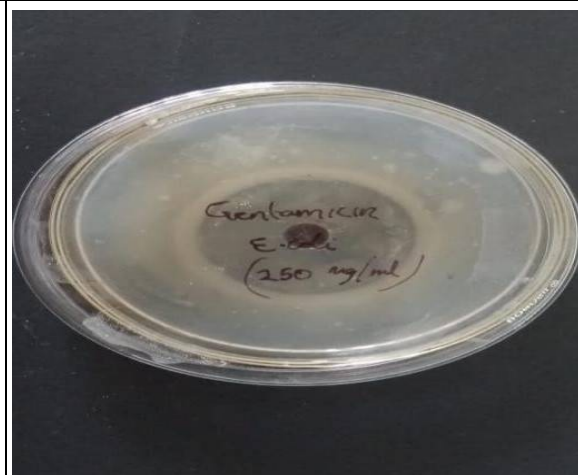
Fig.10: Gentamicin against E.coli [Conc 250µg/ml]

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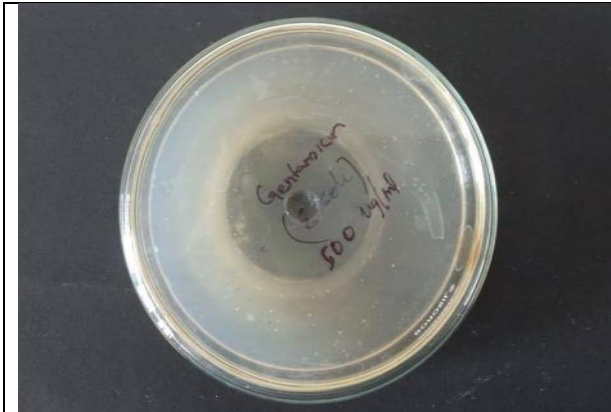
Fig.11: Gentamicin against E.coli [Conc 500µg/ml]

Fig.12: Gentamicin against E.coli [Conc 1000µg/ml]

Fig.13: Gentamicin+ Ethyl acetate extract against E.coli [Conc 250, 500, 1000 µg/ml]

Fig.14: Gentamicin + Ethyl acetate extract against S.aureus [Conc 250, 500, 1000µg/ml]
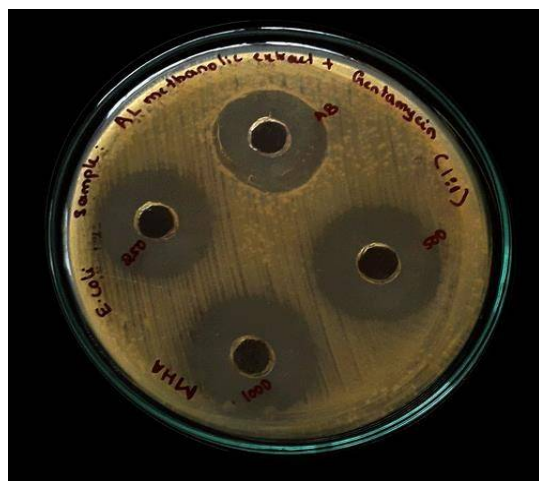
Fig.15: Gentamicin+ Methanol extract E.coli [Conc 250, 500, 1000µg/ml]

Fig.16: Gentamicin+ Methanol extract *S.aureus* [Conc 250, 500, 1000µg/ml]

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Fig.17: Antimicrobial activity of Antibiotic and combination of Antibiotic with plant extracts.

Fig.18: Statical Analysis 1

Fig.19: Statical analysis 2

RESEARCH ARTICLE
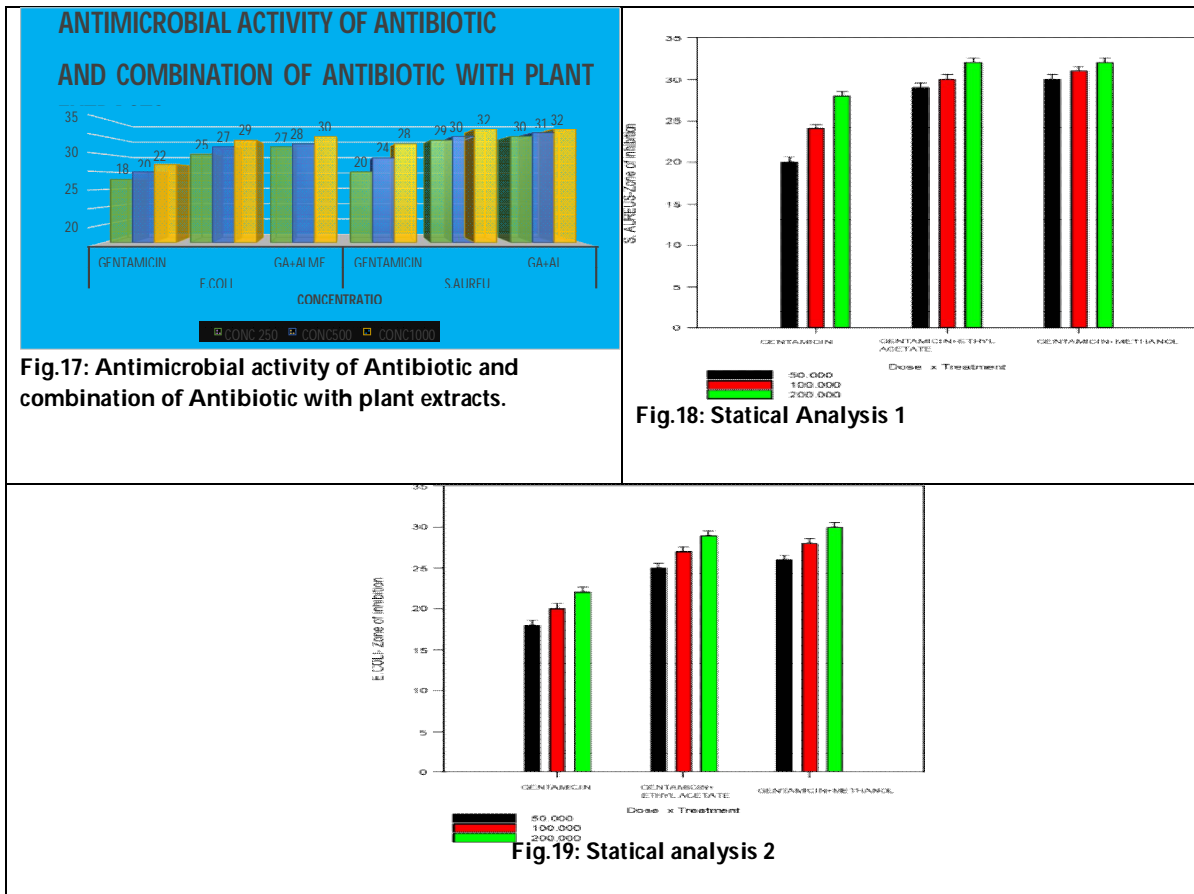
Efficient Digital Signature Scheme for Medical Information Systems Based on Non-Commutative Algebraic Structure

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ABSTRACT

The telecare medical information system (TMIS) aims to establish telecare services and enable the public to access medical services or medical information at remote sites. Authentication and key agreement is essential to ensure data integrity, confidentiality, and availability for TMIS. In this paper, we define a novel and secure digital signature scheme with hash functions for Medical Information Systems. Each user encrypts a data with a digital signature and the receiver can decrypt the data upon the successful verification of the digital signature. The proposed approach is highly secure and the security analysis is based on polynomial symmetric decomposition problem using groups.

Keywords: TMIS, EMR

INTRODUCTION

Telecare, which employs information and communication technologies to provide remote services that assist with certain healthcare activities, is becoming a viable solution to the continuously rising demand in medical and healthcare services. Telecare has the potential to lower social and medical expenses and improve the medical quality and efficiency. It is considered as an economical, time saving, and effective alternative compared with traditional clinical service. A number of telecare services have been proposed in recent years, such as electronic healthcare, remote nursing, and home monitoring [1, 2, 3, and 4].The telecare medical information system (TMIS) aims to

establish telecare services and enable the public to access medical services or medical information at remote sites. The medical server maintains various private data and information of registered users, such as name, address, telephone number, the electronic medical records (EMRs), which are directly interrelated with the user's privacy. However, since TMIS has to provide remote access services over the insecure Internet, it is subject to the same security risks as the Internet. Security and privacy is the main obstacle to be addressed when deploying TMIS [5, 6, 7, 8, 9, and 10]. By analyzing medical records, data recipient can make a large amount of valid statistical analysis to derive the relationship between disease and age. According to medical data mining, data recipients can also predict epidemic diseases. However, electronic medical record contains the patients' privacy information. In practice, most of patients are concerned about the confidentiality of their personal health information, because it might cause troubles for every unauthorized collection and disclosure [11, 12, 13 and 14]. Because accessing the medical records become more and more convenient and faster, protecting patient privacy has become increasingly important and should not be overlooked. Thus, we should set permissions of accessing these information to only authorized persons, which means only authorized health care providers (doctors) can access patient medical records and confirm the patient's identity, and unauthorized people cannot get anything [15,16,17 and 18].

Related Work

Currently, the most common login authentication mode is the "username + password" in electronic medical records system. This approach is simple with no additional costs, but it uses a static password which is not secure. Two-factor authentication, as the name suggests, combines two elements such as "password + physical" and certificates the personnel entering into the system [10]. Personal Identification Number (PIN) adds other authentication mechanisms based physical media to strengthen the approach. This method can enhance the security of the system to a large extent. The staff can enter the EMR systems using similar two-factor authentication mechanism, which can ensure authorized users to access and browse the information within their remit. However, this method is vulnerable to resist malicious attacks such as masquerading attacks, replay attacks, etc.

On the other hand, anonymous identification schemes are emerging through the usage of pseudonyms and other techniques to protect privacy. Lin et al. proposed SAGE, realizing not only the content-oriented privacy but also context privacy resisting strong global adversary [12]. Sun et al. gave a solution to privacy and emergency response based on anonymous credential, pseudo-random number generator and the knowledge proof [17], [20],[18]. They also proposed an anonymous authentication scheme based on zero-knowledge proof in P2P systems. However, when directly applied to the distributed health care systems, this scheme is impractical due to the heavy computational overhead of zero-knowledge proof. Riedl et al. introduced a new pseudonymization architecture to protect privacy in E-health [14]. Salaaming and Stingl integrated pseudonymization of medical data, identity management, anonymous authentication fuzzy metadata to prevent disclosure attacks and statistical analysis and established a security mechanism to guarantee anonymity and privacy in the personal health information transmission and storage [16]. However, the mentioned anonymous authentication schemes were established based on public key infrastructure (PKI), so there are an online Certificate Authority (CA) and one unique public key encryption for each symmetric key to data encryption on authorized physicians, which lead the overhead of the construction to increase linearly along with the group size. In addition, the anonymity level depends on the size of the anonymity set, thus anonymous authentication is unrealistic in the particular environment where patients are sparsely distributed.

Most recently, Chen et al. [13] demonstrated that the user anonymity of Khan et al.'s scheme [14] may be vulnerable to the insider attack because the secret key is shared by all the legal users. They also proposed an efficient and secure dynamic ID-based authentication scheme for TMIS, in which a dynamic identity is generated for each transaction session to avoid the leak of personal information about the user and the risk of ID-theft, and claimed that their scheme achieves user anonymity. Chen et al.'s scheme is superior to the previous solutions for implementation on mobile devices.

Our Contributions

Thus, the increased growth of TIMS increases the need for improved security measures across various public to access medical services platforms. At present, there exists a variety of security standards for TIMS systems. But still, the authenticity and security of the data remain to be the major issue. In order to solve the issue in this paper, we define a secure and novel digital signature scheme for TIMS system. The proposed approach is highly secure and it solves polynomial symmetric decomposition problem using group. Hash functions are used to verify the authenticity of the transferred data content.

Mathematical Background

Complexity Assumptions

Let us consider a group $(R, +, \bullet)$. For any random element $a \in N$, construct a set $p_a \subseteq N$ is constructed by

$$p_a = \left\{ \delta(a) : \delta(x) \in Z_{>0}[x] \right\}.$$

Polynomial Symmetrical Decomposition Problem *(NPSD):*

Instant: Given $(a, x, y) \in G \times G \times G$ and $r, s \in Z$

Objective: Find $z \in p_a$ such that $y = z^r x z^s$.

Proposed Signature Scheme Over Near-Ring

Initial Setup

Let $(G, +, \bullet)$ be the group and it is the underlying work fundamental infrastructure and GPSD problem is intractable on the non-abelian group $(N, \bullet)$. Now let us select two small integers $r, s \in Z, H : N \to M$ defines a cryptographic hash function that maps from N to the message space M. Thus the tuple $\langle N, r, s, M, H \rangle$ denotes the public parameters associated with the system.

Key Generation

First Alice selects two random elements $a, b \in G$ and a random polynomial from group $\delta(x) \in Z_{>0}[x]$ such that $\delta(a)(\neq 0) \in G$ and then takes $\delta(a)$ as her private key, compute $y = \delta(a)^r b \delta(a)^s$ and publishes her public key $(a, b, y) \in G \times G \times G$.

Signature Generation

Alice Performs of the following steps

Step 1

Alice randomly selects another polynomial from group $\delta(x) \in Z_{>0}[x]$ such that $\delta(a)(\neq 0) \in N$ and take $\delta(a)$ as salt.

Step 2

Alice compute following steps

$\sigma = p(a)^r b p(a)^s$

$\psi = \delta(a)^r \left[H(M)\sigma \right] \delta(a)^s$

$\lambda = p(a)^r \psi \, p(a)^s$

$\rho = p(a)^r \psi \delta(a)^s$

$\alpha = \delta(a)^r H(M) \, p(a)^s$

$U = p(a)^r H(M) \, p(a)^s$

Then $(\sigma, \lambda, \rho, \alpha, U)$ is the Alice signature on message M and sends it to Bob for verification.

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Verification

To verify the Alice's signature $(\sigma, \lambda, \rho, \alpha, U)$, Bob do the following

Step 1

To compute $V = \rho y^{-1} \alpha.$

Step 2

Bob accepts Alice's signature if $\sigma^{-1} U = \lambda^{-1} V$ otherwise, he reject the signature.

Example for Proposed Signature Scheme

The Proposed method is described using matrix group $M_2(Z_n)$, where n is large secure prime.

Initial setup

We choose $n = 23, r = 2, s = 3, \mathrm{N} = \begin{bmatrix} a & b \\ 0 & c \end{bmatrix} \in M_2(Z_n)$ where $r, s \in Z.$

The calculation over multiplication modulo 23 is evaluated for simplifying computation and verification.

Key generation

Private Key

Alice chooses two random elements $a = \begin{bmatrix} 1 & 3 \\ 0 & 1 \end{bmatrix}, \mathrm{b} = \begin{bmatrix} 1 & 5 \\ 0 & 1 \end{bmatrix} \in M_2(Z_n)$ and a polynomial randomly

$\delta(x) = 2x^2 + 3x + 1 \in Z_{>0}[x]$ such that $\delta(a)(\neq 0) \in N.$

$\delta(a) = 2 \begin{bmatrix} 1 & 3 \\ 0 & 1 \end{bmatrix}^2 + 3 \begin{bmatrix} 1 & 3 \\ 0 & 1 \end{bmatrix} + \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \bmod 23$

$\delta(a) = 2 \begin{bmatrix} 1 & 6 \\ 0 & 1 \end{bmatrix} + \begin{bmatrix} 3 & 9 \\ 0 & 3 \end{bmatrix} + \begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \bmod 23$

$\delta(a) = \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix} \bmod 23$

As her private key.

Public Key

$y = \delta(a)^r b \delta(a)^s$

$y = \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^2 \cdot \begin{bmatrix} 1 & 5 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^3 \bmod 23$

$y = \begin{bmatrix} 216 & 1911 \\ 0 & 125 \end{bmatrix} \bmod 23$

$y = \begin{bmatrix} 2 & 3 \\ 0 & 20 \end{bmatrix} \bmod 23.$

as her public key.

Signature generation

Step 1

For given $M = \begin{bmatrix} 1 & 6 \\ 0 & 1 \end{bmatrix}$ and then Alice compute

$$H(M) = \begin{bmatrix} 2^1 & 2^6 \\ 2^0 & 2^1 \end{bmatrix} \bmod 23$$

$$= \begin{bmatrix} 2 & 18 \\ 1 & 2 \end{bmatrix} \bmod 23$$

Alice also chooses another polynomial $p(x) = 5x^2 + 3x + 5$ and compute following ways:

$$p(a) = 5\begin{bmatrix} 1 & 3 \\ 0 & 1 \end{bmatrix}^2 + 3\begin{bmatrix} 1 & 3 \\ 0 & 1 \end{bmatrix} + 5\begin{bmatrix} 1 & 0 \\ 0 & 1 \end{bmatrix} \bmod 23$$

$$p(a) = \begin{bmatrix} 13 & 39 \\ 0 & 13 \end{bmatrix} \bmod 23$$

$$p(a) = \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix} \bmod 23.$$

Step 2

$$\sigma = p(a)^r\, b p(a)^s$$

$$= p(a)^2\, b p(a)^3$$

$$= \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^2 \begin{bmatrix} 1 & 5 \\ 0 & 1 \end{bmatrix} \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^3 \bmod 23$$

$$= \begin{bmatrix} 4 & 11 \\ 0 & 4 \end{bmatrix} \bmod 23$$

$$\psi = \delta(a)^r \left[H(M)\sigma \right] \delta(a)^s$$

$$= \delta(a)^2 \left[H(M)\sigma \right] \delta(a)^3$$

$$= \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^2 \begin{bmatrix} 2 & 18 \\ 1 & 2 \end{bmatrix} \begin{bmatrix} 4 & 11 \\ 0 & 4 \end{bmatrix} \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^3 \bmod 23$$

$$= \begin{bmatrix} 15 & 4 \\ 19 & 1 \end{bmatrix} \bmod 23$$

$$\lambda = p(a)^r\, \psi\, p(a)^s$$

$$= p(a)^2\, \psi\, p(a)^3$$

$$= \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^2 \begin{bmatrix} 15 & 4 \\ 19 & 1 \end{bmatrix} \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^3 \bmod 23$$

$$= \begin{bmatrix} 10 & 15 \\ 7 & 21 \end{bmatrix} \bmod 23$$

$$\alpha = \delta(a)^r H(M) p(a)^s$$
$$= \delta(a)^2 H(M) p(a)^3$$
$$= \begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^2 \begin{bmatrix} 2 & 18 \\ 1 & 2 \end{bmatrix} \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^3 \bmod 23$$
$$= \begin{bmatrix} 2 & 21 \\ 1 & 11 \end{bmatrix} \bmod 23$$

$$U = p(a)^r H(M) p(a)^s$$
$$= p(a)^2 H(M) p(a)^3$$
$$= \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^2 \begin{bmatrix} 2 & 18 \\ 1 & 2 \end{bmatrix} \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^3 \bmod 23$$
$$= \begin{bmatrix} 9 & 17 \\ 4 & 21 \end{bmatrix} \bmod 23$$

Alice sends $(\sigma, \lambda, \rho, \alpha, U)$ to Bob, as her signature.

Signature verification

Bob receives $(\sigma, \lambda, \rho, \alpha, U)$ from Alice, computes

$$V = \rho y^{-1} \alpha$$
$$= \begin{bmatrix} 5 & 15 \\ 15 & 12 \end{bmatrix} \begin{bmatrix} 2 & 3 \\ 0 & 20 \end{bmatrix}^{-1} \begin{bmatrix} 2 & 21 \\ 1 & 11 \end{bmatrix} \bmod 23$$
$$= \begin{bmatrix} 5 & 15 \\ 15 & 12 \end{bmatrix} \bmod 23$$

$$\sigma^{-1} U = \begin{bmatrix} 4 & 11 \\ 0 & 4 \end{bmatrix}^{-1} \begin{bmatrix} 9 & 17 \\ 4 & 21 \end{bmatrix}^{-1} \bmod 23$$
$$= \begin{bmatrix} 7 & 3 \\ 12 & 8 \end{bmatrix} \bmod 23$$

$$\lambda^{-1} V = \begin{bmatrix} 10 & 15 \\ 7 & 21 \end{bmatrix}^{-1} \begin{bmatrix} 5 & 15 \\ 15 & 12 \end{bmatrix} \bmod 23$$
$$= \begin{bmatrix} 7 & 3 \\ 12 & 8 \end{bmatrix} \bmod 23$$

Bob accepts Alice's signature if and only if $\sigma^{-1} U = \lambda^{-1} V$ otherwise, he rejects the signature.

Security Analysis

Data forgery

Consider a situation where, Eve replaces the original message M, with the fraudulent message M_f, such that Bob obtains the signature $(\sigma, \lambda, \rho, \alpha, U)$. Using the fraudulent message M_f or $H(M_f)$, the equation is verified as $\sigma^{-1} U = \lambda^{-1} V$ infeasible, due to the reason M_f or $H(M_f)$ is entirely associated with the signature generation process and has no impact towards the verification algorithm.

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Hence $\sigma^{-1}U = \lambda^{-1}V$ holds true only for the original message. Also, the fraudulent of data without signature extraction is

$$\rho = p(a)^r \psi\delta(a)^s$$

$$= p(a)^2 \psi\delta(a)^3$$

$$= \begin{bmatrix} 13 & 16 \\ 0 & 13 \end{bmatrix}^2 \begin{bmatrix} 15 & 4 \\ 19 & 1 \end{bmatrix}\begin{bmatrix} 6 & 21 \\ 0 & 5 \end{bmatrix}^3 \bmod 23$$

$$= \begin{bmatrix} 5 & 15 \\ 15 & 12 \end{bmatrix} \bmod 23$$

highly impossible. The other attempt could be made to find M_f, for valid $H(M)$. But it is infeasible, due to the reason that H is assumed to be cryptographically secure. So that it is impossible to sign an invalid data with a valid signature.

Signature Repudiation

Consider a scenario, where Alice is refused to recognize her signature on some valid data. Such that the valid signature $(\sigma, \lambda, \rho, \alpha, U)$ could be fraudulent by Eve and the message M can be signed by Alice, with the fraudulent signature $(\sigma_f, \lambda_f, \rho_f, \alpha_f, U_f)$ instead. The verification procedure is defined as follows

$$V = \rho_f y^{-1}\alpha_f$$

$$V = \left[p(a)^r \psi\delta(a)^s \right]_f \left[\delta(a)^{-r} b^{-1}\delta(a)^{-s} \right]\left[\delta(a)^r H(M) p(a)^s \right]_f .$$

Since $\left[\delta(a)^r \right]_f . \left[\delta(a)^r \right] \neq I, \left[\delta(a)^{-s} \right]_f . \left[\delta(a)^{-s} \right] \neq I$ where I denotes the identity element in the multiplicative structure of the near-ring. Consequently $\left(\sigma^{-1}U \right)_f \neq \left(\lambda^{-1}V \right)$. So this signature scheme ensures that the non-repudiation property.

Existential Forgery

Consider a scenario where, Eve tries to sign a fraudulent message M_f. Then he should fraudulent the private key in replacement to some $\left[\delta(a)^r \right]_f$. Immediately, there exists a difficult with the public key, as we believe that NPSD is intractable on non-abelian near-ring. Also note that all the structures in this signature scheme are constructed on non-abelian near-ring and based on NPSD. Exact identification of these structures is almost intractable as long as NPSD is so hard on this underlying work structure. Consequently construction new valid signatures, without proper knowledge of private key are impossible. So Eve is not able to calculate fraudulent signatures.

CONCLUSION

The property of data security remains to be the major concern across TMIS systems. In this context, several approaches to protect TMIS security has aroused in recent years. Security remains to be the major concern even though there exists a lot of approaches in the TMIS systems. This is due to an increase in the application of TMIS systems across various domains. In this paper, we define a novel and secure digital signature scheme for TMIS environment. The major objective of this approach is to improve security and authenticity measures across the TMIS. The proposed approach makes use of hash functions and group concepts to define and verify the digital signature. The security analysis of the proposed approach is based on the symmetric polynomial decomposition problem using group. From the analysis, it is observed that the proposed approach provides improved security and authenticity measures across the TMIS environment.

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RESEARCH ARTICLE

Forensic Examination of Writings on Wall – A Preliminary Study

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ABSTRACT

Writings on unconventional surfaces can be very commonly seen anywhere in the form of graffiti, advertisement, direction, information etc but when it comes on authentication and on fixing the authorship of the questioned writings on unconventional surfaces, it has several challenges. Writings on unconventional surfaces may be found in many cases like suicide, murder, rape, ransom, defamation etc.[1] Writings on unconventional surfaces are often written with unconventional writing instruments also. This further poses problem in examination for forensic document examiner. This paper presents the study of variations in writing characteristics on unconventional surface i.e wall, written with unconventional writing instrument i.e lipstick.

Keywords: unconventional, suicide, murder, lipstick, surface, writing instrument

INTRODUCTION

Writer firstly, conceives the picture of forms of letters in the mind and then tries to copy it consciously ,and, after practicing the forms of letters, he writes without consciousness gradually, which develops the developed writing form [2]. Similar process works when a person writes on the unconventional surfaces, but there are many other factors which may affect the writing such as availability of suitable writing instrument, type and condition of writing instrument, type and condition of surface, extrinsic or intrinsic factors [3], mental condition or mood of the writer,

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availability or accessibility [1,4] of surface and writing instrument etc. Cases with unconventional surfaces eg. wall, wooden doors, metal, tiles, marble, plastic sheet, clothes, skin etc or unconventional writing instruments such as cosmetics, body fluids, hard object etc have increased in number in forensic laboratories nowadays. Forensic Document Examiner may come across writings which may have variations in writings characteristics which are beyond the scope of natural variations. Deliberate deviation from the writing style to hide own identity in case of murder, defamation or threat while writing in such cases, may further complicate the examination of case. The exemplars in such cases give further challenge to the Forensic Document Examiner as exemplars are on conventional surface such as paper and usual writing instrument is pen or pencil, but in cases where unconventional writing surface is used for writing, the writing instrument may vary. It may be cosmetics, brick, coal, body fluids etc. Writing is a process and one of the most permanent and unconscious habits [5]. The choice of writing instrument varies according to choice of the writer, mood, availability or accessibility of the writing instrument at the time of occurrence of crime.

MATERIALS AND METHODS

Handwriting samples were collected from 50 individuals both male and female. All the individuals are well educated and learned, and have knowledge of script and language of English. They were asked to write on a wall with lipstick of any color of their choice. They were asked to write *"a quick brown fox jumps over the lazy dog"* three times on a wall. They wrote with standing position. After execution of writing, wall was photographed. The writers were then asked to write on A4 sheet using ball point pen. Then writings were examined on both the surfaces and comparisons were made to examine the effects of writing surface and writing instruments on the writing of individual.

RESULTS

Handwriting on wall was compared with the handwriting on A4 sheets and comparisons were done. Both general and individual writing characteristics and features were examined. Following observations were made:

Size – A noticeable increase in the size of letters was observed in the writings on the wall in comparison to the writings written on paper. The increase in size of the letters of writings is due to the whole arm movement of writer, the availability of space on the writing surface or may be due to vertical position of writing surface. All the changes in the size of writing on the wall do not pose any impact on comparison of writings on two writing surfaces (figure 1&2).

Letter formation – the writings of two surfaces were compared for identifying characteristics but no significant change in the basic formation of letters could be observed. Lipstick being the soft (at tip) writing instrument and wall usually has irregularities on its surface, due to which the movement of writing may not be smooth.(figure 3 & 4)

Slant- the slant of letters found to be almost similar in both the sets of writings.

Connecting stroke- the occurrence of connecting strokes in the writings written with lipstick on the wall found to be less frequent as compared to the writings written on the paper using ball point pen. It is mainly due to the presence of irregularities on the surface of wall [1]. Due to which the continuation of writing or the combination of writing instrument and the writing surface breaks or alters and it causes the pen pauses, pen hesitation, pen lifts etc on the writing surface. (figure 5 & 6)

Space- the spacing between the letters, the spacing between the words and the interlineal space in the writings written with lipstick on the wall were found to be big. It is due to the availability of larger writing surface.

Skill – no significant change in the skill of writer was observed in the writings written on wall with lipstick as compared to the writings written on paper with blue ball point pen. pen.(figure 7 & 8)

Line quality – line quality of writings written on wall with lipstick as writing instruments found to be deteriorated as compared to the writings written on paper with blue ball point pen. (figure 9)

Pen pauses- Increase in number of pen pauses was observed in the writings written on wall with lipstick as compared to the writings written on paper with blue ball pen. pen.(figure 10)

Pen hesitations- Increase in number of pen hesitation was observed in the writings written on wall with lipstick as compared to the writings written on paper with blue ball pen. pen.(figure 11 & 12)

DISCUSSIONS

All the samples were examined, analyzed and compared to examine the effect of unusual writing surface i.e wall and the effect of unusual writing instrument i.e lipstick on various handwriting characteristics such as slant, size, line quality, connections, pen hesitations, pen pauses etc. Due to change in the writing instrument and writing surface, the writing may have some differences in appearance but the minute and inconspicuous details of the handwriting remain unaltered which help in establishment of authorship of writing. The deviation from the normal writing of an individual occurs due to the change in the writing surface and writing instrument. Wall has irregularities on the surface, it is hard, it has large writing surface. The writer usually uses the whole arm movement to write on the wall. The writing instrument lipstick has soft tip which makes the ink deposition at places due to uneven surface of the wall. The introduction of pen hesitations, retouching, pen lifts occur in the writing written with unusual writing instrument on unusual writing surface more as compared to the writing written on paper with ball point pen. The examination of writings on unusual surface is challenging as the examination has to be carried out with the help of photographs usually, as the unconventional writing surface cannot be carried to the laboratory as unconventional writing surface may be skin, door, glass, mirror, floor, wall etc. As such, the Forensic Document Examiner has to depend on the photographs of the writings for examination. Photographs are the best tool for examination after the originals for examination of writings [6]. It is easier to examine the writings with the help of photographs than to remove a part of wall having writings [7].

CONCLUSION

The examination writings written with unusual writing instrument on the unusual surface show differences in many factors but the identifying features which are minute and inconspicuous remain unaffected which helps in the establishment of authorship of writing. This study reveals that if the writings written on the unconventional surface written with unconventional writing instrument are undisguised and original, the definite opinion can be furnished.
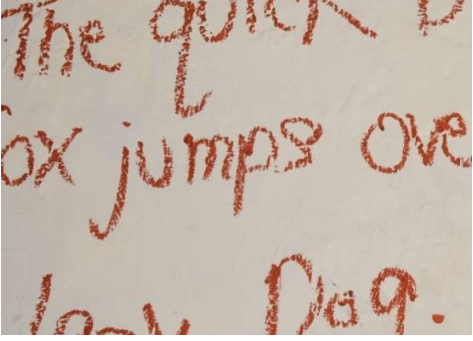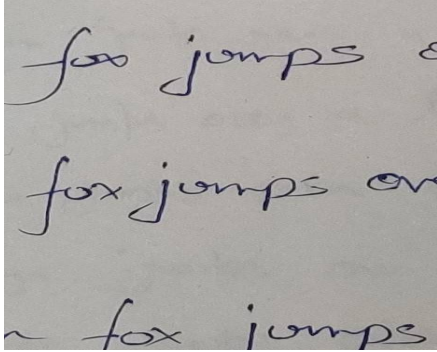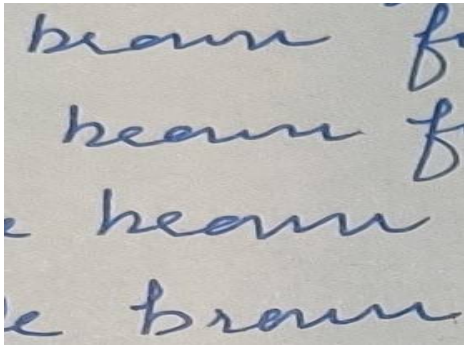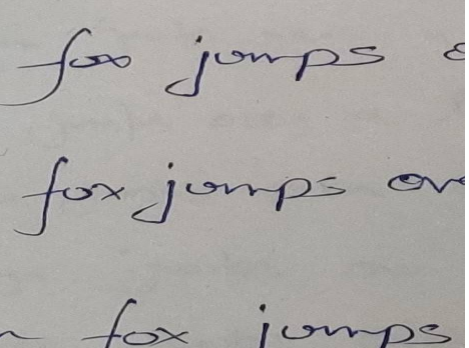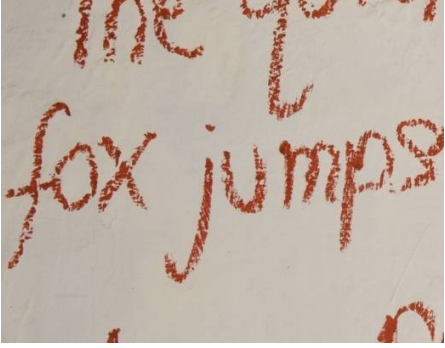
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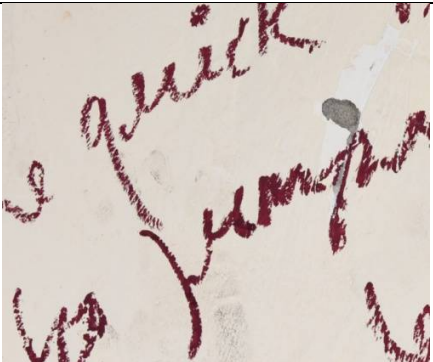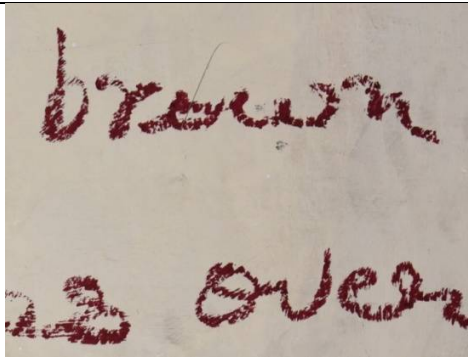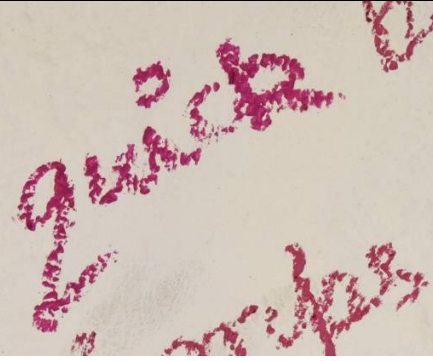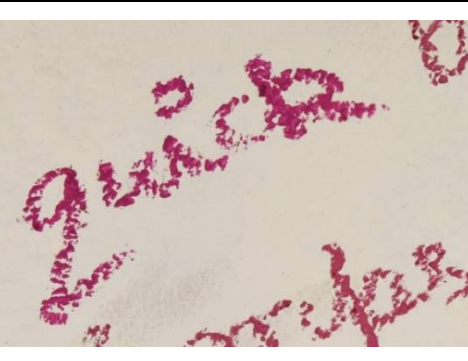
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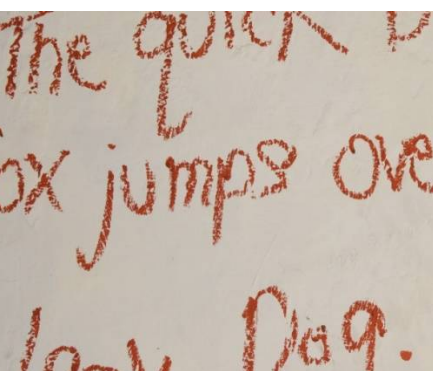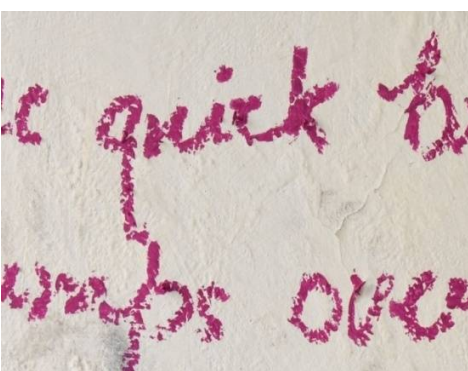
Figure 1	**Figure 2**
Figure 3	**Figure 4**
Figure 5	**Figure 6**

Figure 7	**Figure 8**
Figure 9	**Figure 10**
Figure 11	**Figure 12**

RESEARCH ARTICLE

Problem Solving Strategies in Mathematics used by Secondary School Teachers in Surigao del Norte Division and the Performance of Students in Mathematics: An Intervention Program

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ABSTRACT

Using descriptive correlative design curtained studies on Problem Solving Strategies in Mathematics in the Secondary School Teachers of the Surigao del Norte Division and the performance of Students in Mathematics were analyzed thru some statistical tools such as the Percentage Computation, Weighted Mean and Chi-Square, where findings revealed that the study was deemed appropriate and served as the basis for crafting a program. Eventually, the Find-a-pattern strategy ranked first then follows are Logical reasoning; Using a formula; Using a table or make a list and the strategy on work backward; Guess and check; Cooperative Learning; Team games and Tournaments; Paraphrasing; and the tenth in rank was the Create a diagram. Then, noted that there were 6 (9%) students who had Outstanding performance in Mathematics; 26 (41%) had Very Satisfactory performance; 29 (45%) were Satisfactory; 3 (5%) had Fairly Satisfactory performance and no student who belongs to 74% and below.

Eventually, students' level in Mathematics has an overall standard deviation of 3.44, with a satisfactory rating. It can be inferred that students-respondents in this study had satisfactory performance. Based on the result it was indicated that students needed better strategies in improving their level of achievement, helping them get an excellent mark, this result was inclined to Cristobal (2001) study that the Philippines ranked lowest in both science and mathematics due to teachers' factor in primary and secondary schools that can interpret their students' achievement. Teaching Mathematics was positively and moderately correlated with teachers' frequency of problem-solving strategies. The significant relationship was tested and resulted in 0.0004, which described significantly. Such that students' level of achievement in Mathematics was influenced by the frequency of use of the strategies. Thereby, it is emphasized that Mathematics teachers in the Secondary Schools of the Division of Surigao del Norte were more acquainted with the strategies like Find a pattern, Logical reasoning, and using a formula strategy referred to as best in math problems. And the only strategy which was considered oftentimes used was to

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create a diagram. Results revealed that teaching strategies were important tools in facilitating effective learning for Mathematics concepts and principles. In this manner, learners' needs are properly taken care of. Thus, Junior High School Mathematics of the Division of Surigao del Norte had a satisfactory performance concerning knowledge of Mathematics concepts and principles.

INTRODUCTION

The educational system assumes the responsibility of taking innovative measures both in teaching methodologies and in curriculum contents because of these certain facts. The present educational system has geared all concerted efforts towards the improvement of mathematics education programs. Thus, greater emphasis has been placed on the methods and strategies of teaching. It is desired that mathematics education in secondary schools can be useful and effective if learners can cope with and adjust themselves in the learning process. As expected Filipino teachers in the 21st century are more advanced in the field during the teaching-learning process, with the advancement of the curriculum. And professionally, should demonstrate their responsibilities in helping the learners reaching their idea by extending a helping arm, hoping to mold the children into a desirable outcome for their future, and the nation.

As observed and experienced it was difficult to teach pupils to solve math problems out of context. Normally students with advanced knowledge are more active than pupils who are unfamiliar with the topics introduced most probably if the problem is the first-hand topic. Anyway, in the same manner, teaching math could strategize on how to make it easier like to any other subject (Rubel, Lim, Hall-Wieckert & Sullivan, 2016). Problem-solving is a process that proves what we have discovered this was explained by researchers that the process generates hypotheses testing to arrive at solutions [(Taspinar & Bulut, 2012); (Treffinger, Solomon & Woythal, 2012); (Vijayan & Joshith, 2018); (Perlman, 1957). Students took responsibility for their learning in solving problems, resolving conflicts, and discussing alternatives with a focus on thinking as a vital element of the curriculum. Provides students with the opportunities to use knowledge, skills in real-life activities and in working at higher levels of thinking [(Hiebert, Carpenter, Fennema, Fuson, Human, Murray & Wearne, 1996); (Webster-Stratton & Reid, 2004); (Stacey, 2005); (Shinn, 2002)].

Teachers have many obstacles in teaching problem-solving, they even need to read more books in acquiring modern knowledge being mathematicians. This is consonant to Montague as cited by Klingler, K. L. (2012), when students were taught the process of solving mathematical problem less complicated, then they can learn those strategies, and derive the correct answers. Purposively, understanding by identifying what students' learned (visually and auditory), will provide them the opportunities in plying (e.g. showing their concepts/skills, real-life situation, etc.), and this purpose has turning students interest, to be more proactive, and learning has relevance/meaning for their immediate lives [(Pritchard, 2017); (Bybee,1989); (Tularam & Hassan, 2018)]. In this emerging view of Mathematics, students can be educated meaningfully with positive thoughts regarding Mathematics, students can be trained to remember but rather involves the intellectual structures and processes, students developed as they engaged in activities they have endowed with a sense [(Carpenter & Lehrer, 1999); (Hiebert & Grouws, 2007); (Pea, 1987); (Boaler, 2002)]. The researcher was motivated to study the teaching of Mathematics teachers using different strategies in problem-solving, a way to enrich their students' skills in mathematical problem-solving. He even assumed that the appropriate strategy in teaching is problem-solving that would be able for them to discover means to solve mathematics problems successfully and that students' performances attributed to the teachers' factor in teaching Mathematics.

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Theoretical Background

One should understand how to solve problems, otherwise, solving problems puzzled one's understanding. It believed that students need prior knowledge, not just ones but some routine problems, also complex higher-order thinking problems, making this way would help them, solving problems they will encounter, students should learn Mathematics through problem-solving [(McDonald, 2017); (BROWN-LOPEZ, P. R. I. S. C. I. L. L. A., & ALVA, 2010); Kowalski, 2009); (Monogiou, 2010); (Newton & Sword, 2018)].

Aims of Numeracy Professional Learning

Mathematics through real-world experiences and the classroom is one way that brought students to be more critical thinkers. Classroom setting and its conditions affect students' learning behavior (Facilitator's Handbook). Effective Teaching-Learning in Mathematics is reflective of classrooms and emerging situations within the surroundings. Students engaged in solving problems needs sufficient time and support or mentoring from their teachers, they need structured lectures to build prior knowledge before solving any problem thereby. Effective Teaching in Mathematics needs varied problems and solutions to develop a deeper understanding. Students consolidated their knowledge through shared and independent practices. Teachers select and/or organized students' solutions for sharing to highlight the Mathematics learning e.g., bansho, gallery walk, math congress [Fisher & Frey, 2013); (Garfield & Ben-Zvi, 2008); (Kunter, Klusmann, Baumert, Richter, Voss & Hachfeld, 2013)].

Teachers should need specific knowledge and pedagogy to teach effectively or else ironically, he/she is inadequate for teaching. It requires the capacity to criticize one's knowledge and makes it refined where outcomes were visibly realistic. Teachers concretized his idea and prove his knowledge with his unquestioned skills in solving mathematics problems: work backward from a mature and compressed understanding of the content to unpack its constituent elements and make mathematical ideas accessible to others. Teachers worked with content for students while it is in a growing and unfinished state. Teachers' obstacles in teaching problem solving can be resolve through reading more strategies and practice it as well as good mathematicians. Noticed that when students are given strategies, simplified the process to less complicated, then they could learn those strategies and become successful problem solvers [Krawec, Huang, Montague, Kressler & Melia de Alba, 2013); (Montague & Dietz, 2009)].

Jausovec cited by Eissa & Mostafa (2013) expressed that each problem has three components: first, undesired initial situation, second, desired end situation, last, an obstacle in the middle of both. Every student must encounter these three parts in a mathematical problem or otherwise it is considered a mathematical exercise. It expounded a significant difference between a mathematical exercise and a mathematical problem. A mathematical exercise involves reading the directions and using one to two steps to solve the exercise. The mathematical problem does not always present itself with a cut and dry solution at the surface. The student has to delve into the problem to think of a process with which they solve the problem. Mathematical problems have a higher complexity level of thinking than mathematical exercises from a workbook or textbook. In mathematical word problems, students also identify these three parts to find a solution.

According to Lambdin as cited by Rhodes (2019), a problem is defined as a situation in which disequilibrium and perplexity occur. A mathematical problem causes a student to become uncomfortable when they cannot find a solution. When individuals encounter a problem and find themselves in an uncomfortable situation, if no changes at all, they do not overcome the situation (Godbey, 1997). It is in this instance that students realize what they have encountered is not an exercise in mathematics, but rather a mathematical problem.

Schroeder and Lester cited by Mamona-Downs & Downs (2005) explained that the role of problem-solving is developing students' mathematical skills via problem-solving as if this is the most appropriate approach. They argued that advocates of this approach consider problem-solving not as a topic, but rather as a pedagogical position. This approach has come to be referred to today as teaching through problem-solving (NCTM 2003). His research influenced the mathematical curriculum in today's classroom. The keyword in his research was teaching through

problem-solving. Giving students the problems to work through, rather than randomly solving problems helps students develop a better understanding of problem-solving. It shaped problem-solving looks like, and what teachers need to do to influence the teaching process for problem-solving. Teachers' influences provide scaffolding information this is called the Zone of Proximal Development.

According to Gabel (2003) that researchers and teachers have become interested in the learning cycle designed for instruction and procedure for curriculum development.

Strategies are groupings of actions, mental or physical, designed to solve problem Biddlecomb, B., & Carr, M. (2011). Moreover, there are strategies that students used and find the correct answer. In learning mathematics, students have the opportunity in discovering themselves along the way in solving problems (Cotič & Zuljan, 2009)). Students should learn how to properly use strategies for problem-solving. With their advanced knowledge, students are more capable of solving the problem that may arise.

Teachers' strategies, a part of his/her teaching-learning process to make off teaching-learnings will be facilitated as it will help them understand the concepts being inculcated. As a mathematics teacher, it is always the concern that every, knowledge being imparted will be solely absorbed on the learners' mind to be able to understand and independently sustain learning skills. Teachers basic needs in Mathematics is to use varied strategies, as the learners' coach and facilitator, they must support their proficiency as a role model. First, they become better diagnosticians. As (Artzt, 1999) emphasized that Teachers valid procedures that enable them to access, use information in making their assessment acceptable to the learners, their teaching must be within the scope of the prepared curriculum, and that enable to share proof data with students in making instructional decisions. Materials should be provided during the assessments, which are cited by known authors would potentially productive in their instruction. And one way to encourage students is to be more active in assisting in solving problems and supporting illustrative ideas, and concrete solutions to reflect on their work and the teachers to reflect on theirs.

There is no best strategy as posited by Debnatha & Gupta (2005) because instructional strategy depends on the instructional goal. He stressed that teachers should be encouraged to use suitable procedures in the teaching-learning process. It should be of good communication and activity method that will suit the learners' need and has relevance to the community.

Teaching Strategies in Problem Solving in Mathematics

Create a diagram. Using a drawing or a diagram is a manipulative act that helps to solve problems and be understood. It is more accurate and helpful to anyone if materials are used to find the solution to the problem. Remember different pictures might lead to finding a correct solution. It is more advantageous if steps are labeled as you work out the problem (Roam, 2013).

Guess and check. Think of the answer that might work and then test your guess, if not right you can change it closer to the answer. Then, write down all your guesses, and so, you can see the patterns and use them to revise guesses until you derive to correct answer (Capraro, An, Ma, Rangel-Chavez & Harbaugh, 2012).

Use a table or make a list. Decide what information needs to go on your chart. List all the possibilities in a table or chart to make sure pieces of the problem don't get lost organize and record the information so it makes sense (Sulak, 2010; Carson, 2007).

Logical reasoning. Use your information, examine relationships, and think about your information to form a logical answer generalize each relationship to reach a logical conclusion (Nunes, et.al, 2007; Schoenfeld, 1988; Peter, 2012; Masuhay, 2019).

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Find a pattern. List the information you know, identify the information you found, make an organized list or table, examine your list for a recognizable pattern use pattern to find the missing information (Stacey, 1989; Wilson, Fernandez & Hadaway, 1993).

Work backward. Start with a problem, and work step by step toward the beginning to get a solution. Write down what you don't know and what you do know. Write down each step as you get close to your answer. The last step will verify that the solution works (Barham, 2020); Chi, 983; Gick, 1986).

Using a Formula. Students used problem-solving strategy to answer the problems, they choose the suitable formula and substitute data in the correct places with the chosen formula (Tan, 2018).

Paraphrasing strategy. A design to help students in restating their own words strengthens their comprehension (Swanson, Kong, Moran & Orosco, 2019).

Cooperative learning. Students for a purpose assigned by the teacher in groupings, for them to share, and learn from among them (Davidson, 1990).

Mathematics teachers were using group work in problem-solving, probably to assess what goes in group work, this strategy finds not only what mathematicians have been learned, but finds also how they worked together, this is also established data on how to use assessments of group work, and in giving grades individually. Recent studies that employed group work but not assessing their work had found that students don't recognize its significance. Interviews on Mathematics Education indicated that Group work is an integral part of instruction. Whereas, developers constructed high-quality assessment tasks that can be conducted in groups and subsequently scored fairly (Stacey, 1992). Team games and Tournaments. A strategy in which the students are group into teams and compete with each other (Salam, Hossain & Rahman, 2015).

In exemplifying Mathematics learning students performances will be assessed to find his level of performances, an indicative to students' achievements, specified their learnings: to learn to perform mathematical operations; to use those mathematical operations along with mathematical reasoning in solving mathematical problems; and to solve mathematical problems makes mathematics learning more realistic. Mathematics assessments improved students and teachers monitoring and evaluating their progress. Assessing students pushing them aware of what they learn, and discovered the importance of Mathematics. Their consciousness, self-monitoring, and self-regulation are important characteristics to promote their level of confidence (Kenney & Silver, 1993).

Conceptual Framework
The study was formulated from the various concepts and information cited in the review of related literature. Mainly, the concept of this study stemmed from the comprehensive understanding of the teaching strategies used in teaching problem solving among the students in mathematics. High-quality Mathematics assessment should focus on learning and teaching. The fundamental principle of assessment in Mathematics is an integral part, an opportunity for teachers and students to identify areas of understanding and misunderstanding. With this, students and teachers build their concrete understanding and transform themselves as Mathematician. Assessment requires more time to spend in improving the students' mathematical skills in solving problems (National Research Council & Mathematics Learning Study Committee, 2001; National Research Council, 2002).

A teacher is the main factor to consider in improving students' level of achievement, pointed out that high-level performance by students could be attained through the existence of teachers who are more advanced in strategizing to enlighten the learners about the subject taught (Falk, 2002; Ahn & Choi, 2004).

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Problem-solving is part of the curriculum, presupposes that students have its responsibility for their learning, and took it to solve problems, to resolve conflicts, and thinking as a vital element of the curriculum. This event provides students the opportunities to use their knowledge in real-life activities, thus, assist them in working at higher levels of thinking skills (Hiebert, et.al, 1996). Based on the Expert's opinion, students easily understand a problem if they felt comfortable within the environment. And there were the five-stage model that students should easily memorize, which has direct applications to their existence: First, List all related relevant facts; Second, Make a list of all the given information; Third, Restate their own words; Fourth, List specified problem and Fifth, Describe known problems.

To younger students, illustrations can help them organize data, manipulate information, and outline the limits of a problem and the possible solution(s). They look at a problem from many different perspectives: Understanding the problem, pupils understand a problem, by framing a problem in their own words; Describing any barriers, pupils aware of the causes of the problem, this encourages them to verbalize these impediments, and this is always an important step. Identifying various solutions, a problem will be understood, students will tend to select one or more appropriate strategies to help solve the problem. They need to understand that there are possible strategies in solving the problems, among these strategies are: Creating visual images. Some of the problem-solvers find potential solutions before working on the problem. Photos and any visual images would help the students to map out many dimensions of a problem, as their guiding procedure on solving it.

Guesstimate, this allows students more opportunities in engaging trial-and-error approaches to problem-solving. This strategy gathers some preliminary results. Creating a table, students have opportunities to design and create tables of information, designed to arrange data, group, and organize to be easily understood, and solve a problem. Using manipulatives, any moving objects developed patterns that would be recognizable to visualize satisfying components. Work backward this frequently observed that students took data at the end of a problem, and then used a series of computations out from that data to trace where the problem begins. Look for a pattern, this strategy are similar and fall into predictable patterns. A pattern has its meaning, a systematic, logical, and numerical. Create a systematic list, this is a process to map out a plan, defining and solving problems. This inspired students to record their ideas through listing determinants, regularities, patterns, or similarities between problem elements. Try out a solution, working with strategies keeps accurate, and conversant records of their thoughts, reports, and measures this even forecasts a process.

Students should feel comfortable in rejecting potential strategies at any time during their quest for solutions, Teachers must monitor with great care the steps undertaken as part of a solution, it is a natural tendency for students to "rush" through a strategy to arrive as quick the answer, but teachers should encourage them to carefully assess and monitor their progress. Let students felt comfortable to set aside for some time the problem, and undertaking it at a later time. Students must have the privilege to assess their problem-solving skills, and their solutions should be evaluated. Normally, students are dependent on teachers in the classroom. Self-assessment is not easy, though it is necessary for certain students' independence. It is more effective if students are asked the question "How do you feel with your answers?" "Are you sure about it?" and "Do you have proof "(Webster-Stratton & Reid, 2004). Given assessing. The Teaching Strategies used by Mathematics Teachers in Teaching Problem Solving and their Students' Level of Achievement: An Intervention Plan. These variables are shown in box 1 of Figure 1 as the input of the study. The gathering of data on teaching strategies is indicated in box 2 of Figure 1 as the process. The result of this study was the basis for an intervention plan shown in box 3 of Figure 1 as the output of the study.

The Problem
Statement of the Problem
The study assessed the Problem Solving Strategies in Mathematics used by Secondary School Teachers in the Surigao del Norte Division and the Performance of Students in Mathematics. The findings of the study served as the basis for a program. The specific questions sought answers to the following:

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1. To what extent are the following problem-solving strategies used by the teachers in teaching Mathematics?
1.1 Create a Diagram
1.2 Guess and Check
1.3 Use Table or Make a List
1.4 Logical Reasoning
1.5 Find a Pattern
1.6 Work Backwards
1.7 Using Formula
1.8 Paraphrasing Strategy
1.9 Cooperative Learning
1.10 Team Games and Tournament
2. What is the students' level of performance in Mathematics based on the Grade Point Average?
3. Is there a significant relationship between the problem-solving strategies used by the teachers in teaching Mathematics and the students' level of performance in Mathematics based on the Grade Point Average?
4. What intervention program can be proposed based on the findings?

Hypothesis

H_0: There is no significant relationship between the extent of problem-solving strategies used by the teachers in teaching mathematics and the students' level of performance in mathematics based on Grade Point Average.

Significance of the Study

The result of this study will contribute new knowledge to education specifically in the area of intensifying the teaching strategies in Mathematics. It would particularly benefit the following agencies and groups:

Department of Education. The study will hopefully inspire the DepEd officials to allocate a budget to spend intended for Mathematics teaching.

Educators and Curriculum Planners. Results of this study will guide educators and curriculum planners in the development of curriculum materials that will best suit the teacher's competencies in imparting knowledge on mathematics teaching.

Principals. Knowledge of the results of the study will provide principals information on the adequacy and efficiency of Mathematics teaching. They will then see into what areas they will channel the greater bulk of their supervisory functions and enable them to design ways and means to carry them out.

Teachers. Results of this study will provide teachers innovative techniques in improving the teaching-learning processes on Mathematics teaching.

Pupils and Students. The pupils and students are the end beneficiaries of this study, for they would be provided with the best learning atmosphere by their teachers through the principal's follow-up and monitoring activities to enhance Mathematics teaching.

Definition of Terms

Terms may differ in their meaning according to the situations in which they are used. To understand better the terms are defined conceptually and operationally as follows:

Mathematics Achievement obtained by the students represents the academic performance of the student in mathematics which could be affected by the teaching strategies utilized by the teacher.

Problem Solving Strategies is an approach that requires a student to work actively in the solution of difficulty or undesirable condition, there involves the following:

1. Teaching Strategies. Refers to a technique used in conducting classroom instruction that involves investigating episodes using intellectual and physical manipulative techniques, not just to verify principles and concepts but more to develop and arrive at these principles and concepts to facilitate an effective teaching-learning process.

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2. Create a diagram. Refers to draw a picture, have people play roles, or use manipulative to act the problems out. Manipulate your materials to find the solution to the problem. Remember different pictures might all lead to the correct solution. Label the steps as you work out the problem.

3. Guess and check. Means to think of the answer that might work and then test your guess. However, if your guess is not right, think about another idea to get closer to the correct answer. Guessing simply starts from seeing patterns, and use the information to make revised guesses. Repeat until you reach the exact answer.

4. Use a table or make a list. It has decided what information needs to go on your chart. List all the possibilities in a table or chart to make sure pieces of the problem don't get lost organize and record the information so it makes sense.

5. Logical reasoning. Meaning using one's information. Examine relationships, and think about your information to form a logical answer generalize each relationship to reach a logical conclusion.

6. Find a pattern. Making a list of the information, you need to find an organized list or table by examining your list for a recognizable pattern, and use the pattern to find the missing information.

7. Work backward start with the end of a problem. To work step by step toward the beginning to get a solution. Write down what you don't know and what you do know. Write down each step as you get close to your answer. The last step will verify that the solution works.

8. Using a Formula. Students used this approach to find answers to math problems, choosing precise formula would simply constitute to arise data.

9. Paraphrasing strategy. A design to help students restate their own words, therefore strengthening their comprehension of the problem.

10. Cooperative learning. It refers to students work in groups for a purpose assigned by the teacher. It will allow the students to work together and learn from each other.

11. Team games and Tournaments. It refers to a strategy in which the students are group into teams and compete with each other.

12. Performance of Students. It is the extent to which a student has achieved their educational goals through the learning measured by grade point average.

RESEARCH METHODOLOGY

Research Design

The descriptive correlative design was used in the study, investigated a current condition on Problem Solving Strategies in Mathematics of the Secondary School Teachers in Surigao del Norte Division and the Performance of Students in Mathematics. Furthermore, the study attempted to describe the significant relationship between the extent of the strategies used by the teachers in teaching Mathematics and the students' level of performance in Mathematics based on the Grade Point Average.

Research Environment

This study was conducted in Secondary Schools in the Division of Surigao del Norte, Surigao City this place is strategically located because it is accessible to all forms of transportation such as air, sea, and land transportation. Surigao is considered as the gateway to Mindanao because it used to be the old entrance to major navigations even during the time of exploration of Ferdinand Magellan in 1521. The major source of livelihood of Surigao is farming and fishing. Recently, however, Surigao has become a major tourist destination in the region because of its cultural heritage and natural resources. Furthermore, Surigao is the mining capital in the region being the host to major mining industries such as gold, nickel, and copper. Surigao is one of the major sources of nickel, ore, and gold. These natural resources contributed much to the progress and development of this locality which eventually helped improve the educational program in this locality.

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Research Respondents

The respondents are the Secondary Mathematics Teachers of the Division of Surigao del Norte. The name of the schools (refer to table 1) were identified randomly based on the 40 percent of the total number of public secondary schools of the division. Then identified 50 percent of the number of teachers handling grade 7 mathematics regardless of its sexual orientation. The total numbers of respondents were 64 Grade 7 teachers SY 2017-2018. Table one show the distributions of participants and their gender per school.

Research Instrument

Gathering data were made possible by the researcher, he prepared his questionnaire, validated by the experts, and revision was incorporated. The questionnaire was focused on the teachers' teaching strategies, in a form of a checklist, as to find out the participants' responses. The level of achievement of studs in Mathematics was considered and determined based on their Grade Point Average (GPA) as reflected in Form 137-A.

Data Gathering Procedure

Research protocols are imposed, secured a permit from the Schools Division Superintendent, Surigao del Norte for the conduct of the study in the public secondary schools. After securing a permit, the researcher had a courtesy visit to the respective school principals. The respondents were given the questionnaire for them to answer. After they have answered, the researcher retrieved the filled up questionnaire.

Statistical Tools

The following statistical tools were used to analyze the data. Simple Percentage and Mean were used to answer the problem on the frequency, use of teaching strategies in mathematics.

Formula

$\% = f/N \times 100$

Where: % = Percent

f = Frequency

N = Number of cases

Sample Mean   Population Mean

$x = \Sigma x/n$ $\mu = \Sigma x/N$

Where:

Σx is the sum of all data values

N is the number of data items in the population

n is the number of data items in the sample

$WM = \dfrac{\Sigma f w}{N}$

Where:

WM	=	the weighted mean
Σ	=	summation
f	=	frequency of the responses
w	=	weight of the responses
N	=	number of case

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The chi-square test is used to determine whether there is a significant difference between the frequency of problem-solving strategies in mathematics employed by teachers and the students' level of performance in mathematics based on Grade Point Average.

Where:

$$\chi_c^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

"C" is the degrees of freedom
"O" is the observed value
"E" is the expected value

RESULTS AND DISCUSSION

This section presents the analysis and interpretation of data gathered in this study. The presentation follows the sequence of specific problems posed in the study.

To what extent are the following strategies used by the teachers in teaching mathematics; Creating a diagram, Guess and check, Use a table or make a list, Logical reasoning, Find a pattern, Work backward, Using the formula, Paraphrasing strategy, Cooperative learning, and Team games and tournament?

Table 2 presents the extent of different teaching strategies in teaching mathematics. This explained that problem-solving was designed to simplify Mathematics teaching as if this method is the most appropriate approach.

Legend:

Scale	Verbal Interpretation
4 (3.26-4.00)	*Very Extensive*
3 (2.60-3.25)	*Extensive*
2 (1.76-2.59)	*Moderately Extensive*
1 (1.00-1.75)	*Not Extensive*

Among the teaching strategies in problem-solving in Mathematics class which were reflected in Table 2, find a pattern ranked first with a Mean of 3.70, Standard Deviation of 0.9, and Verbal Interpretation of Very Extensive. This strategy used the following approaches; list the information you know, and identify the information you need to find; make an organized list or table; examine your list for a recognizable pattern, and use the pattern to find the missing information.

Logical reasoning ranked second with a Mean of 3.63, Standard Deviation of 0.60, and Verbal Interpretation of Very Extensive. This method refers to a strategy that uses information; examines relationships; and thinks about your information to form a logical answer; generalize each relationship to reach a logical conclusion.

Another strategy is using a formula that was ranked third with a Mean of 3.55, Standard Deviation of 0.56, and Verbal Interpretation of Very Extensive. This strategy refers to a problem-solving strategy that students can use to find answers to math problems. Students must choose the appropriate formula and substitute data in the correct places of a formula.

The teaching strategy, Use a table or make a list ranked fourth with a Mean of 3.53, Standard Deviation of 0.67, and Verbal Interpretation of Very Extensive. It refers to the decision on what information needs to go on your chart, list

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all the possibilities in a table or chart to make sure pieces of the problem don't get lost, organize and record the information so it makes sense.

The strategy of Work backward ranked fifth with a Mean of 3.48, Standard Deviation of 0.64, and Verbal Interpretation of Very Extensive. The strategy has to start with the end of a problem and work step by step toward the beginning to get a solution, write down what you don't know and what you do know, write down each step as you get close to your answer and the last step will verify that the solution works.

The problem-solving strategy, Guess and check ranked sixth with a Mean of 3.45, Standard Deviation of 0.69, and Verbal Interpretation of Very Extensive. This strategy uses the following teaching approaches; think of the answer that might work and then test your guess, if your guess is not right, think about how you can change it to get closer to the answer, write down your guesses so you can start seeing patterns, and use the information to make revised guesses and repeat until you reach the exact answer.

The ranked seventh of the problem-solving strategies is Cooperative learning with a Mean of 3.38, Standard Deviation of 0.70, and Verbal Interpretation Very Extensive. It refers to the Students work in groups for a purpose assigned by the teacher. It will allow the students to work together and learn from each other.

The problem-solving strategy, Team games, and Tournaments ranked eighth with a Mean of 3.37, Standard Deviation of 0.65, and Verbal Interpretation of Very Extensive. This strategy means the students are group into teams and compete with each other.

Paraphrasing strategy was ranked ninth with a Mean of 3.34, Standard Deviation of 0.69, and Verbal Interpretation Very Extensive. This strategy mentions a design to help students restate the math problem in their own words, therefore strengthening their comprehension of the problem.

The only strategy which was interpreted as Extensive was to Create a diagram that was ranked tenth with a Mean of 3.18, Standard Deviation of 0.61. This strategy uses drawing a picture, have people play roles or use manipulatives to act the problems out, manipulating the materials to find the solution to the problem, remembering different pictures that might all lead to the correct solution, and labeling the steps as the work out the problem.

Strategies are groupings of actions, mental or physical, designed to solve the problem. There are many strategies to find the correct answer. Students need to know these strategies and utilize them. In learning Mathematics, the students should have the opportunity to discover by themselves how to properly use strategies for problem-solving. With the background knowledge of mathematical problem-solving strategies, students are better able to solve a problem that may arise.

What is the students' level of performance in mathematics based on the Grade Point Average (GPA)?
It represents the average value of the final grades earned in courses over time, commonly called a GPA, a student's grade calculated by adding up all accumulated final grades and dividing that figure by the number of grades awarded. This calculation results in a mathematical mean—or average—of all final grades. The GPA in Mathematics in this study was based on their grades in the Third Grading Period. Table 3 presents the students' level of achievement in Mathematics. It can be noted in Table 3 that there were 6 (9%) students who were Outstanding; 26 (41%) Very Satisfactory; 29 (45%) were Satisfactory; 3 (5%) Fairly Satisfactory and no student belonged to 74% and below. The overall mean of 84 implied that the students' level in Mathematics in this study was Satisfactory with a standard deviation of 3.44. It can be inferred that most of the students-respondents in this study had Satisfactory Performance. These results indicated that these students need better strategies to improve their level of achievement in Mathematics to help them get an excellent mark. It was revealed that a study based on the Third International Mathematics and Science Study (TIMSS) that the Philippines ranked lowest in both Science and Mathematics due to

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a lack of qualified Mathematics teachers in both elementary and secondary schools. Thus, Mathematics teachers shall find ways and means to improve the teaching-learning processes in their classes (Cristobal cited Vistro-Yu & Villena-Diaz, 2009).

Assessment methods and results can be used not only to provide feedback directly to the individual student. They can also be used to monitor the performance of teachers and schools and as a method to make priorities on the allocation of resources to schools. Other ways assessment results can be used are to make decisions about students' retention or promotion to the next steps of the educational program concerned and to inform parents about their child's progress (Adeyele & Yusuff, 2012).

Is there a significant relationship between the extent of the strategies used by the teachers in teaching mathematics and the students' level of achievement in mathematics based on Grade Point Average?

It can be inferred from Table 4 that students' performance in Mathematics was positively and moderately correlated with teachers' frequency of use of problem-solving strategies based on the observed value of 35.197 and its critical value of 17.132. The significant relationship was tested and resulted in 0.0004, which was significant at 0.05 level. Thus the null hypothesis was rejected. As such, students' level of achievement in Mathematics was influenced by the frequency of use of the strategies which can be noted that the frequent use of the strategies by the teacher in teaching Mathematics would help in students' achievement.

It was posited by Falk (2002) that the high level of performance achieved by the students depends on the existence of teachers who are knowledgeable about critical elements of teaching and the strategies needed to connect to the understanding of the students. Additionally, the better use of the problem-solving strategies in teaching mathematics by the teachers in their classes, the higher is the level of students' performance in mathematics.

Most of the teachers think that they can improve their teaching practices through developing a sound knowledge of content this is a major drawback in many schools, for better learning opportunities for the students. Furthermore, both, how and what are linked together but still far different and unique.

ACKNOWLEDGEMENT

Recommendation

Based on findings and conclusions, the following recommendations are offered: Firsts, Mathematics teachers should pursue postgraduate courses, attend seminars, in-service training, and workshops to gain new insights and more knowledge and skills in facilitating the teaching-learning process; Second, Mathematics teachers should use appropriate strategy to help student gains knowledge of Mathematics concepts and principles and should encourage

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them to participate in class discussion; and Third, there must be proper supervision by the administrators to assure quality Mathematics education. Evaluation of teachers' performance must be regularly done to improve students' level of achievement.

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Table 1. Distribution of Participants

School	Male	Female	Total
Alegria NHS	1	4	5
Amando A. Fabio Sr. MNHS	1	3	4
Bacuag NHS	1	2	3
Campo NHS	1	2	3
Claver NHS	3	2	5
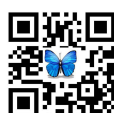
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Gigaquit NHS	2	2	4
Mainit NHS	2	4	6
Malimono NHS	1	2	3
Matin-ao NHS	1	2	3
Placer NHS	2	3	5
San Francisco NHS	2	3	5
Surigao Norte National NHS	3	7	10
Taganaan NHS	1	3	4
Toledo S. Pantilo NHS	1	3	4
Total	22	42	64

Table 2. Problem Solving Strategies in Mathematics Teaching

Problem Solving Strategies Indicators	Mean	Standard Deviation	Verbal Interpretation
1. Create a diagram a. Draw a picture, have people play roles or use manipulatives to act the problems out. b. Manipulate your materials to find the solution to the problem. c. Remember different pictures might all lead to correct solution. d. Label the steps as you work out the problem.	3.18	0.61	Extensive
2. Guess and check a. think of the answer that might work and then test your guess. b. if your guess is not right, think about how you can change it to get closer to the answer. c. write down your guesses so you can start seeing patterns, and use the information to make revised guesses. d. repeat until you reach the exact answer.	3.45	0.69	Very Extensive
3. use a table or make a list a. decide what information needs to go on your chart. b. list all the possibilities in a table or chart to make sure pieces of the problem don't get lost. c. organize and record the information so it makes sense.	3.53	0.67	Very Extensive
4. logical reasoning a. use your information b. examine relationships, and think about your information to form a logical answer. c. generalize each relationship to reach a logical conclusion.	3.63	0.60	Very Extensive
5. Find a pattern a. list the information you know, and identify the information you need to find. b. make an organized list or table c. examine your list for a recognizable pattern. d. use pattern to find the missing information.	3.70	0.49	Very Extensive

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6. work backwards a. start with the end of a problem, and work step by step toward the beginning to get a solution. b. write down what you don't know and what you do know. c. write down each step as you get close to your answer. d. the last step will verify that the solution works.	3.48	0.64	Very Extensive
7. using a formula A problem solving strategy that students can use to find answers to math problems. Students must choose the appropriate formula and substitute data in the correct places of a formula.	3.55	0.56	Very Extensive
8. paraphrasing strategy A design to help students restate the math problem in their own words, therefore strengthening their comprehension of the problem (Montague, 2005).9.	3.34	0.69	Very Extensive
9. cooperative learning Students work in groups for a purpose assigned by the teacher. It will allow the students to work together and learn from each other.	3.38	0.70	Very Extensive
10.Team games and Tournaments A strategy in which the students are group into teams and compete with each other.	3.37	0.65	Very Extensive
Overall Mean	3.46	0.63	Very extensive

Table 3.Students' Level of Performance

Description	Interval	Frequency	Percentage	Mean	Standard Deviation
Outstanding	90-100	6	9%	84	3.44
Very Satisfactory	85-89	26	41%		
Satisfactory	80-84	29	45%		
Fairly Satisfactory	75-79	3	5%		
Did not meet expectation	Below 75	0	0		

Table 4. Significant Relationship between Frequency of used of teaching strategies in mathematics and Students' Level of Performance

Chi-square (Observed Value	Critical Value	DF	p-value	alpha	Significance	Decision
35.197	17.132	3	0.0004	0.05	p < a	Reject the null hypothesis

INPUT	PROCESS	OUTPUT
1. Extent following mathematics teaching strategies used by the by the teachers in teaching mathematics? 1.1 Create a Diagram 1.2 Guess and Check 1.3 Use Table or Make a List 1.4 Logical Reasoning 1.5 Find a Pattern 1.6 Work Backwards 1.7 Using Formula 1.8 Paraphrasing Strategy 1.9 Cooperative Learning 1.10 Team Games and Tournament 2. The students' level of achievement in mathematics based on Grade Point Average. 3. There a significant relationship between the frequency of used of the teaching strategies in mathematics by the teachers in teaching problem solving and the students' level of achievement in mathematics based on Grade Point Average.	Gathering of Data 1. Library Research 2. Internet Research 3. Research Instrument Statistical Treatment 1. Simple Percentage 2. Mean 3. Chi - Square	INTERVENTION PROGRAM

Figure 1. Conceptual Framework

RESEARCH ARTICLE

Effect of Malathion Toxicity on the Histology of Commercial Important Freshwater Fish *Oreochromis mossambicus*

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ABSTRACT

Present study dealt on the effect of sub-lethal toxicity of malathion on the tissue histopathology of commercial important freshwater fish *Oreochromis mossambicus.* The malathion treated *O.mossambicus* result the changes in architectural loss, necrosis, desquamation of epithelial layer hyper plasia and lamellar telangiectasia. The exposure of 0.306 ppm sub-lethal concentration of malathion can cause significant changes in lamellar shortening, lamellar necrosis, telangiectesis and lamellar clubbing. The findings of the present histological investigations demonstrate a direct correlation between exposure to malathion compounds and histopathological observations indicated that exposure to sub-lethal concentrations of malathion caused destructive effect in the gills, liver and kidney tissues of *O. mossambicus.* The observed abnormal behavior and altered histopathology of vital organs demonstrate the severe adverse effects to exposure of malathion toxicity in *O. mossambicus.*

Keywords: Malathion, *Oreochromis mossambicus,* Telangiectesis, Histopathological,

INTRODUCTION

Aquatic toxicology by definition, is concerned with the effects of toxic chemicals on a single species or multispecies. In ecotoxicology, the main objective of testing procedures is to evaluate the toxicity of chemical, the outcomes of which might be serious not only in respect to aquatic ecosystems, their ecology and organisms, but also with human beings, as a component of food chain in the ecosystem. Aquatic Ecotoxicology of any chemical is based on a multidisciplinary research involving environmental, chemical, toxicological and ecological aspects (Adams and Rinne, 1982; Khan *et.al.,* 2000).During the last three decades there has been an unparallel expansion of chemical industries. In India, fish mortality has been reported due to water pollution. Pesticides contaminate the aquatic

ecosystem easily. When a pesticide reaches the aquatic environment, it may be present there for several days or weeks, depending upon its solubility, producing mass mortality, morphological, physical, biochemical and behavioral changes in the organisms. On the other hand, the accumulation of pesticide in the organisms may result in the delayed mortality, reduced reproductive capacity, altered growth rate and reduced ability to withstand the changes in the environment (Heath, 1987). Not only the ichthyo fauna but also the zooplankton and other aquatic invertebrates are affected by the pesticide contamination. Since fish and other aquatic organisms are economically important, their conservation and management of their natural habitats are essential prerequisites to obtain fish productivity and to maintain proper ecological balance of the aquatic ecosystem (Gopalakrishnan, 1990)

Pollution due to pesticide toxicity needs considerable attention because of harmful effects on beneficial organisms like fishes in aquatic bodies. Even sublethal concentration of a toxicant, which lacks the capacity to dramatically increase the rate of mortality in the exposed population, may cause their ecological death after a longtime of exposure, probably as a result of cumulative effect of improved tissues or organs (Jha and Sharma 2002). Among the pesticides, Malathion is a man-made organophosphate insecticide that is commonly used to control mosquitoes and a variety of insects that attack fruits, vegetables, landscaping plants, and shrubs. It can also be found in other pesticide products used indoors and on pets to control ticks and insects, such as fleas and ants. Short-term exposures to high levels of malathion can affect the nervous system causing a variety of symptoms, including headaches, nausea, dizziness, weakness, cramps, diarrhea, excessive sweating, blurred vision and increased heart rate. The aims of the present paper was to evaluate the pesticide malathion toxicity at the tissue level after its intoxication at sublethal dose in the freshwater fish, *Oreochromis mossambiscus.*

Effects of acute (short term) and chronic (long term) exposures in the selected body tissues were investigated. In a nutshell, in the present study, an attempt was made to understand the toxicity of malathion on histological changes in freshwater fish *O.mossambicus.*

MATERIALS AND METHODS

The major steps involved in histopathological analysis are fixation, tissue processing, decalcification, section cutting (Steedman, 1960) and staining (Humason, 1972).

Fixation

Fixation is the process of preserving, hardening and preventing postmortem changes in the tissues. Tissues were placed in fixative immediately after removal from the body. Tissue blocks were then cut to thickness of about 5 mm so that the fixative could readily penetrate throughout the tissue in a reasonably short time. The volume of fixative employed was 15-20 times so that tissue was fixed. The duration of fixation was 24 h. They were then washed in running tap water overnight and was then stored in 70% alcohol.

Tissue processing

This step involves dehydration, clearing and infiltration of the tissue with paraffin. Dehydration using 50-70% dilution of alcohol prevents distortion that would occur to the tissues. Clearing helps in bringing about miscibility between alcohol and paraffin. The tissue was then impregnated and embedded with molten paraffin. The following time schedule was used to make paraffin wax blocks for histological studies as per the technique described by Gurr (1959). In brief, Tissues were washed overnight in running water. A sudden change of the tissues from aqueous medium to alcohol concentrations of 30%, 50% and 70 % was carried out. The tissues were stored in fresh 70% alcohol. At this stage tissue can be stored until further processing. Tissues were then dehydrated by transferring them sequentially to 70%, 80%, 90%, 95% alcohol for one hour each. Transferred to absolute alcohol (2 changes) for one hour each. Placed the tissues in 1:1 mixture of absolute alcohol and xylene for 30 minutes. Tissues were then placed in acetone for complete dehydration for one hour. Cleared in xylene until the tissues became translucent. Tissues were transferred to a mixture of xylene and paraffin wax and left overnight. Infiltrated the tissues in 2 – 3

changes of molten paraffin wax of melting point 60 - 62°C for 1 h each. Embedded in paraffin wax of melting point 60-62°C.The blocks were trimmed and sections of 3-4 µm thickness were cut with a rotary microtome.

Decalcification

Decalcification is the term applied to organic tissues which have been infiltrated with calcium salts. These salts were removed to assure that the specimen is soft enough to allow section cutting. In brief, Gill tissues were cut into small pieces with fine saw. After sufficient fixation, pieces were placed in decalcifying solution containing 10% EDTA. Stirring and heating hastens decalcification. They were then suspended in the upper $1/3^{rd}$ of fluid during decalcification, so that calcium salts sink to the bottom of the container. Since the decalcifying solution contains acid, the gill tissues were washed thoroughly to remove acid from subsequent processing.

Section cutting

Sections were cut at 5 µm thickness and were floated in a water bath between $38-49^{0}C$. The sections from water were then mounted on clean glass slides smeared with Mayer's egg albumin. They were then dried on a hot plate at about $50^{0}C$ for 30 minutes. The sections on the slides were kept ready for staining

Staining

Staining was made according to the procedure of Haematoxylin and Eosin (Gurr, 1959). In brief, the slides containing the section were processed serially as follows: The slides were transferred to xylene: absolute alcohol (1:1) (xylol) and were subjected to two changes for 5 minutes each. They were hydrated by passing through a descending series (95%, 90%, 80%, 70%, 50% and 30%) of alcohol for 5 minutes each. The slides were washed in running tap water for 5 minutes. They were stained using haematoxylin for 10 minutes. The stained slides were washed in running tap water for 10 minutes. The slides were counter stained by keeping in Eosin working solution ranging from 15 seconds to 5 minutes. The stained slides were dehydrated by passing them through an ascending series (30%, 50%, 70%, 80%, 90%, and 95%) of alcohol for 3 minutes each. They were subjected to 2 to 3 dips of 95% alcohol in which two changes were provided. They were followed by 100% alcohol. Two changes were provided for 1 to 2 minutes each. The slides were then placed in acetone. Two changes were provided for 3 minutes each. The slides were dipped in xylene: absolute alcohol (1:1). Two changes were provided for 3 minutes each. Finally the slides after clearing with xylene (2 changes) were mounted in DPX medium. They were examined under microscope (Krempt Wetzlor type) with camera attachment and were photographed at both high as well as low power resolutions. The nuclei stained blue and cytoplasm in various shades of pink. Results were expressed as mean $\pm$ Standard error of mean (SEM) of three observations. Statistical analysis of data was performed using 't' test. P values of less than 0.05 were considered significant (Bailey, 1959).

RESULTS

Effect of Malathion Toxicity on the Gill Histology of the Fish, *O. mossambicus*

General histology of gills in control (Fig 1a & Fig 1b)

Teleost fishes have five pair of gill arches. In the front, four pairs of the slender gill filaments form two lines facing towards the back 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. Numerous semicircular secondary gill lamellae are lined up along both sides of the gill filament. The surface of the gill lamellae is covered with simple squamous epithelial cells and many capillaries separated by pillar cells run parallel along the surface. Numerous semicircular secondary gill lamellae are lined up along both sides if the primary gill lamellae. 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. The blood cells of the secondary filaments. The

blood cells of the secondary gill lamellae have a single nucleus, which are flattened in appearance. The region between the two adjacent secondary gill lamellae is known as inter lamellar region.

Histopathology of gills of *O. mossambicus* treated with malathion (Fig. 1c-1f)

In the malathion treated *O. mossambicus* the changes observed were architectural loss, necrosis, desquamation of epithelial layer, hyper plasia and lamellar telangiectasia. On exposure to 0.306 ppm sublethal concentration of malathion, the most significant changes observed were lamellar shortening, lamellar necrosis, telangiectesis and lamellar clubbing.

Effect of malathion toxicity on the liver histology of *O. mossambicus*

General histology of liver in control (Fig. 2a-2b)

Liver of *O. mossambicus* is a bilobed gland comprising of two tissue compartments, the parenchyma and stroma. The parenchyma comprising of hepatocytes and the stroma comprising of hepatopancreas, bile duct, blood vessels and connective tissue. The parenchymatous cells forming hepatic cords lie irregularly and get separated by blood sinusoids. Hepatocytes are polygonal cells with a prominent spherical central nucleus and a densely stained nucleolus. Each sinusoid consists of an outer peripheral connective tissue and an inner tissue and an inner lining of endothelial cells. In the control group, the liver exhibited a normal architecture with hepatocytes presenting a homogenous cytoplasm and a large central or sub central spherical nucleus.

Histopathology of liver of *O. mossambicus* treated with malathion (Fig.2c-2f)

The important histopathological changes observed in the malathion lethal treated groups were pyknotic nuclei and clear cellfoci. In the sublethal treated group the changes observed were pyknotic nuclei and necrosis.

Effect of malathion toxicity on the kidney histology of *O. mossambicus*

General histology of kidney in control (Fig. 3a-3b)

The structural details of the kidney of control *O. oreochromis* are shown in Fig. 3a & 3b. Sectioned kidney of control fish revealed Bowman's capsule and basal epithelial cells. Renal tubule cells are normal showing nucleus and cytoplasmic granules. Blood capillaries are seen in and around Bowman's capsule and renal tubules. The architecture of kidney is clear cut with its components and it is evident that no histological alterations were totally absent.

Histopathology of kidney of *O. mossambicus* treated with malathion (Fig. 3c-3f).

Since kidney is the organ that filters large volume of blood, malathion present in the blood caused some pathological changes in *O. mossambicus* treated with sublethal concentrations of malathion. The histological results indicate that the fish was negatively affected at the tissue level on exposure to both lethal (96 hrs) and sublethal contentration (15 days) of malathion. Important changes observed were in the treated fish were in Bowman's capsule, such as cell proliferation and thickening of basal lamina leading to the reduction of Bowman's capsule. Occlusion of proximal and distal tubules were noticed by the accumulation of some materials in lumen and also consequence of epithelial cells swelling. Most significant changes observed was vacuolation of tubular epithelium and necrosis.

DISCUSSION

The results from the present study suggest that the histopathologic lesions observed in the organism might be due to exposure to insecticide, malathion (Table 1). Histopathological characteristics of specific organs express condition and represent time-integrated endogenous and exogenous impacts on the organism stemming from alterations at

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lower levels of biological organization (Kutz*et al.*, 1980). Therefore, histological changes occur earlier than reproductive changes and are more sensitive than growth or reproductive parameters and as an integrative parameter, provide a better evaluation for the health of the organism than a single biochemical parameters (Tazeen Arasta and Bais, 1996).

Gills are highly susceptible to toxic chemicals of environmental pollutants, because of direct contact between gills and environmental pollutants. The absorption of toxic chemicals through gills is enhanced by increasing the permeability to water and ions of gill epithelium and by inhibition of ions exchange activity of the chloride cells. The measurements of the effect of aquatic pollutants in marine and freshwater habitats have frequently been tested in gill. Histological study of the gills shows a typical structural organization of the lamella in the untreated fish. However, fish exposed to malathion shows several histological alteration, included hypertrophy, lifting of the epithelial linings from the surfaces of secondary lamellae, and at few places, degeneration of lamellar epithelium. Similar observations were reported due to exposure of other fresh water fish to pesticides may interfere with vital operations including respiratory chain, compete with essential metals for transport with consequences for the osmotic balance and for active centers of enzymes. Lifting and hyperplasia of lamellar epithelium could be interpreted as defense responses of the fish, as these alterations increase the distance across which waterborne irritants must diffuse to reach the blood stream. The most common cause of cellular degeneration in gill filaments is Oxygen deficiency as a result of gill toxicity.

Another important histopathological change observed in the malathion greater group was hyperplasia. Morphologically, hyperplasia refers to an increase in the number of normal cells that constitute a given tissue. Gill alterations such as hyperplasia of the epithelial cells can be considered adaptive, since they increase the distance between the external environment and blood, serving as a barrier to the entrance of contaminants. Hyperplasia observed may be the fish's response (1) to ward off or block something that irritates its tissues, whether externally or internally, or (2) to quickly heal an injured or irritated site. Hyperplasia, however, may play a role in the early stages of neoplasia. Gill hyperplasia might serve as a defensive mechanism leading to a decrease in the respiratory surface and an increase in the toxicant-blood diffusion distance. Increased mucus production and fusion of lamellae were obvious on exposure to both the sublethal concentrations of malathion. Mucus cells contain mucins, polyanions composed of glycoproteins that can be effective in trapping toxicants and aid in the prevention of toxicant entry into the gill epithelium (Natarajan 1981; Ram and Sathyanesan 1987; Tilak *et al.*, 2001a,b; Figueriedo fernnandes 2007). Extensive epithelial desquamation was also observed in the malathion treated group. It is well known that changes in fish gill are among the most commonly recognized responses to environmental pollutants (Vijayalaksmi and Tilak 1996; Tilak *et al.*, 2005). After acute exposure to pesticides or toxicants, *Channa punctatus* and *Punctius conchonius* fishes exhibited marked degenerative changes in the histology of gills, liver and kidney tissues (Tilak *et al.*, 2005).

Generally pesticide concentrations are toxic which may be lethal or sublethal in aquatic environmental lethal concentrations cause the death of organisms directly. But sublethal concentration effect and disturb metabolic and histological changes (Jha and Verma, 2002).The gills of both sublethal malathion treated groups exhibited telangiectesis (localized dilation of blood vessel). This appearance of the secondary lamellae results from the collapse of the pillar cell system and breakdown of vascular integrity with a release of large quantities of blood that push the lamellar epithelium outward (Alazerni *et al.*, 1996). Shortening and clubbing of ends of the secondary epithelial lamellae and clubbing of adjacent lamellae were well marked in the malathion treated group. Complete lamellar fusion may have reduced the total surface area for gas exchange. Otherwise, they increase the distance of the water-blood barrier, which together with epithelial lifting and the increase in mucus secretion may drastically reduce the oxygen uptake.

As fish gills are critical organs for their respiratory and osmoregulatory functions, the injuries in gill tissues observed as a result of exposure to malathion pesticide may have reduced the oxygen consumption and resulted in the disruption of the osmoregulatory function of the fish. As gills are the major site of osmotic and ionic regulation

in fish, any change in gill morphology may result in perturbed osmotic and ionic status which was observed as decreased ATPase activity in the present investigation. Also the histopathological alterations could be attributed to increased peroxidative damage to gill membrane in fishes exposed to malathion toxicity. It is important to stress that lamellar fusion and disappearance of secondary lamellae can lead to a notable reduction in the respiratory surface, which consequently can hinder gas exchanges (Figheirdo Fernandes *et al.*, 2007). The defense responses will take place at the expense of the respiratory efficiency of the gills and eventually, the respiratory impairment must outweigh any protective effect against pollutant uptake.

We have observed significant deformations in liver on exposure to both the sublethal concentrations of malathion. Liver being the main organ of various key metabolic pathways, toxic effects of chemicals usually appear primarily in the liver. This, in turn, provides important data on the chemicals toxicity and mode of action. Also it is a principal site of detoxification based on the fact that in teleosts it is the major site of cytochrome P450 which inactivates some chemicals and activates others. Furthermore, nutrients derived from gastrointestinal absorption are stored in hepatocytes and released for further metabolism by other tissues (Hawkes, 1980), bile synthesized by hepatocytes aids in the digestion of fatty acids and carries conjugated metabolites of toxicants (Gingerich, 1982) into the intestine for excretion or enterohepatic recirculation and the yolk protein vitellogenin is synthesized within the liver (Hawkes, 1980). Many organic compounds induce toxicopathic lesions in the liver of fish species. Stressor-associated alterations of hepatocytes may be found in the nucleus or cytoplasm or both.

An important observation in the current study on exposure to malathion was clear cell foci which exhibited an altered staining pattern. Foci lesions precursors to the development of hepatocellular neoplasm indicating a reduced capacity to metabolize xenobiotics. Chris *et al.*(2000) suggest that there are strong and consistent associations among all of the putatively preneoplastic foci of cellular alteration (basophilic, eosinophilic, and clear cell foci), between focal lesions and the different types of neoplasms, and among the various neoplasm types. Hepatocellular foci of altered hepatocytes have been suggested as an early stage in the stepwise formation of hepatic neoplsia and as such provide an excellent example of a histopathological biomarker for contaminant exposure (Das and Mukerjee 2000). Histologic examination of mosquito fish (*Gambusia affinis*) from a thiodon contaminated site in the Elizabeth River, Virginia, revealed high incidences of hepatic neoplastic lesions (Cengiz *et al.*, 2001); Cengiz and Unlu (2006) observed that on chemical contamination exposure deltamethrin on the mosquito fish, *Gambusia affinis* showed toxicopathic liver lesions such as neoplasms, preneoplasms, specific degeneration/necrosis and non-neoplastic proliferative lesions.

Another important change observed in the liver of treated groups was necrosis. Necrosis, which is a passive mode of cell death shows that the capacity to maintain homeostasis was affected. Thus occurrence of necrosis may be one of the important reasons for decreased lysosomal membrane stability observed leading to the leakage of lysosomal marker enzyme acid phosphatase to the soluble fraction. Also the increased level of the important marker enzyme ALT in liver (Adams 1990; Agrahari and Gopal 2009) indicates the stress induced by the malathion toxicity in this tissue.In both malathion sublethal treated group shrunk and photic nuclei were observed in liver. Pyknotic nuclei observed indicate that the cells became hypofuctional. Pyknosis results in irreversible condensation of chromatin I, the nucleus of a cell. Acute toxic injury usuall includes cloudy swelling or hydropic degenerations and pyknosis, karyorrhexis and karyolysis of nuclei (Ram and Sing1988; Rao 2006; Sanjoy Deka and Rita Mohamed, 2012). Cloudy swelling, bile stagnation, focal necroatropy and vacuolization have been reported in the *Gambusia affinis* exposed to thiodon (Cengiz *et al.*, 2001).

Cengiz and Unlis (2006) reported hypertrophy of hepatocytes, increase of kupffer cells, circulatory disturbance narrowing of sinusoids, pyknotic nuclei, fatty degeneration and focal necrosis in the liver of *Gambusia affinis* exposed to deltamethrin. The cellular degeneration in the liver may be also due to oxygen deficiency as a result of gill degeneration and /or to the vascular dilation and intravascular haemolysis with subsequent stasis of blood (Mohamed *et al.*, 2013). The kidney is a highly dynamic organ in most of the vertebrates. Kidney receives about 20%

of the cardiac output. Any chemical substances in the systemic circulation are delivered in relatively high amounts to this organ. Thus a nontoxic concentration of a chemical in plasma could become toxic in the kidney. The kidney of the fish receives largest proportion of postbranchial blood, and therefore renal lesions might be expected to be good indicators of environmental pollution (Baby Joseph and Justen Rayj, 2011). In the present study, the most evident changes observed in the kidney of malathion treated groups were glomerular congestion, pyknotic nuclei and renal tubular architectural loss m-resol treated group showed histopathological alterations such as necrosis and vacuolation of tubular epithelial cell. It was also observed that in both the treated groups epithelial cells have become swollen and basophilic.

Hypertrophy of renal cells, changes in the nuclear structure, formation of vacuoles, necrosis and degeneration of renal components were noticed on the renal cells of *Cyprinus carpio* exposed to malathion (Farook Ahamed Mir *et al.*, 2012). Nagarajan and Shasi Kumar (2002) reported dilation of tubules, necrotic changes characterized by karyorrhexis and karyolysis at the nuclei of affected cells of *Labeo rohita* exposed to sago effluent. The exposure to fish to toxic agents such as pesticides induce histological alterations in several components of the trunk kidney (Tilak *et al.*, 2001b; Tilak *et al.*, 2007). Virk *et al.* (1987). observed lesions in the kidney tissues of fish exposed to endren and carbaryl characterized by degeneration in the epithelial cells of renal pyknotic nuclei in the hematopoietic tissue, dilation of glomerular capillaries, degeneration of glomerulus, intracytoplasmatic vacuoles in epithelial cells of renal tubules with hypertrophied cells and narrowing of the tubular lumen. Ramalingam *et al.*, (2002) observed histopathological changes in liver and kidney of freshwater fish, *Cirrhinus mrigala* in Vargai reservoir contaminated with organochlorine pesticide residues. They noticed characteristic changes such as mononuclear pyknosis in liver and kidney.

CONCLUSION

As a conclusion, the findings of the present histological investigations demonstrate a direct correlation between exposure to malathion compounds and histopathological observations indicated that exposure to sub-lethal concentrations of malathion sub-lethal doses caused destructive effect in the gills, liver and kidney tissues of *O. mossambicus.* The observed abnormal behavior and altered histopathology of vital organs demonstrate the severe adverse effects to exposure of malathion toxicity in *O. mossambicus.* The current study reinforces the application of histopathology as a powerful tool for monitoring anthropogenic contamination within aquatic environments. Whilst links between such pathologies and contaminants are not definitive, such as surveillance provides a useful insight into individual, population and overall ecosystem quality. When these pathological endpoints are assessed in conjunction with other parameters such as parasite community structure, sediment and water chemistry, enzyme responses, bile metabolite levels and molecular damage indices, a clearer picture of the complex interactions between anthropogenic and natural.

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CONFLICTS OF INTEREST

We declare that we have no conflict of interest
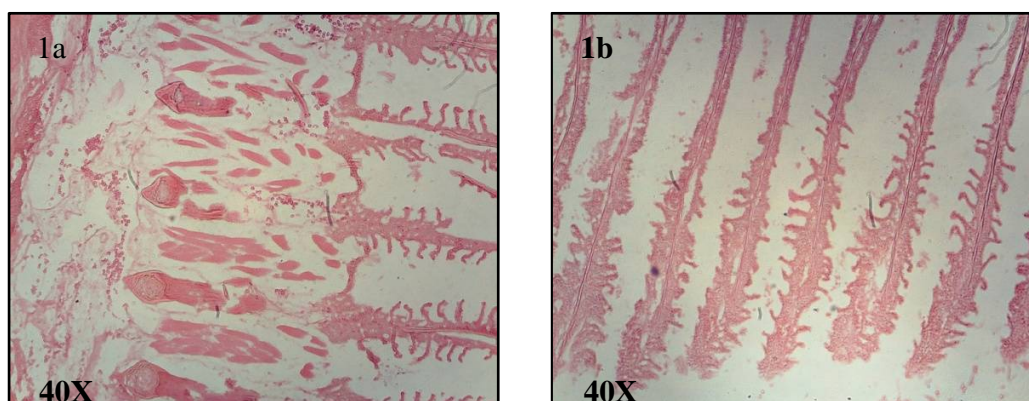
Fig. 1a & b. Photomorphograph of control gills in *Oreochromis mossambicus*

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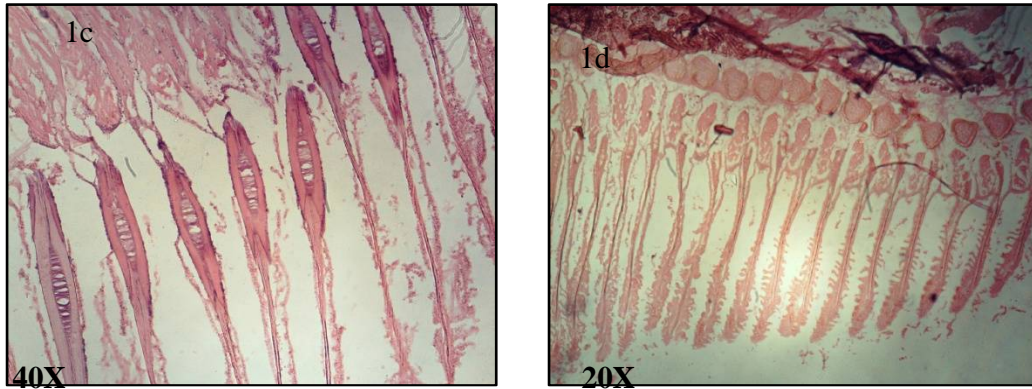
Fig. 1c&d. Photomorphograph of 0.5 ppm treated gills in *Oreochromis mossambicus*
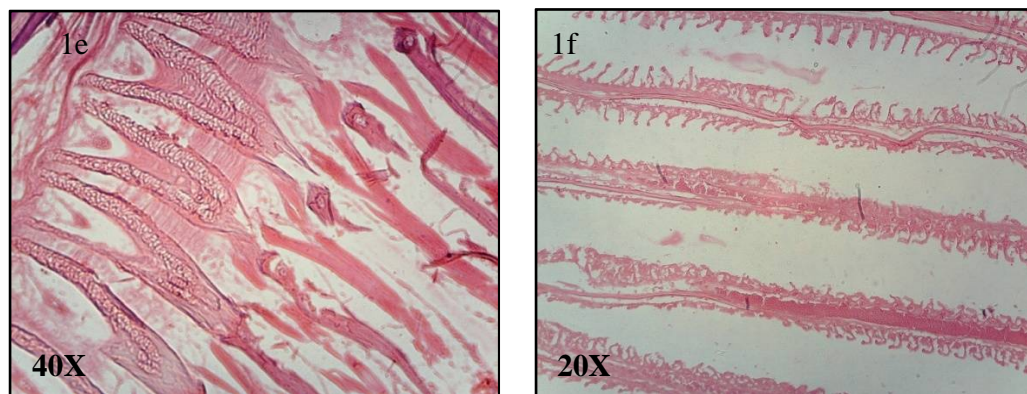
Fig. 1e&f. Photomorphograph of 0.7 ppm treated gills in *Oreochromis mossambicus*
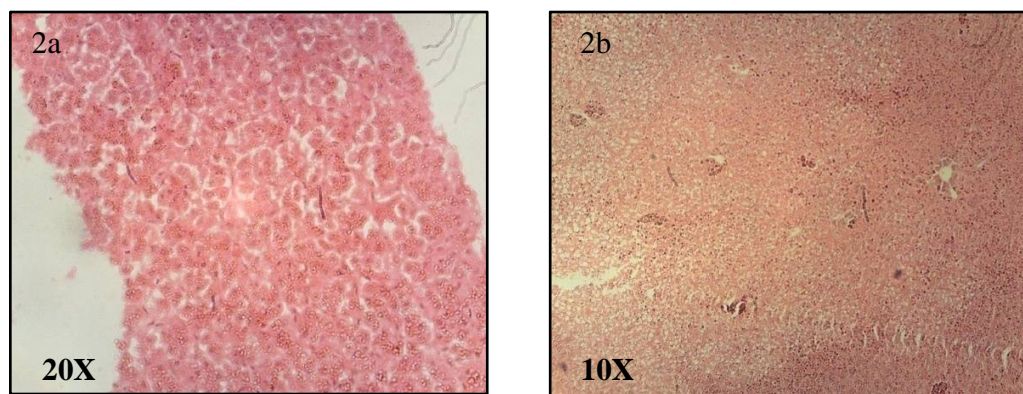
Fig. 2a & b. Photomorphograph of control liver in *Oreochromis mossambicus*

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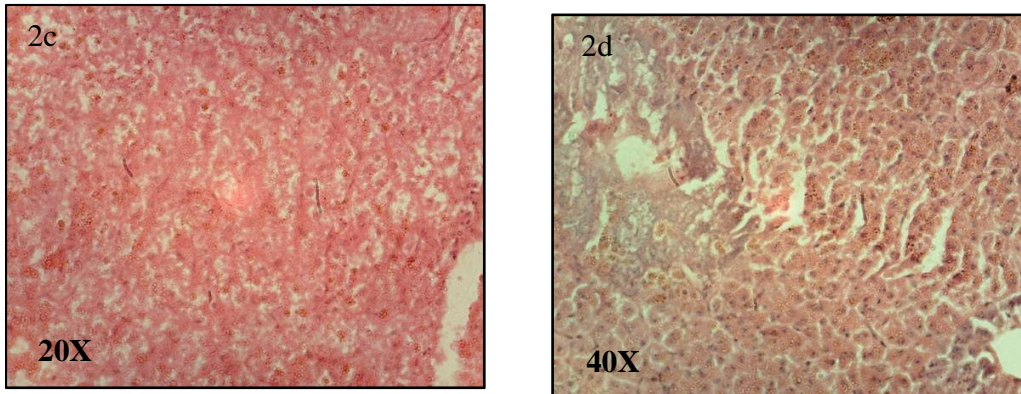
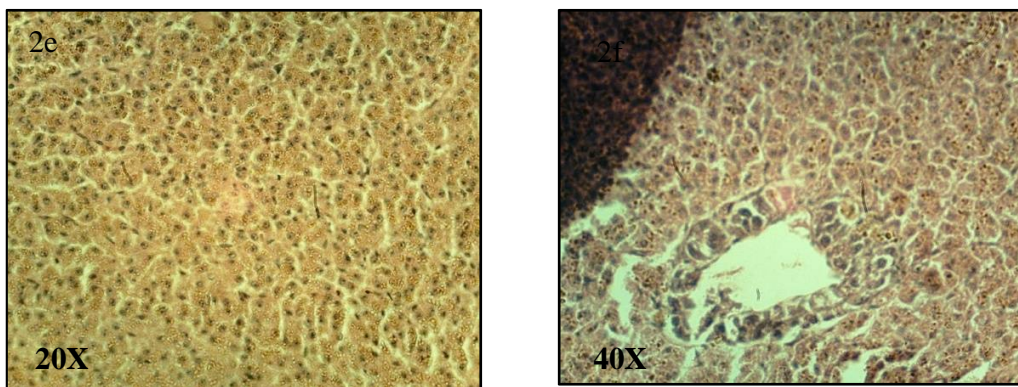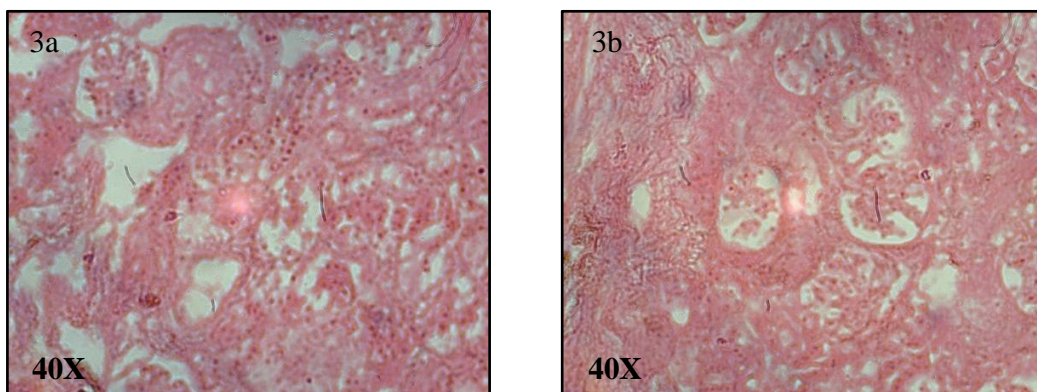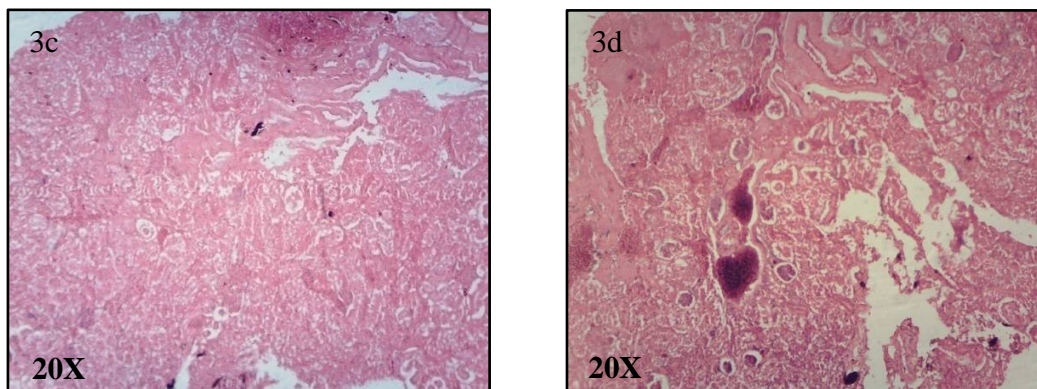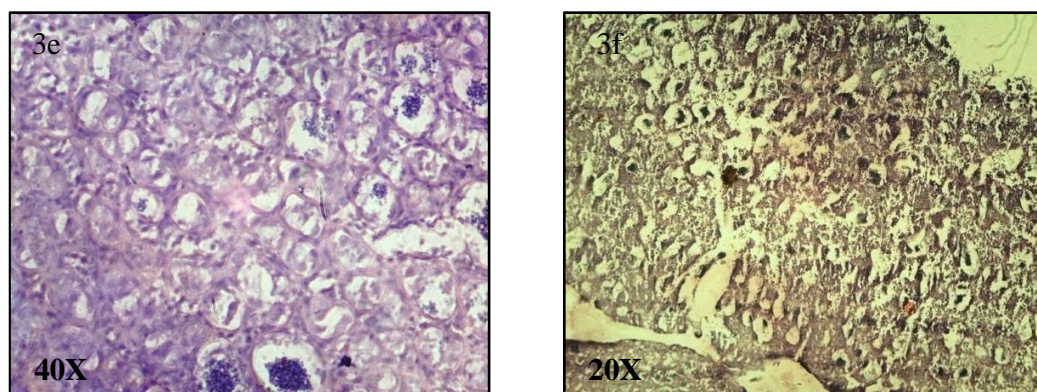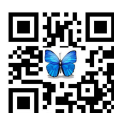**Fig. 2c&d. Photomorphograph of 0.5 ppm treated liver in** *Oreochromis mossambicus*

Fig.2e&f. Photomorphograph of 0.7 ppm treated liver in *Oreochromis mossambicus*

Fig. 3a & b. Photomorphograph of control kidney in *Oreochromis mossambicus*

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Fig. 3c&d. Photomorphograph of 0.5 ppm treated kidney in *Oreochromis mossambicus*

Fig. 3e&f. Photomorphograph of 0.7 ppm treated Kidney in *Oreochromis mossambicus*

Table 1. Histological alterations of gills, liver and kidney in common Tilapia, *Oreohromis mossambicus* in each group (n=10) indicating the respective stage of tissue damage and frequency of occurrence, Stage 1: do not alter normal histology of tissue; stage II: More severe and causing abnormal histology of tissue; Stage III: marked severe and causing irreparable damage.

Body tissues	Stage	Exposure period						
		24h	48h	72h	96h	5d	10d	15d
Gills								
Hyperplasia of gill epithelium	I	+	+	+	++	++	++	+++
Hypertrophy of gill epithelium	I	0	+	+	0	+	+	++
Blood congestion	I	+	+	+	+	0	+	+
Lifting of lamellar epithelium	I	0	+	+	++	+	++	++
Lamellar fusion	I	0	0	+	+	++	+	+
Lamellar disorganization	I	0	+	0	0	0	++	++
Lamellar aneurysm	II	0	0	+	++	++	++	++
Rupture of epithelial cells	II	0	+	+	+	+	+	+

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Liver								
Nuclear hypertrophy	I	0	0	0	+	++	+	++
Cellular hypertrophy	I	0	0	0	+	+	+	++
Irregular shaped nucleus	I	0	0	0	0	+	++	+++
Irregular shaped cell	I	+	+	+	+	+	++	+++
Cytoplasmic degeneration	II	0	+	0	0	0	+	+
Nuclear vacuolization	II	0	0	0	0	0	+	+
Cellular rupture	II	0	0	0	0	0	+	+
Kidney								
Nuclear hypertrophy of renal tubule	I	0	0	+	+	+	++	+
Cellular hypertrophy of renal tubule	I	0	0	+	+	+	+	+
Narrowing of tubular lumen	I	0	0	+	+	+	+	+
Enlargement of glomerulus	I	0	0	+	+	+	+	+
Dilatation of glomerulus capillaries	I	+	+	0	0	0	0	0
Reduction of Bowman's space	II	0	0	0	0	0	0	0
Occlusion of tubular lumen	II	0	0	0	0	0	0	0
Tubular necrosis	III	0	0	0	0	0	0	0

Note: absent (0); rare (+); frequency (++); very frequency (+++)

REVIEW ARTICLE

Naturopathy Can Convalesce Dental Health: A Review

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ABSTRACT

Naturopathic system of medicine is a healing science which stimulates the internal ability to restore health by utilizing the natural components like Water, Earth, Fire, Ether and Air. Naturopathy is a call to "Return to Nature" and an opportunity to live in a simplified and balanced way with environment, self, and society. For the management of the disease it plays an important role not only in providing practical approach in a simplified way but also provide a firm basic to holistic medical health care. It seeks attention towards health foundation which provides a frame work for medicine of forthcoming generations. Naturopathy is an approach to healing using "natural" means such as diet and lifestyle. In dentistry, various modalities are available therefore, supporting dental treatment. For the same purpose, this paper is intended to have an overview of other dental treatment modalities available via i.e. Naturotherapy.

Keywords: Naturopathy, Holistic dentistry, Internal Medicine, Alternative therapy, Dentistry.

INTRODUCTION

Ayurveda, Unani, Siddha, Homeopathy, Amichi, Yoga and Naturopathy are the seven known systems of medicine in India. During British rule western medicine has dominated over Indian medicine systems despite of faith. After

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that Indian medicine systems were discouraged where as western system of medicine was recognized as legitimate. In early 20th century, with the arousal of Indian nationalism, arise interest in Indian art and science and due to gradual increase in the growth of ISM. Revolution in Indian health system was initiated by Bhore Committee and Mudaliar Committee, they recognize the significance of Indian System of Medicine. Thereafter, government of India establishes many eminent committees for the recommendation of methods which should be adapted in accordance to Indian System of Medicine [1,2]. Alliance of Indian system of medicine and modern medicine is reported by chopra but allopathic professionals had influence on health system design, and suggestion concerning the inclusions of naturopathic practitioners into national health care services was simply not achieved [3]. Subsequently potency of established system of medicine was recognized internationally. Naturopathy is a discrete type of basic medicinal care which amalgamates traditional healing with current research and scientific advances. It is directed accompanied by unique set of principles that identify inherent capacity of healing, accentuate prevention of disease, and inspire the individual to maintain health of optimal level. Modalities utilized by naturopathic physician like clinical nutrition and diet, hydrotherapy, behavioural change, botanical medicine, homeopathy, pharmaceuticals, minor surgeries, and physical medicine [4,5]. In the 19th century, European used naturopathic medicine to cure disease in a natural way like air, diet, herbs and water. Beginning of 20th century, naturopathic medicine formulated in the Canada and US, combining nature cure, homeopathy, spinal manipulation and other therapies [6]. Dorland's Illustrated Medical Dictionary defines Naturopathy as "a drugless system of therapy, making use of physical forces such as light, message, air, heat, water, etc [7].

Background (Table 1)
Basic Concepts of Naturopathic Medicine
There are number of references in the ancient literature like Vedas where cure of disease by natural methods is a way of life. In the ancient time it is mentioned in the literature that concepts of vital force, morbid matter theory and other concepts were used to cure a disease in a natural way and these methods were used extensively in the ancient time [8,9].
Cure of disease in a natural way is based on the following three principles
1. Abnormal composition of blood and lymph
2. Lowered vitality
3. Accumulation of morbid matter

Naturopathic Medicine Principles
- The Healing Power of Nature (Vis Medicatrix Naturae) – Naturopathic medicine identifies the healing power of the body and believes that body's native acumen to provide a healing ability if proper tools and guidance are given.
- Identify and Treat the Causes (Tolle Causam) –Naturopathic dentists identify the cause of the disease and also treat it, instead of focusing on symptoms of the individuals.
- First Do No Harm (Primum Non Nocere) – Naturopathic dentists starts with lowest and then progress to higher level of interruptions as decided.
- Doctor as Teacher (Docere) – Naturopathic dentist involved in the healing process, instruct the patients and accentuates the doctor-patient relation.
- Treat the Whole Person – All aspects of person's health were taken into account by naturopathic medicine which includes mental, physical, genetic, mental, spiritual , social and environment factors.
- Prevention – Naturopathic medicine accentuate prevention from disease and wellness of the individual [10].

Therapeutic Order of Naturopathic Medicine

Establish the conditions for health
- Disturbing factors can be identified and removed

• Institute a more healthful regimen

2. Stimulate the healing power of nature (vis medicatrix naturae): the self-healing processes

3. Address weakened or damaged systems or organs

• Strengthen the immune system

• Decrease toxicity

• Normalize inflammatory function

• Optimize metabolic function

• Balance regulatory systems

• Enhance regeneration

• Harmonize life force

4. Correct structural integrity

5. Address pathology: Use specific natural substances, modalities, or interventions

6. Address pathology: Use specific pharmacologic or synthetic substances

7. Pathology can be suppressed or removed surgically [11].

Modalities (Table 2)

Naturopathy and Dentistry

When a body restore to health in a natural way by strengthening or supporting the natural healing properties found internally this restoring process is focused by the naturopathic medicine. Naturopathic medicine, including holistic dentistry, looks for safe and nontoxic materials which have additive healing assistance. Holistic dentists and naturopathic medicine give credence that healthier the gingiva and teeth healthier the body or vice versa. Now a day, ADA states that Oral health is a part of overall health; in fact, oral health is frequently correlated with other conditions like heart disease, diabetes and stroke. It may be because many of us visit general physician for overall health and dentist for oral care that we differentiate the oral health from rest of body. Both medical and naturopathic or holistic practitioners admit that cardiovascular, endocrine, immune and oral health are interrelated. Generally, holistic dentistry takes in account due to its non metallic and nontoxic solutions for the promotion of oral hygiene and increasing oral health. While holistic dentists provide conventional services, like cleanings and examinations, treatment for crowns and bridges, fillings, gingivitis and gingival diseases, they may also look at issues around sleep apnea, TMJ and snoring, and halitosis. Most of the holistic dentists may conduct screening for allergic reactions, or lower resistance to those components which are found in the crown, bridge and filling materials.

From the naturopathic perspective essential oils are one of the aspects of dentistry. They may be correlated with herbal and aroma therapy preparations. Essential oils are utilized in strong and purified form of 'fragrances' for aroma therapy and also used in cosmetics and perfumes. Tinctures are herbs extracts strained usually in vinegar or alcohol. Confidence and trust increases when few drop of rose oil was toss on the pillowcase. For apprehensive new patient it is quite admirable but will not overcome brashness, attitudes or inappropriate chair side effects. In case of headaches a mixture of 2/3 ethanol [95% grain alcohol –Ever clear], 1/3 Peppermint oil is of great effect. When a drop placed and rubbed into temporal and middle of forehead region which will diminish most headaches almost completely within 90 seconds. In addition to above when drops were placed on mastoid process and occipital also helps. Instead of all this headache may not be diminished but its severity may be decreased. Physically, mentally and emotionally invigorating behaviour is shown by Peppermint. Muscle knots or triggers pain relieved when lavender was rubbed and also assist in lymphatic drainage. It prevents scarring and also good for burns, mouth abscesses, sprains, skin. When directly applied on the concerned area it works well. Antiviral and antiseptic activity is show by lemon. Astringent activity is also shown by it. Through diffusing action it purify air and it also purify water by adding few drops in a glass of water. Another use is mentally refreshing, for cleaning skin, remove grease and oil.

Sedative for toothaches is clove oil. In case of gingivitis and periodontal infections Rose, Myrrh, Lavender and Frankincense are useful. Pain after periodontal surgery is reduced by the use of Helichrysum which is applied every 15 minutes after surgery. There is no alternative for impeccable oral hygiene when periodontitis and tooth decay was

noticed by professionals. Healthy gingiva does not bleed, collect plaque, have pockets, or generate bad breath and clean teeth do not decay. Mainly fluoride is used in mouthwashes, toothpastes, and also in drinking water which is avoided by many naturopathic dentist. Few of the researchers propose that ingestion of fluoride may be allied to cancer and bone issues. While others propose that there is false benefit from use of fluoride in dentistry that's why most of the naturopathic dentists opt not to use it. Propylene glycol [hard abrasive] might be used in the conventional toothpaste. For maintaining the oral health and hygiene with the help of natural mouthwashes and toothpastes are become more popular and can also be used as an alternative to conventional one [15,16].

These are the products of choice for
• Fighting tartar- sea salt, baking soda, citrus acid
• Fighting bacteria- peppermint oil and green tea oil
• Whitening teeth- papaya plant extract, zinc oxide
• Blocking acid- grapefruit seed extract

Those who are sensitive or allergic– sulphate free, gluten free and low in sulphates holistic products are also available for them [15,16].

CONCLUSION

Treatment by a "holistic" or "natural" or "biological" or "naturopathic" dentist is not costly as compared to conventional treatment but naturopathic dentist may charge more due to the additional care, skill, judgment and time involved. Punctilious follow-up with additional home care and periodical visits by the patients for maintenance and prevention, instead of all this treatment is more demanding. Holistic dentists give credence that healthier the gingiva and teeth healthier the body or vice versa. Holistic dentistry identifies the diseased condition of the teeth and after that disease was treated symptomatically.

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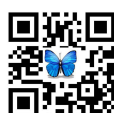
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Table 1: Historical background

Author	Year	Invention
Vincent priessnitz	1798-1852	Founder of "nature cure", and well-known for his hydrotherapeutic institution in Grafenberg, Germany.[6]
Sebastian Kneipp	1824-1897	Known worldwide for his successful nature cure techniques, which integrated hydrotherapeutic treatment with herbs.[6]
Ernst schweninger	1850-1924	Established the first nature cure hospital in Grosslichterfelde, Germany.[6]
Heinrich Lahmann	1860-1905	The first nature doctor who graduated from medical school. Dr. Lahmann founded a hydrotherapy sanatorium, which incorporated raw vegetarian diets.[6]
Henry Lindlahr	1862-1924	Naturopath who established a successful sanitarium for nature cure and osteopathy in Chicago, Illinois. Among other scientific contributions, Dr. Lindlahr wrote Nature Cure, which at its time was considered "the best work ever published in Nature Cure Literature".[6]
Franz Schonenberger	1865-1933	The first university professor who introduced nature cure methods into the Priessneiz Hospital in Berlin, Germany.[6]
Louisa Lust	1868-1925	Known as the "Matriarch of Naturopathy", as she was a successful naturopath specializing in the treatment of women.[6]
Benedict Lust	1872-1945	Known as the "Father of Naturopathy" for his combination of massage, nature cure with homeopathy, therapeutic electricity and spinal manipulation.[6]
Otis G. Carroll	1879-1962	Dr. of chiropractic medicine who invented constitutional hydrotherapy and developed the first means for discerning food sensitivities.[6]
Alfred Brauchle	1898-1964	Conducted "The Great Nature Cure Experiment" in the Johannstadter Hospital in Dresden, Germany. This was the first collaboration between natural and orthodox medical providers.[6]
John Bastyr	1912-1995	Dr. of chiropractic and naturopathic medicine who is known as the "Father of Modern Naturopathic Medicine". Dr. Bastyr founded Bastyr University, located in Seattle, WA.[6]

Table 2: Modalities under Naturopathic Medicine

Natural Modalities	Naturopathic Treatment
Massage	Massage is a generally employed for sedative, stimulant and tonic effects. It is an effective substitute for exercise. [12]
Air	Fresh air is essential for good health. Air therapy is utilized at varied temperatures and pressures while treating various diseases. [12]
Mud	Mud dissolves, absorbs and removes the toxins and rejuvenates the body. It plays an

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	important role in the treatment of diverse disease like skin disease, constipation etc. [12]
Fire	"Agni" (Fire) on which existence of all the forms and creatures depend. In nature cure treatment, production of specific effects different temperatures are employed through various heating techniques. [12]
Chromo Therapy	Sun rays have seven colors -violet, indigo, blue, green, yellow, orange and red. These colours are applied via body or irradiation or by administrating charged oil, motor and pills for treatment. [12]
Hydrotherapy	Hydrotherapy is the use of water in any form (steam, ice, water) either internally or externally for treatment of disease or health promotion. Before it was well established as the traditional European water cure, it was used widely in ancient cultures, which includes Persia, Egypt, India, Israel and China.[13] Most of the treatments can be done at home which makes it cost effective and also participatory for the patients.[10]
Magnet	Magnets North and South poles of various shapes and powers are employed in treatment which influence health of an individual by applying directly or through charged up water/oil on different body parts. [12]
Food	Many diseases are controlled with the help of food therapy. There are slogans of nature cure which are true and quite effective one of such is your food is your medicine, and another is as you eat so will you be mentally as well as physically fit. [12] Diets rich in vegetables and fruits are related with decreased risk for chronic disease.[14]
Space	Fasting is the best way of therapy to diminish congestions of mind and body because congestion causes disease. [12]
Acupressure	For getting rid of the ailments related to the different organs, there are certain points on our feet, body and hands which are correlated with these organs on which pressure is applied. [12]

RESEARCH ARTICLE

Kakaulit O Kauulit: An Issue on Repetition and Reduplication in Writing News on Selected Tabloid

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ABSTRACT

Using Google translate application this Filipino version of an Article interpreted that this paper attempts to clarify some issues regarding the orthography of the Filipino language especially in spelling using words repitition or reduplication of words. It will also provide additional knowledge in the syllable and inflection of words as part of developing spelling rules. The data came from selected Tabloids and deliberately recorded words with repition. The words are classified and the misspellings are identified which leads to deviation from the usual rules written in the guides or manuals of Filipino orthography published by KWF and even SWF-Diliman, From this we will identify the styles or methods of writing as well as the reason to know the origin of the spelling type using reduplication. Despite the modernation of the era that influenced the Filipino language, it can still be seen that rules written in Filipino grammar books are still valued, but they have been fully followed due to the many changes. The usual rules are forcibly maintained and used but there are times when change takes place and the will of the majority is what the setting does. Thus there are two types of use of each of the rules stated in the books to cover current changes especially in reputation using the word borrowed. Based on this analysis, the implications that may help to solve the problem of spelling confusion in the modernization of Filipino orthography have been discovered in this analysis. This may serve as a guide in evaluating the writtenrules in the grammar books. Also the discovery of a few changes and inconsistencies in the rules to be followed may pave the way for a change and general rule in the use of repetition or reduplication based on the current use of the Filipino language.

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INTRODUCTION

Language is one of the most important tools in the life of any creature in the world. Because of language, it is possible to achieve peace, unity, understanding and love of every person in this world. On the other hand, language can be a source of confusion and disunity due to its misuse and irresponsible use.

Therefore, because of the language, the development of activities in all fields has become very fast, man has recorded his past, various thoughts and wisdom for the benefit of his fellow man, man can express his thoughts to those events seen in the society in which he lives. Most of all, people can see the trend and change of language that people use in a particular place or society.

According to Barker and Barker (1993), language connects the past, the present and the future. It also preserves our culture and traditions. He added that the older generation may be lost, but through language, they are still able to communicate their ideas, successes, failures, and even their future plans or aspirations. Through this, future and subsequent generations learn or can learn from past experience and thus avoid re-mistakes or otherwise correct or correct past mistakes and add any shortcomings that need to be added in its development and may it be a solution to the emerging new grammatical issues.

In the Filipino language, even if there are rules or regulations that linguists follow or agree on, it is inevitable that there will be changes especially in the grammatical aspect. One of these changes is the inevitable expansion of words into vocabulary, meaning and use of words. This is just proof that the Filipino language, like other languages, is alive. Let us not lose sight of the fact that language is dynamic, changing, increasing and cultivating. Along with change, change is not harmful if you think about it. This change reflects how lofty and vibrant our language is today. It just shows how healthy and active our language is. One of the characteristics of language is its continuous growth or development over time according to Semorlan (2010). He added that language is alive so it is only natural for it to change, expand and grow.

According to Santiago and Tiangco (1991), words have four structures; such as simple, simple, repeated and compound where each of them has its own - its rules how to use it in the right way according to human needs. But there are times when an object or equipment that is already used but over time needs to be replaced or changed not just because it has just been discovered to be wrong but because of the need of time and opportunity. This is why new forms and unrecorded words have come out that can cause confusion nowadays, but it can also be a way to solve the needs of the ongoing development of our language.

The father of Paraluman Santos-Aspillera (1938) said of the Grammar of the National Language that he wrote before he passed away "That Grammar is not God-made. After about ten years of teaching, you can see what needs to be changed and in that book. " As in the case of the word repeated or reduplication. According to the Grammar book, the root word and the word with the suffix may be repeated completely or incompletely according to its principle. But when you look at what is written in the news and articles of the selected tabloids, it repeats the words that are repeated if looked at carefully contrary to the principles stated in Lope K. Santos' book, such as homosexual, borrowing, contagious, deadly etc. This is probably a change in the present in the part of Filipino grammar that can be considered uncontrollable. Many users do not follow grammatical rules but understand each other and this is true in our society.

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It now poses a challenge to researchers and advocates of the Filipino language to open this topic in many studies to correctly analyze spelling using the reduplication in the orthography of the Filipino language and provide definite steps to clarify the issue facing orthography Filipino. It also serves as the focus of this paper as the beginning of the analysis of the modernization of the Filipino language.

Presenting a Problem

This paper attempts to clarify some issues regarding the orthography of the Filipino language especially in spelling using words with repetition or reduplication of words. It will also provide additional knowledge on the syllable and inflection of words as part of developing spelling rules.

In order to carry them out, it is also good to answer the issues raised on this issue. Here are some important questions to answer in this review.

1. How are the traditional rules written in grammar books about spelling words with repetition or reduplication found in selected Tabloids valued?
2. In the analysis of words with reduplication or repetition found in selected Tabloids, how can it help solve the problem of spelling confusion in the modernization of Filipino orthography?

Subject Environment

Repetition of words or reduplication that is often used where there is confusion in the way it is used or written can still be considered an issue in the field of Filipino grammar. It has been a challenge for researchers to focus on the issue. By discussing the current trend of its use in the Tabloids it may bring a positive reception to consumers. It is also good to start this analysis before starting this paper on the terms and clarifications of some linguists of the country.

In a lecture by Dr. Aurora E. Batnag in 2015 on Emerging Grammar in Filipino, she mentioned and focused on the issue of spelling of words with repetition or reduplication that has a new spelling. It is believed to deviate from traditional grammatical rules and develop vague rules which in turn cause confusion for many. According to him, there are those who say that there are new grammatical changes that have not been recorded in grammar books or that have not yet been evaluated and this was later called emerging grammar by some. Some observations on the trends of language changes.

It just keeps changing a living language and he emphasizes that, What is wrong today can be acceptable tomorrow. So language guards should have nothing to fear. We need to be open-minded to change. Lope K. Santos says, the (mouth of the people) has the final decision, embarrassing or embarrassing? It has long been debated which should be repeated, the root word or the suffix? The problem was resolved in 1987 with the new alphabet and spelling rules issued by the then Philippine Language Institute (KWF) through the then DECS, the problem was solved by accepting two forms: both correct the embarrassing and the embarrassing. But it is not explained why both forms are correct.

According to the rule in grammar books, in the conjugation of the verb, the first syllable of the root word is repeated. To show that the rule is wrong, the ironing is given as an example. The first syllable is a plan, then, to be a planner-without any users. This was later corrected: what is repeated is the first consonant and the first vowel of the root word so the whole word will be like this.

The word embarrassing / embarrassing:
Root word: shame
Word base: shame
Suffix: ma-

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If we look at this analysis, what is repeated is not the root word but the first and first vowel (first K and first P) of the base which is also called the word stem and not the root word. But the use of some ordinary people who do not consider grammatical rules becomes embarrassing rather than embarrassing.

It used to be taught in old grammar books that suffixes are ma-, mag-, maka-, maki-, makapag-, and so on. In modern terms they are divided: ma- + ka- + pag- + and only the last syllable is considered verbal finance.

When this is the case, the scope of the rule will be wider and we will see other examples enter the rule.

fighting, and not fighting
using, and not using
departing, not departing

However, the repetition of the first K and the first P of the root word is widely used especially in written communication. This is the observation of the words attached to na- + ka - +, both of which are used in two forms:
embarrassing - embarrassing
sad - sad
frustrating - frustrating
senior - senior

But because it is probably rare and 'not used', only one form has been observed; such as the word penetrating (nothing intrusive) and disgusting (nothing disgusting) when something like this is observed.

Can be sent can be sent
Explained explained
Expressable expressive

There are two forms, then, when pa- in the word base. In the case of (assumed) and (assumed) it can be said that there is a difference in meaning between the two words: assumed (opinion); assumed (take something somewhere). But for most users, the two have no distinction. This is exactly what happens in a modernized language - the scope of a single word becomes broader.
It will also be noticed that many borrowed words enter the Filipino vocabulary which further expands its use. The need for the Filipino language to borrow from the surrounding languages has become a reality and it revolves around the society in which it operates such as English and Spanish, vernaculars and others. They respond to widespread stress

The Tabloid
The tabloids come from a name given by a London pharmacy or pharmaceutical company BURROUGHS WELLCOME & COMPANY on a miniature tablet they sold called taboid pills in the late 1880s. Such a name was later used in things smaller or smaller than the usual. An item published in 1902 in the London's West Minister Gazette was recorded. This is what the owner wants to use the tabloid method in all the news published by other journals. So tabloid journalism in 1901 means a paper containing stories / stories that are simplified and easily understood by the readers. The term was also used as a reference in small newspapers containing short stories in 1918.

Tabloids or tabloid newspapers have different meanings. Technically, it is a type of newspaper measuring 11x17 inches, which is smaller and shorter than a broadsheet newspaper. On the one hand, it is a newspaper whose focus is on revealing amazing stories about artists, crime and more.

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Currently the word tabloid means weekly news release in a shorter format. The tabloid method of publishing news contains important and honest (unambiguous) news and is often preferred by the reader especially the people on the go or commuters because they are often short of space. This newspaper is different from large newspapers because it provides an alternative perspective because the editor has extensive knowledge of the local scene or setting.

Theoretical Basis

Grammatical theories of Prescriptive, Descriptive and Functional grammar form the basis of this paper. In order to fully clarify and articulate the concepts surrounding the situation occurring in repeated words some grammatical theories have been used to construct them.

The study first considered the PRESCRIPTIVE GRAMMAR theory which gives value to established rules related to grammar. It emphasizes the accuracy and appropriateness of following the rules and grammatical rules of language use. In this study, reference and basis were used for customary rules related to repeated words. The search for the issue of repetitive words - whether complete or imperfect in selected tabloids and other grammar books, began.

Apart from the said theory, DESCRIPTIVE GRAMMAR can also be very helpful in clarifying the current situation of repeated words. Based on the theory of descriptive grammar, it is up to the person using the language how to use it correctly and appropriately for the opportunity to interact with society because it considers the grammar that depends on the person who uses it correctly and acceptable.

The PRESCRIPTIVE GRAMMAR theory, on the other hand, contradicts the previous theory because it follows the standard of proper use of grammar and when it achieves a high level of accuracy rather than accepting what is customary.

Apart from the previous two theories, this study also gave consideration to FUNCTIONAL GRAMMAR, which is a universal theory of indigenous languages developed by Simon C. Dik, et al. This theory is based on the data and descriptions of other languages so it maintains a high degree of accuracy. FG offers policy or guidelines to theoretical linguists who plan representation, formalism and descriptive linguists in data analysis.
More about theoretical

The Conceptual Basis

This paper focuses on discussing the rule of words with repetition or reduplication in the Filipino language. Emphasis will be placed on repetition of consonant and vowel or consonant clusters (words). With the modernization of language, it is inevitable that any domain will also discuss its development and one of these is the spelling part of grammar. The use of the rule in repetition or reduplication has become a source of confusion, especially when using borrowed and lapi words. In order to fix this, the Filipino language authority has outlined the proper Terms of use so that the reason can be stated correctly and it will have spelling abnormalities and will not be buried in confusion.

But as time progresses and technological change enters the country particularly in the field of journalism using the Tabloids that are adapted to the Filipino language. With the survival of the Filipino language, its many uses evolved in various ways of repeating or reduplication of the use of words. As a result, there are many changes in the field of spelling to adapt to the norms of society and the field of speech whether written or oral.
There was a new way and style of expressing words with reduplication in the field of journalism using the Tabloids. Due to the over-simplicity of the news makers, it is getting new to the spelling of the Filipino language using modern words that can develop or break the orthography already set in the books.
To better understand the concept mentioned, it is best to look at it in the developed paradigm scheme shown below.

The picture shows the concept followed in this study. At the center of the study are the Tabloids who hold the review. From this there is an arrow focused on the grammatical rule of reduplication and spelling that shows what the rule follows in writing or spelling words and long arrow directly focused on the spelling method placed in a heart shape. The heart-shaped symbol is used because the spelling or writing still follows the grammatical rule that what is pronounced is the spelling which in fact can be said to be in the heart or the writer's decision what he will follow in the rules.

Issue Analysis

In this part of the study the results generated from the collected data will be reviewed and discussed. The data came from selected Tabloids and deliberately recorded words with repetition. The words are classified and the misspellings are identified which leads to a deviation from the usual rules written in the guides or manuals of Filipino orthography published by KWF and even SWF-Diliman. From this we will identify the styles or methods of writing as well as the reason to know the origin of the spelling type using reduplication.

Recorded over a hundred words that appear repetitively used in news articles in select tabloids. Words are classified according to the form of reduplication or repetition. Word analysis considers the rule of repetition specifically in the repetition of the first K and first P of the root word and word based. The review is based on the rules stated in the KWF and SWF-Diliman manual. That there is less conflict between the two or so inherent change.

The rule issued by KWF states that words will be repeated when: (1) the word begins with a vowel and only the vowel is repeated like the word /a.ak.yat/ (ascending). And (2) when the word starts with the KP structure, only the first syllable is repeated like the word /la.la.kad/ (walk). This rule when applied to the tabloid news articles reviewed shows that they are almost compliant but with words like this.

Table 1 shows the words that have repetitions that are not commonly used. Note that the words that have been used correctly in accordance with the set rule will have a repetition of the first syllable of the root word such as words that can be solved and watched. It both agrees with the rule stated in the repetition but it becomes new to the taste of other readers as it becomes unused or becomes unconventional. In the word solver it is common to use solver that has a repetition in the word base and not in the root word it contains and also in other words in the table.

In a word that is uplifting and helpful, it will also be noticed that repetition occurs in its word base which also becomes deviated from the usual word formation. Repetition of its root word, which is uplifting and helpful, is considered a commonly used word. The occurrence of the word mentioned has the same condition as in the other examples.

Such a scenario of repetition of words has become a strange looking incident. In this repetition of words can be asked to any user who, what should be more appropriate? If we go back to the terms issued, it can be said that it has been clarified in terms of terms such as SWF-Diliman stating that either of the two can be used and only select the most appropriate method according to your needs. And in the KWF rule it is clearly stated that repetition occurs only in the first vowel and first syllable of words that both refer to the root word.

Here there is confusion as to who is more authoritative in setting the rules. It will be remembered according to Dr. It has been clarified before, but it is still up to the user to decide what he intends to use so now there is a rule that both forms of repetition are acceptable as long as the meaning is not changed. KWF, the proponent of the Filipino language, also cannot ignore this scenario of language change. It can be made a clear rule to be the basis for having a solution to the confusion of repetition in the root word or word based which to this day still does not reveal the true order that is still based on the user's wishes of language.

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Table 2 shows words with repetition or reduplication of borrowed words using -ka-. It will be noted in the recorded words that there is a repetition of words in its word base that is -ka- and not in the root word which is set by the grammatical rule specifically in the KWF manual released. And based on the words obtained in the Tabloids used it can be said that they prefer the repetition of -ka- in the sample words. In fact, such a scenario becomes inappropriate but this is where Dr. Who knows who should prevail, the usual rule or the current flow of language change.

Repetition of the word using -ka- has become a grammatical issue that is still a mystery in society. It will be remembered that its rule based on tradition, has a repetition in the first syllable of the root word and not the second syllable of the prefix maka- or naka- is repeated in the formation of the verb in the contemplative or progressive aspect (in the case of maka -) or adjective (in the case of already). It is noteworthy that the repetition of the second syllable of the prefix or ng -ka- (SWF-Diliman, 2008) is also widespread today. And in order to correct the confusion here, the two forms of repetition that can occur in the first syllable of the root word and the second syllable of the prefix maka- or naka- are also considered to be both correct without giving the correct explanation to consistency correct.

With the introduction of borrowed words, the process of this rule has become more complicated just like the word kaka-video which if followed in terms of proper repetition in the root word will be like this form of repetition kavi-video as well with words like kaka-picture - kapi-picture, kaka-live in - kali-live in, nakaka-text - nakate-text, and makapag-move on - makapagmo-move on.

Changes that occur in words such as kaka-air instead of kai-air will also be noticed if the correct rule is followed. Also in words that are striking, insecure, scheduling, and interesting that will be stressful, insecure, scheduling, and interesting if followed in the prescribed repetition rule written in grammar books. It can also be seen in these words that the root word of a vowel also begins with a vowel the repetition that takes place.

These words may be correct and follow the rules stated in the books but it can be said that some do not like it because for some especially young people it is more acceptable to use the repetition of -ka- because of its consistency in spelling of borrowed words that are joined by maka or naka that have repetition kaka. Based on this analysis, it can be said that the field of tabloid news is also in line with the current state of the Filipino language change and speaks according to the Filipino masses. So with regard to this style of repetition, it can be said that the preference of the user of the language should be the one to be followed but it must be supported by the rules that he himself must have an adequate explanation for all the changes.

Table 3 shows the repetition of borrowed words and words with a double consonant. It is noticeable that they have a repetition system based on how it is used in the Tabloid. Also noteworthy is the widespread entry of borrowed words in the field of tabloid news where there is a new dress of development in the Filipino language. It will also be noticed that most of the words that have repetitions used are those that are based on the English language. And the pro-Filipino spelling method is used to repeat words. So it can be said that the Tabloids contain the words currently spoken by the people, although it is painful to accept the fact that the English language has a great influence even in changing the Tabloids.

It will also be noted that they follow the set rules of repetition using borrowed words. According to the usual rule that when there is a repetition the will happen in the first syllable of the root word. In the case of borrowed words listed in table 3 they can be seen following the usual rules of repeating the first syllable of the root word such as words to manipulate, tap, admire, push, male-late, resign, link, bond, suggest, decide and patent. But it will also be noted that what follows is the first sound of the first syllable of the root words. This change should be stated in the grammatical rules of the Filipino language that when a borrowed word is repeated it will make the first syllable sound so that there will be no confusion in the first syllable just for example in the words mentioned.

Also in this table can be seen the repetitions of borrowed words with primary consonants such as words promote, shoot-shoot, bless, prioritize and gym. The form of these words can be said to be wrong if we follow the basic rule in repeating the first syllables of the root word, which is also referred to by Dr. Batnag with something wrong like the words promote, shoot-shoot, bless, prioritize and go to the gym. The forms they should follow according to the stated rules are as follows: promote, shoot, bless, prioritize and go to the gym but if examined carefully it does not conform to the rules listed in the grammar book because the first syllable is incorrect and does not sound like the first syllable of the root word.

The current KWF manual identifies this error and corrects it by stating that the repetition of two consonants and vowels can be considered as similar repetitions of the words mentioned above to promote, promote- shooting, nable-bless, prioritize and go to the gym. These words are also considered right or wrong today. These two methods of repetition of borrowed words can be used but in reality it is still up to the user of the language what spelling of repetition of the borrowed word he will prefer. And there is still a hole of error and even change the rule issued. The rule may have solved the confusion on this issue but there is no definitive explanation as to why they have become the same.

Table 4 shows the words read in the selected Tabloids that can be said to have misused words. Words starstruck, apply, early, extend and expect will show a lack of information in the terms set out here. It may be that the user has not been open to the rule but these words may also have become commonplace to the user. They can also be seen as informal types of writing because they are used more in the form of speech than in writing.

The scenario of this change of repetition of words especially in borrowed words may be the way to come up with another rule that will determine its proper use. This type of word repetition is characterized by loosening of set rules and sometimes lack of control over words. This is just proof that the grammatical rule also changes according to the use of language in its current state. It can be said that the user of the language still prevails over what is said in the grammar books. So it can be said that the spelling of Filipino orthography is still true in the spelling of Filipino orthography, especially in the spelling of What is pronounced is the spelling.

Findings

Based on the analysis of words with repeated repetition or reduplication found in selected Tabloids, the following were found:

1. It was found that the words with repetitions found in the news articles of the selected Tabloids almost complied with the rules set by the books on Filipino grammar but there were words that showed non-compliance with the rules and causes confusion.

2. It is found that there are words that contain repetition that are not customary to use. It will be noted that the words that have been used correctly in accordance with the rule set there is a repetition in the first syllable of the root word and repetition in the word base and not in the root word that contains the word which also deviates from the usual word formation. And it becomes new to the taste of other readers as it becomes unused or becomes unfamiliar.

3. There is also the discovery that there are words with repetition using -ka- in words borrowed from its word base and not from the root word that is in the rules in the grammar book. Although correct and in accordance with the rules stated in the books but it can be said that some do not like it because it is more acceptable to use the repetition of -ka- because of its consistency in spelling the borrowed words used by maka or naka having recurrence kaka. It can be said that the field of tabloid reporting also adapts to the current state of the Filipino language change and speaks according to the wishes of the majority.

4. Also found out that there are ongoing repetitions of borrowed words and the repetition of the first double consonant. It is noteworthy that borrowed English words are widely introduced in the field of tabloid news where

there is a new form of development in the Filipino language. It will also be noted that they follow the set rules of repetition using borrowed words in the first syllable of the root word but also note that it follows the first sound of the first syllable of the root words. This does not agree with the rule recorded in the grammar books because the first syllable is incorrect and does not sound like the first syllable of the root word.

5. But at present this error is acknowledged and corrected by the revelation that the repetition of two consonants and vowels can be considered variant. These two methods of repetition of borrowed words can be used but in reality it is still up to the user of the language what spelling of repetition of the borrowed word he will prefer.

6. It was also discovered that borrowed words with repetition that are added to the word base can be found in selected Tabloids and it can be said that they have the wrong application. This can be seen in the lack of information on the terms set out here and the prevalence of language users. They can also be seen as informal types of writing because they are used more in the form of speech than in writing.

CONCLUSION

From the above findings the following general implications for the analysis are formed:

Despite the modernization of the time that influenced the Filipino language, it can still be seen that the rules written in the books of Filipino grammar are still valued but they have not been fully complied with due to the many changes. The usual rules are forcibly maintained and used but there are times when change takes place and the will of the majority is what the setting does. Thus there are two types of use in each of the rules stated in the books to cover current changes especially in repetitions using the word borrowed.

Based on this analysis, the implications that may help to solve the problem of spelling confusion in the modernization of Filipino orthography have been found in this analysis. This may serve as a guide in evaluating the written rules in the grammar books. Also the discovery of a few changes and inconsistencies in the rules to be followed may pave the way for a change and general rule in the use of repetition or reduplication based on the current use of the Filipino language.

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Table 1.Some selected words have repetitions that are not commonly used.

Tabloid words	Method of repetition (root / word base)	Form of repetition commonly used
makalulutas	salitang-ugat	makakalutas
nakalulungkot	salitang-ugat	nakakalungkot
makasasagot	salitang-ugat	nakakasagot
nakatatawag	salitang-ugat	nakakatawag
napapakialaman	word base	napakikialaman
napanonood	salitang-ugat	napapanood
nakakaangat	word base	nakaaangat
maipalalabas	salitang-ugat	maipapalabas
makakatulong	word base	makatutulong
nakakukuha	salitang-ugat	nakakakuha
nakaloloka	salitang-ugat	nakakaloka
makasasama	salitang-ugat	makakasama

Table 2.Words with repetition using -ka- in borrowed words

Tabloid words	Method of repetition (root / word base)	Forms of word repetition based on grammatical rules
kaka-video	word base	kavi-video
kaka-love team	word base	kala-love team
makakapag-move on	word base	makapagmo-move on
kaka-picture	word base	kapi-picture
kaka-live in	word base	kali-live in
nakaka-text	word base	nakate-text
nakaka-striking	word base	nakai-istriking
nakaka-insecure	word base	nakai-insecure
kaka-air	word base	kai-air
nakakapag-schedule	word base	Nakapag-i-schedule
nagkaka-interest	word base	nagkai-interest

Table 3.Repetition of borrowed words and primary double consonants.

Tabloid words	Method of repetition (root / word base)	Form of word based repetition in grammatical terms
magpro-promote	salitang-ugat	magpro-promote/magpo-promote
magsho-shooting	salitang-ugat	magsho-shooting/magso-shooting
nabe-bless	salitang-ugat	nabe-bless/nable-bless
magpa-priority	salitang-ugat	magpa-priority/ magpri-priority
nagdi-gym	salitang-ugat	nagdi-gym/nagdyi-gym
magma-manipulate	salitang-ugat	magma-manipulate
magti-taping	salitang-ugat	magti-taping
nagma-mature	salitang-ugat	nagma-mature
naa-admire	salitang-ugat	naa-admire
maipo-push	salitang-ugat	maipo-push
male-late	salitang-ugat	male-late
magre-resign	salitang-ugat	magre-resign
nagre-recover	salitang-ugat	nagre-recover

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nali-link	salitang-ugat	nali-link
magre-renew	salitang-ugat	magre-renew
nagba-bonding	salitang-ugat	nagba-bonding
nagsa-suggest	salitang-ugat	nagsa-suggest
nagde-decide	salitang-ugat	nagde-decide
napa-patent	salitang-ugat	napa-patent

Table 4.**Borrowed words with repetition added to the word base.**

Tabloid words	Form of word based repetition In Filipino grammar
nagi-starstruck	nag-i-starstruck
maga-apply	mag-a-apply
nagi-early	nag-i-early
nagi-extend	nag-i-extend
magi-expect	mag-i-expect
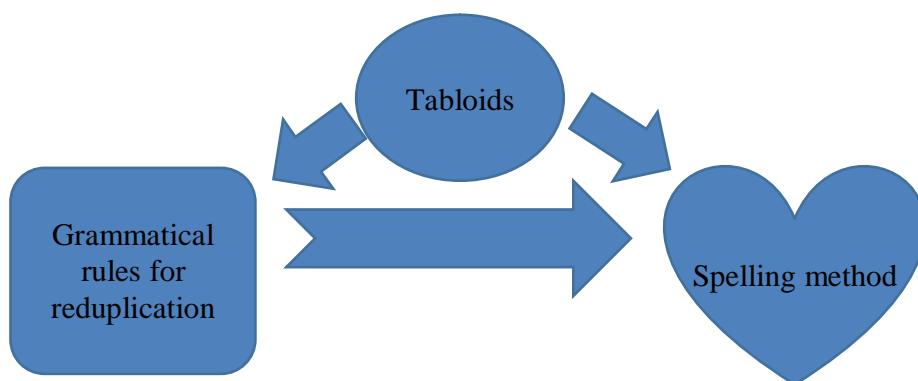
Fig. 1.The Scheme of the Conceptual Paradigm of analysis.

RESEARCH ARTICLE

Parallel Operation of Resonant Converter for Renewable Energy Source Applications

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ABSTRACT

In this article proposes the parallel operation of converters for renewable energy source applications in datacenters**.** A new control approach is proposed for switch controlled capacitor LLC converter. The output voltage regulation is achieved by the switching frequency control. It can give the good frequency variation range and peak gain range compared to conventional converters. To attain load sharing the half wave switch controlled capacitor is used to control the resonant frequency of each LLC stage. The simulation results are compared with experimental results. A 600w prototype model is developed to prove the feasibility.

Keywords: Resonant converter, frequency control, zero voltage switching (ZVS), zero current switching (ZCS).

INTRODUCTION

The switch controlled capacitor (SCC)-LLC converter is proposed in this paper. The resonant frequency is controlled by using switch controlled capacitor. Because of this, the regulation of output voltage is attained in constant switching frequency control. This gives good solution for phase shedding of each converter, load sharing of converters by the parallel operation, and interleaving operation of converters. But some limitations there in fixed switching frequency operation of resonant converters; they are variation range in load current and input voltage compared with the traditional converters in switching frequency control. The new control strategy for switch controlled capacitor (SCC) resonant converter is focused in this chapter. This control method has achieved the interleaving operation of converters at the same time holds the benefits of variable switching frequency control. This article discusses about the following points.(i).compares the modulation between switching frequency and resonant

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frequency. (ii).proposes the new control technique and analysis and design of that control technique.(iii).compares the practical results with theoretical and simulation results.(iv).summary.

Comparison between the Modulations of Switching Frequency with Resonant Frequency

The various literatures discuss about the constant switching frequency operated resonant converter. That technique is used to solve the load sharing problem and interleaving operation. Therefore that control technique is used in high current applications with higher efficiency. The constant switching frequency LLC converters present the different characteristics from traditional converters due to it uses resonant frequency modulation in it is place of switching frequency modulation. The comparison between the modulations of switching frequency with resonant frequency is as follows. The equation (1) shows the voltage gain of LLC resonant converter derived from The Fundamental Harmonic Approximation (FHA) method.

$$M = \frac{K}{\sqrt{\left[\left(\frac{\omega_r}{\omega_z}\right)^2 - K - 1\right]^2 + \frac{\pi^4 \omega^2 L_p^2}{64 N^4 R_L^2}\left[\left(\frac{\omega_r}{\omega_z}\right)^2 - 1\right]^2}} \tag{1}$$

$$M = \frac{N V_0}{V_{in}}, \tag{2}$$

$$N = \frac{N_p}{N_z}, \tag{3}$$

$$K = \frac{L_p}{L_z}, \tag{4}$$

$$\omega_s = 2\pi f_s, \tag{5}$$

$$\omega_r = \frac{1}{\sqrt{L_r C_r}} \tag{6}$$

Where

$\omega_r = $ The resonant frequency in radians.

ω_s = The switching frequency in radians

R_L = The Load resistance

K = The load resistance

L_p = The parallel inductance

N = The transformer turns ratio

M = Resonant tank gain

The gain curves resulted from resonant frequency modulation (FrM) and switching modulation (FsM) are plotted using the equation (1) with set of values of N, R_L, K,L_P, respectively as depicted in the figure .1. The resonant frequency modulation (FrM) curve obtained when the switching frequency (ω_s) is fixed and resonant frequency (ω_r) is varied. The normalized frequency in resonant frequency modulation is defined as the ratio between the resonant frequency to the switching frequency (ω_r/ω_s).The switching frequency modulation (FsM) curve drawn when resonant frequency (ω_r) is constant and the switching frequency (ω_s) is varied. The figure 1 shows the Normalized frequency versus gain of resonant frequency and switching frequency modulations. The switching frequency modulation gives higher efficiency compared with the resonant frequency modulation. Resonant frequency modulation has less effective in impedance point of view compared to the switching frequency modulation. Two MOSFET'S with gate driver circuits are used to control the SCC, which increases the conduction loss and complicated circuit. Therefore a half wave SCC can be utilized, this is more attractive. The next section discuss about the half wave SCC.

Switching Frequency Controlled LLC Converters
SCC – LLC Converter with Switching Frequency Control

The interleaving operation of SCC-LLC converters are connected in parallel, the switch frequency of all phases must be same. And at the same time the switching frequency modulation has more helpful than resonant frequency modulation. So the proposed the innovative control technique is in the subsequent paragraphs.

(i).All SCC-LLC converter phase output voltage controlled by the switching frequency and all SCC-LLC phases operates same switching frequency but its variable based on the output voltage requirements.
(ii).The output current ripple cancellation is done, by applying interleaved gate driving pulse to each phase of SCC-LLC converters.
(iii).The load current sharing is obtained by SCC control method, because of the component toleranceThe figure 2 shows the improved topology. This new topology is suitable for interleaving operation of SCC-LLC converters.

Comparison between Half Wave and Full Wave Switch Controlled Capacitor (SCC)

The half wave switch controlled capacitor operating waveform are shown in the figure.3.Here the control angle (α) is varied from 0 to π and there is only one MOSFET, but the control method is same as in the full wave switch controlled capacitor.

The switch controlled capacitor MOSFET is turn on when the Capacitor (Ca) voltage discharging and reaches to zero for diminish the power loss of MOSFET's body diode. The *Ca*voltage is fully discharging and reaches to zero, then immediately the SCC MOSFET is ON. The MOSFET acts like a synchronous rectifier from the capacitor (Ca) voltage zero crossing point to the resonant current zero crossing point. This is not affecting the modulation of equivalent capacitance. The expression (7) shows the equivalent capacitance value of half wave SCC, and that expression is derived based on fundamental harmonic estimation method.

$$C_{SC,HW} = \frac{2C_a}{2-(2\alpha - sin2\alpha)/\pi} \quad (7)$$

The two extreme conditions to design the SCC equivalent capacitance are,
 (i). SCC switches always ON,
(ii). SCC switches always OFF.
The equivalent capacitance value is large when the SCC switches ON. And the equivalent capacitance value is equal to (C_a) when the SCC switches OFF. Hence other conditions do not affect the overall design accuracy.

Design and Analysis of Variable Switching Frequency Controlled Resonant Converter

In this resonant converter, the voltage output is regulated by the variable switching frequency; hence the design process is similar to the traditional resonant converters, the only difference is the unknown value of SCC capacitance (C_a).The current sharing performances of parallel connected converters are discussed in this section, and design procedure for selecting the SCC capacitance (C_a) corresponding to the circuit element tolerance values.

Performance of Load Sharing

The resonant frequency in each phase of LLC converters are slightly varied because of circuit element tolerance value, resulting various switching frequency versus output current curves, the figure 4 shows simulated characteristic curve between the switching frequency and output current with circuit elements tolerance values. The following detail gives the specification and tolerance values of circuit elements related to the figure 4.The theoretical and simulation peak output current is equal to the resonant tank maximum capacity. The figure. 4. represents the output current (A) versus switching frequency (kHz).In this figure the center curve indicates the relationship between output current and switching frequency when the circuit elements with no tolerance. The left corner curve indicates the relationship between output current and switching frequency when the circuit elements with maximum tolerance value. The right corner curve indicates the relationship between output current and switching frequency when the circuit elements with minimum tolerance value. This graph indicates the performances of two converters are connected in parallel. When the switching frequency 170 kHz, the first resonant LLC converter output current is 50A, the second converter output is 0A.
The SCC can be used to decrease the value of equivalent resonance capacitance of less current output phase, for attain load sharing, Hence the lower phase output current will rises and equal the greater output current phase. The

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important character of the SCC is mainly rise the resonant frequency and not decreases it. So, the greater resonant frequency phase automatically acts as reference phase in interleaving operation of converters. The SCC act as a compensator for all the lesser resonant frequency phases to equalize the reference phase. The load sharing is most terrible when the converters in the two extreme tolerance conditions. At this situation, the left corner performance curve must be motivated towards the right corner performance curve still the two performance curves are in line. The figure 5 shows the compensated performance curves for the input voltage 400V, output voltage as a 12V and turns ratio of transformer is 20:1. The load sharing performance of converters from no load to full load is obtained by reducing the resonant capacitance value. Further the resonant capacitance value is approximately constant. In this pattern, the 19% decreases of C_s value gives the most excellent match at higher loads;18 % decreases of C_s value gives the close match of the output current and 17% decreases of C_s value results in lesser output current than the higher resonant frequency phase. The 19% decreases of C_s value is the most horrible case of SCC design, this is known from this example.

EXPERIMENTAL RESULTS

The feasibility and the advantages of the proposed control strategy are verified using practical model of a 600Watts two phase interleaved variable switching frequency half wave switch controlled capacitor (SCC)- LLC converter. The specification details are listed in the table 2. In order to test the load sharing performance of the converter, resonant inductors are implemented with the transformers leakage inductance, and are purposely made non identical. The digital controller is implemented using a microchip DSCdsPIC33FJ32GS606.Load current (A) versus efficiency (η %) with phase shedding and without phaseshedding of LLC converter are shown in the fig.4.4.The 25A (50%) load efficiency is improved from 94% to above 95.5%, and the 5A (10%) load efficiency is improved from 81% to 90%. When the load current is below 50%, one phase is shut down.

CONCLUSION

A new control approach is proposed for switch controlled capacitor LLC converter. The output voltage regulation is achieved by the switching frequency control. It can give the good frequency variation range and peak gain range compared to conventional converters. To attain load sharing the half wave switch controlled capacitor is used to control the resonant frequency of each LLC stage. The advantages of half wave switch controlled capacitor are less cost, less conduction loss, and simple circuit. The switch controlled capacitor LLC converter load sharing characteristics are studied. To determine the optimal switch controlled capacitor value a visual assist design method is proposed. A two phase interleaved switch controlled capacitor LLC converter with power rating of 600watts prototype is developed. It shows the efficiency improvement in light load, cancellation of current ripples, and good load sharing performance. The proposed converter is suitable for renewable energy source applications.

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Table 1. Specification and tolerance's of circuit elements

Transformer turns ratio	20:1
Parallel inductor(L_p)	± 7%(87µH nominal)
Capacitor (Cp)	± 5%
Series capacitor(Ls)	50nF
Output voltage	12V
Input voltage	395V
Resonant inductor(Rs)	± 7%(12µH nominal)

Table 2.Specification details of experimental model

SR MOSFET	BSC011N03LS
Switch controlled capacitor MOSFET	BSC060N10NS3G(100V,6mΩ)
Full bridge MOSFET	IPB60R190C6
Output capacitance	1800µF
Switch controlled capacitor	160nF ± 5%
Series capacitors	38nF±5%
Resonant inductance	12µH(phase I),14µH(phase II)
Magnetizing inductance	87µH(phase I),85µH(phase II)
Turns ratio of transformer	18:1,Centre tapped
Output power	550Watts,(225Watts per phase)
Output voltage	12V
Input voltage	430V Maximum/330V Minimum
Switching frequency	Variable switching frequency around 200kHz

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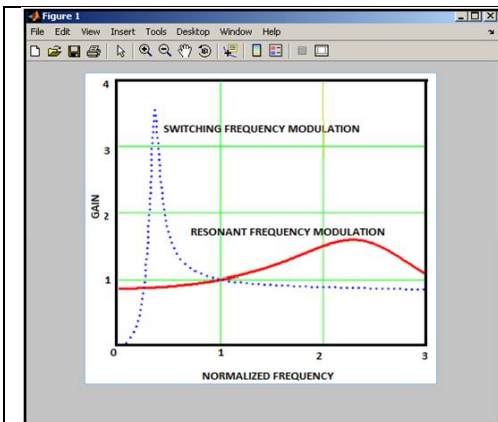
Fig.1.Gain versus normalized frequency with resonant frequency and switching frequency modulations
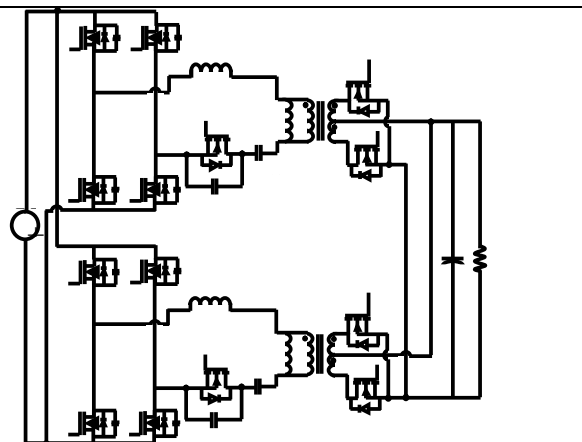

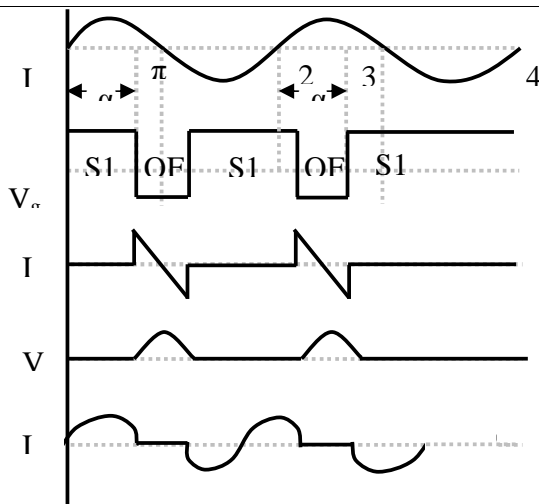**Fig .2. Variable switching frequency control strategy for interleaved SCC – LLC converter.**

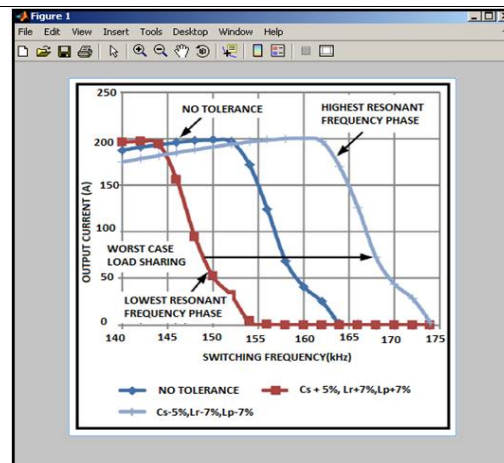**Figure.3.Half wave SCC, Operating wave forms.**

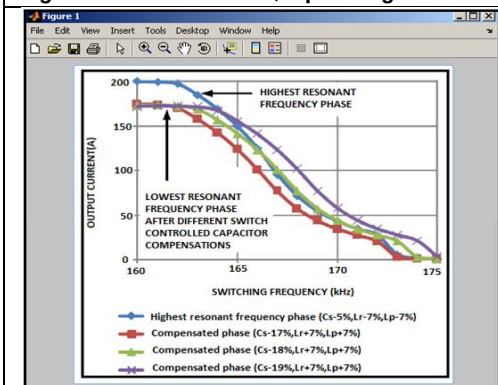**Figure 4. The switching frequency (kHz) versus output current (A) corresponding to table 1**

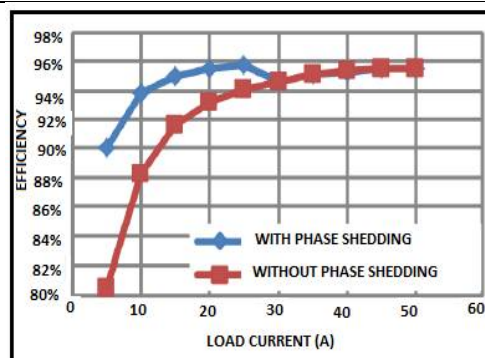**Figure 5 The switching frequency (kHz) versus output current (A) after SCC compensation.**

Fig. 6. Load current (A) versus efficiency (%) with phase shedding and without phase shedding of LLC converter

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RESEARCH ARTICLE

Effect of Sub-lethal Toxicity of Malathion on Respiratory Activity and Haematology of Common Edible Fish *Oreochromis mossambicus* (Peters, 1852)

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ABSTRACT

Aquatic toxicity is relatively a new and still evolving discipline, originating from the concern for the safety, conservation and protection of aquatic environmental and also as an off shoot of toxicology, since degradation of water mass is caused mainly by anthropogenic toxicants dumped in to the water bodies by human activities, directly or indirectly. The present study was attempted to understand the impact of pesticide malathion on the physiological (Respiration) and haematological (Blood) characteristics of common edible freshwater fish *Oreochromis mossambicus.* Effect of malathion toxicity on respiratory activity was studied in *O. mossambicus.* Total Oxygen consumption and rate of Oxygen consumption were measured in *O. mossambicus*, when exposed to two sublethal concentrations both at short duration (24, 48, 72 and 96hrs) and long duration (5, 10 and 15 days). Results of respiratory physiology showed significant decreases in both total Oxygen and rate of Oxygen consumption. Dose-response in these physiological activities was observed, with increasing exposure durations, there were concomitant decreases both in the amount of total Oxygen consumption and rater of Oxygen consumed by the fish *O. mossambicus.* Haematological study showed that there were decreases in RBC, WBC, Haemoglobin and Haemocrit values in fishes treated with two sublethal concentrations of malathion, than the normal fish, where the amounts of ESR, MCH and MCHC were found to be increased. Pesticide toxicity in fish causes diverse effects including behavior (Respiratory activity) and hematological (Blood) changes. It is, therefore, a matter of great environmental and Fisheries Scientists to regularly monitor the pesticide residues in fish foods and humans in order to assess the population exposure to this pesticide.

Keywords: Malathion, Toxicity, *Oreochromis mossambicus,* Respiratory activity, Haematology

INTRODUCTION

Pesticides contamination of surface water from agriculture use is a problem of worldwide importance. Once a pesticide is introduced into the environment there is a reasonable chance that it will eventually finds its way into water. Therefore aquatic systems probably represents one of the most important complex environment as far as describing the fate and behavior of pesticides. Bio accumulation of pesticides in aquatic species is increasing alarmingly, thus posting a threat to aquatic life. Fish forms one of the important target organisms in any aquatic systems, and are one of the major sources of cheap protein for human beings. A variety of fish species show uptake and accumulation of many contaminants such as pesticides (Heath 1987; Gill *et.al.,* 1991). Pesticides have been found to be highly toxic not only to fishes but also to fish food organisms, thus the threatening the life of the fish (Lal *et.al.,*1986; and Zaheer khan and Francis Law 2005).

Malathion (O, O-dimethyl-S1, 2-bis ethoxy carbonyl ethyl phosphorodithionate) is a non-systemic, wide spectrum pesticide. It is widely used throughout the world for agricultural, residential, and public health purposes, mainly to enhance food production and to provide protection from disease vectors. Malathion preference over other organophosphate pesticides relates to its low persistence in the environment as it is highly susceptible to hydrolysis, photolysis and biodegradation. However, numerous malathion poisoning incidents including acute and chronic cases have been reported among pesticide workers and small children through accidental exposure. Pollution due to malathion toxicity needs considerable attention because of harmful effects on beneficial organisms like fishes in aquatic bodies. Even sub-lethal concentration of a toxicant, which lacks the capacity to dramatically increase the rate of mortality in the exposed population, may cause their ecological death after a longtime of exposure, probably as a result of cumulative effect of improved tissues or organs (Jha and Verma, 2002). There are numerous reports are available on the toxicity of different kinds of pesticides on fish biology (Ilavazahan *et.al.,* 2010; Nikam *et.al.,* 2011; Thenmozhi *et.al.,* 2011; Farook Ahamed Mir, 2012; and Shankar murthy *et.al.,* 2013). Even though vast amount of scientific information available on the toxicity effect of malathion on *Oreochromis* in India, limited information is only available on the effect of this pesticide on *Oreochromis mossambicus.* Therefore the present attempt is made on to understand the effect of sub-lethal toxicity of malathion on respiratory activity and haematology of common edible fish *Orechromis mossambicus.*

MATERIALS AND METHODS

Collection and maintenance of test fish

Healthy living specimens of freshwater fish, *Oreochromis mossambicus* weighing 20 ± 1 gm and 10 ± 0.5 cm in length were collected from the ponds in and around Thanjavur, Tamil Nadu, India. They were brought to the laboratory in well aerated containers to avoid hyperactivity- physical injuries and stress. The fishes were screened for any pathological symptoms and washed with 1% $KMNO_4$ solution. The healthy specimens (n=10) were transferred to each glass aquarium (50 x 25 x 25 cm) containing tap water. The fishes were acclimatized to the laboratory conditions (Temperature: 28 ± 2^0C; pH 8.0 ± 0.02 & D.O. 6.2 ± 0.4) for 15 days prior to experimentation. During acclimatization fishes were fed with artificial pellet diet; water was replaced with clean water whenever necessary to remove feces and food remnants. For toxicity studies in *O.mossambicus* an insecticide namely Malathion (C_{10}, H_{19}, O_6, PS_2) was selected. The chemical characteristics of malathion are given in Table 1.

Fishes and Treatments

50 healthy specimens of *O.mossambicus* with uniform body size (weight 20gm and 10.0 cm length) were selected from the rearing aquaria in the laboratory. They were divided equally into three groups of 10 specimens each. The fishes were transferred from the initial acclimation aquaria, to exposure aquaria (74L capacity). Two experimental exposures were executed. The first group acts as a control group. The second and third groups are exposed 10% and 30% sublethal concentration of 96hr LC $_{50}$, respectively for 15 days. Physico-chemical characteristics such as Temp:

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(Mean $\pm$ SD = 28°C $\pm$ 2°C); dissolved oxygen (Mean $\pm$ SD = 6.2 mg/L $\pm$ 0.4); pH (Mean $\pm$ SD = 8.0 $\pm$ 0.02) and photoperiod (12:12 Light: Dark) were maintained during the experimental period and results were recorded.

Effect of sublethal toxicity of malathion on oxygen consumption (Respiratory activity)

A series of rectangular jars, each with (one Liter capacity) dissolved with each sub-lethal concentration were used as aquaria. Only one healthy fish was introduced into each aquarium and a thick layer of coconut oil was spread on the surface of the medium to prevent the fish from reaching to trap atmospheric air. The control aquarium contained one healthy fish without the toxic sublethal concentration. Both control and experimental fishes were allowed to respire for one hour in their respective respiratory chambers (aquaria). After one hour, water samples from respiratory chambers were taken into sample flask of known volume through a siphon system and the dissolved oxygen was estimated. Dissolved O_2 content in water samples collected before and after experiment according to modified Winkler's iodometric method (Welsh and Smith 1953) in control and malathion was exposed at different exposure periods. Average of five fishes were taken per exposure period and the mean values of O_2 consumption both in control and exposed fishes were calculated and the results were tabulated.

Determination of oxygen consumption

Dissolved oxygen consumption by the fishes were measured by the method of Winkler as described by Welsh and Smith (1953) for control and malathion exposed fishes and results were expressed as mg/lit.

Effect of sublethal toxicity of malathion on Haematology

Blood biochemistry test indicates what is happening in the body of fishes exposed to insecticide malathion. All blood parameters were studied according to the procedures described by Dacie and Lewis (1984).

Blood sample collection

Fishes were collected from the experimental aquaria for haemotological studies. Blood samples were collected from sublethal treated individuals at 24h 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 watch glass containing EDTA an anticoagulant. The blood was mixed well with the EDTA solution by using a glass rod and this sample was used for the estimation of following blood parameters.

Blood cell counting

Counts of erythrocytes, leukocytes, and platelets were expressed as concentrations-cells per unit volume of blood. The unit of volume for cell counts originally was expressed as cubic millimeters (mm^3) because of the linear dimensions of the hemocytometer (cell counting) chamber. The International Committee for standardization in Hematology recommends that the unit of volume be the liter. Since 1 mm^3 = 1.00003 μl, the preferred mode of expressing blood cell counts for the examples below is on the right.

Erythrocytes
$5.00 \times 10^6/mm^3$ = $5.00 \times 10^6/\mu l = 5.00 \times 10^{12}/L$
Leukocytes
$7.0 \times 10^3/mm^3$ = $7.0 \times 10^3/\mu l = 7.0 \times 10^9/L$
Platelets
$300 \times 10^3/mm^3$ = $300 \times 10^3/\mu l = 300 \times 10^9/L$

Except for platelet counts and low leukocyte counts, the hemocytometer is no longer used routine blood cell counting in any but the smallest of laboratories. Yet it is still necessary for the technologist to be able to use this method effectively and to know its limitations. Any cell counting procedure includes three steps: dilution of the blood, sampling the diluted suspension into a measured volume, and counting the cells in that volume.

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Erythrocyte Indices

Wintrobe introduced calculations for determining the size, content, and Hb concentration of red cells. These erythrocyte indices have been useful in the morphologic characterization of malathion exposed fishes. They were calculated from the red cell count, hemoglobin concentration, and hematocrit.

Mean cell volume (MCV)

The MCV is the average volume of red cells and was calculated from the hematocrit (Hct; packed cell volume) and the red cell count (RBC). MCV = Hct x 1000/RBC (in millions per μl), expressed in femtoliters or cubic micrometers.

Mean Cell Hemoglobin (MCH)

The MCH is the content (weight) of Hb of the average red cell; it was calculated from the Hb concentration and the red cell amount which was expressed in pictograms.

Mean Cell Hemoglobin Concentration (MCHC)

The MCHC is the average concentration of Hb in a given volume of packed red cells. It was calculated from the Hb concentration and the hematocrit. Indices are determined in the coulter counter model S (p.594) somewhat differently. The MCV was derived from the mean height of the voltage pulses formed during the red cell count, and the Hb was measured by optical density of HiCN. The reference values for the indices was depend on whether they are determined from the centrifuged hematocrit or the coulte model S. The values in normal individuals was similar if both are corrected for trapped plasma. Because of increased trapped plasma in hypochromic anemia's and sickle cell anemia, however, the MCHC calculated from the micro-naemocrit was significantly lower than the MCHC derived from the Coulter Model S. With the coulter Model S, calibrated with correction for trapped plasma, our 95 percent reference intervals for normal adults are: MCV = 80 to 96 fl; MCH = 27 to 33 pg; and MCHC = 32 to 36 g/dl.

Estimation of Haematocrit (Hct)

After adequate mixing of the sample to ensure even distribution and oxygenation of red cells, the hematocrit tube was filled. The tip of the pipette was introduce to the bottom of the tube. As filling proceeds, the tip of the pipette was raised, but it remains under the rising blood meniscus in order to avoid foaming. The level of the blood should be noted and the rubes capped to avoid evaporation during the required centrifugation for 30 minutes at 2500 g. Reading was done without distributing the specimen.

Determination of Haemoglobin (Hb)

Haemoglobin determination is the quickest means for detecting anemia. However, many factors are known to influence the haemoblobin level. The sahlis Hellige method was followed for haemoglobin determination. Sahlis pipette was filled slightly above the 20 mm mark, the pipette was wiped with a filter paper of cotton to remove excess blood and the volume was adjusted to exactly 20 mm^3 by blotting the trip. The blood was expelled into a calibrated (transmission) test tube containing 2 ml of 0.1 N HCl. The pipette was rinsed several times in the acid solution. The sample was allowed to stand for 15 minutes. The principle behind the method is the conversion of haemoglobin to acid haematin. The acid haematin was then diluted with distilled water till colour matched with the colour of the standard in the haemoglobinometer. The height of the column at which the colour match obtained gives the value of haemoglobin in g%. Oxygen carrying of blood was calculated by multiplying the haemoglobin content with 1.25, oxygen combining power of Hb/g (Johansen, 1970).

RESULTS

Toxicity Bioassay (LC$_{50}$ Determination): The 96 hrs LC$_{50}$ values of fish, *Oreochromis mossambicus* was determined in the laboratory studies as 1.02ppm (Table: 2 and 3). In this study, fish were exposed to 0.102 ppm and 0.306ppm sub-lethal concentrations which corresponded to 10% and 30% of the 96hrs LC$_{50}$ respectively.

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Effect of malathion toxicity on respiratory activity of *Oreochromis mossambicus*

Results on the total oxygen consumption and rate of oxygen consumption in *Oreochromis mossambicus* exposed to two sub-lethal concentrations (1/10 and 1/30 of LC_{50}) are presented in Figs. 1 - 4. Total Oxygen consumed (ml of O_2/gm/hrs) by the fish *O. mossambicus* were found to be 2.34, 1.71, 0.86 and 0.76 at 24, 48, 72 and 96 hrs exposure of sub-lethal (0.012 ppm) concentration of malathion. The total O_2 uptake in control fish recorded was 2.78 ppm. Rate of consumption (ml of O_2/gm Wet wt.) also declined in the treated fish with 0.012 sub-lethal concentration of malathion. A significant negative dose-response was obtained with increasing exposure durations. There were corresponding decreases both in total Oxygen consumption and rate of Oxygen consumption (Fig. 1). Total Oxygen consumption and rates of Oxygen consumption were found to be declined due to sub-lethal (0.036 ppm) concentration of malathion at 24, 48, 72 and 96 hrs of exposure. 2.07, 1.96, 1.01 and 92 ml of O_2/gm/hrs were found to be values for O_2 uptake at 24, 48, 72 and 96 hrs respectively when compared to 2.78 in control. Likewise, rate of O_2 consumption values were decreased as 0.081, 0.073, 0.064 and 0.058 as against 1.01 ml of O_2 in controls at 24, 48, 72 and 96 hrs of exposure (Fig. 2).

In the present investigation, total Oxygen consumption was 2.86, 2.04 and 1.86 ml (C.C) of O_2/gm/hrs during 5, 10 and 15 days respectively in sub-lethal (0.102 ppm) treated fish whereas in control total Oxygen consumption was 3.12 ml (C.C) of O2/gm/hrs. This indicated decreasing trend when compare to control. The rate of O_2 concentration were 0.076, 0.052 and 0.41 ml (C.C) of O_2/gm/Wet wt. during 5, 10 and 15 days of exposures respectively when compared to 1.14 ml of O_2 in control fish (Fig. 3). Total Oxygen consumption values obtained in another sub-lethal (0.036 ppm) treated group were 2.14, 1.08 and 0.93 during 5, 10 and 15 days respectively. In control, total Oxygen consumption was 3.12 ml of O_2/gm/hrs.   Rates of Oxygen consumption were found to be 0.048, 0.032, and 0.021 ml of O_2 at 5, 10 and 15 days of exposure respectively as against 0.97 ml of O_2 in control fish (Fig. 4).

Effect of malathion toxicity on blood characteristics of *Oreochromis mossambicus*

Experimental data obtained on blood characteristics of *O. mossambicus* exposed to two sub-lethal concentrations (1/10 and 1/30 of LC_{50}) for 24, 48, 72 and 96 hrs are presented in Figs. 5a-c, Fig. 6a-c and 7. The results showed a decrease in total RBC's with reference to increases in exposure periods (hours). The amount of RBC in the blood of the fishes exposed to 0.102 ppm malathion for 24, 48, 72 and 96 hrs was found to contain 1.35, 1.45,1.46 and 1.65 and mean control was found to be 1.5, 1.6,1.7 and 1.80 x 10^6/mm^3 respectively. Likewise, decreased amount of RBC in the blood of sub-lethal treated 0.306 ppm malathion. The recorded RBC counts were 1.18 hrs, 1.36, 1.20 and 1.50 when compared to control values 1.5, 1.6, 1.7 and 1.80 x 10^6/mm^3 for 24, 48, 72 and 96 hrs respectively.

The amount of WBC in the fish blood was declined in both the sub-lethal treatments for 24, 48, 72 and 96 hrs exposure. 2.8, 2.9, 2.7 and 2.6x10^4/mm^3 were the values obtained in the sub-lethal treatment (0.102 ppm) as against control values such as 3.1, 3.3, 3.0, 2.8 x 10^4/mm^3 for 24, 48, 72 and 96 hrs respectively. WBC amounts were also found deceased in the fish blood which exposed to another sub-lethal treatment of 0.306 ppm of malathion and the values were 2.70, 2.72, 2.50 and 2.60x10^4/mm^{3}compared to control values which were 3.1, 3.3, 3.0, 2.8x10^4/mm^3 for 24, 48, 72 and 96 hrs respectively. The level of haemologlobin in the fish *O. mossambicus* which exposed to sub-lethal (0.102 ppm) concentration of malathion were 56, 65, 65 and 68 g/L whereas the mean control values were 63,67,69 and 71 g/L at 24, 48, 72 and 96 hrs exposure. Similar decreases were also found in another sub-lethal concentration of 0.306 ppm malathion treatment. The recorded haemolobin values were 49, 62, 63 and 66 g/L as against 63, 67, 69 and 71g/L for 24, 48, 72 and 96 hrs exposure.

Haematocrit (%) values also decreased in both sub-lethal treated fishes. The least level of 21, 22, 21 and 19 percentages were noticed in treated group (0.102 ppm sub-lethal treatment) when compared to control fish which results 23, 24, 26 and 29 percentage respectively. Similar trend of results were found in another sub-lethal (0.306 ppm) treated groups and the percentages obtained were 20, 21, 20, and 16 compared to control Hct percentages such

as 23, 24, 26 and 29 at 24, 48, 72 and 96 hrs exposure. The amount of ESR (mm/H) in the blood of exposed fishes at 0.102 ppm sub-lethal malathion found to contain 9.4, 12.4, 14.2 and 17.3 compared to control fish which shows ESR values such as 10.0, 11.0, 13.7 and 16.2 at 24, 48, 72 and 96 hrs exposures. This indicate increasing trend with respect to increases in exposure period in hours. The ESR values also showed increases in the blood of exposed fishes to 0.306 ppm sub-lethal malathion. ESR values were 12.0, 12.8, 15.6 and 18.2 mm/H as against control ESR values viz. 10.0, 11.0, 13.7 and 16.2 at 24, 48, 72 and 96 hrs exposures respectively.

MCH (g/L) and MCHC (g/L) showed an elevated trend in respect to increased exposure periods (hours) in both sub-lethal treatments of malathion. The amount of MCH in the fish exposed to 0.102 ppm was found to contain 309, 320, 365 and 412 g/L when compared to control fish with MCH values i.e. 218, 208, 310 and 380 g/L at 24, 48, 72 and 96 hrs of exposures respectively.   The amount of MCHC (g/L) in the blood of 0.102 sub-lethal treated fishes were found to contain 17.4, 19.0, 23.0 and 36.0 g/L when compared to control MCHC values of 16.8, 17.0, 21.0 and 31.0 g/L at 24, 48, 72 and 96 hrs treatment.   Likewise, increases in the amount of MCHC were found as 18.6, 21.0, 25.0, 38.0 g/L whereas in control fishes MCHC values were 16.8, 17.0, 21.0, 25.0, 38.0 g/L as against in control fishes with MCHC values of 16.8, 17.0, 21.0 and 31.0 g/L for 0.306 sub-lethal treated group at 24, 48, 72 and 96 hrs exposures respectively.

The results on the blood characteristics of *Oreochromis mossambicus* exposed to two sub-lethal (0.102 and 0.306 ppm) concentrations for 5, 10 and 15 days are presented in Fig. 8a-c, Fig. 9a-c and Fig.10. Experimental data on the studies of blood characteristics in the exposed fish results the values of RBC (10^6 X mm^3), WBC (10^4 X mm^3) Haemoglobin (g/L) and Haematocrit (%) decreased in both sub-lethal treatments compared to control fishes. Decreases in their amount were found lesser with reference to the exposure periods (5, 10 and 15 days). Haematocrit (%), ESR (mm/H), both MCH and MCHC (g/L) were found to increase in their levels in two sub-lethally treated fishes as against in control fishes. The amount of RBC in the blood of the fishes exposed to 0.102 ppm of malathion for 24, 48, 72 and 96 hrs were found to contain 1.3, 1.2 and 1.3 and the mean control was found to be 1.7 x 10^6/mm^3 RBC amount recorded in the blood of 0.306 ppm exposed fishes was found to contain 1.1, 1.0 and1.1 x10^6/mm^3 compared to the control value of 1.7 x 10^6/mm^3 at 5, 10 and 15 days respectively.

The amount of WBC in the blood of fishes exposed to two sub-lethal concentrations of malathion were found to contain 4.1, 3.7 and 3.6 x 10^4/mm^3 and 3.8, 3.4 and 3.0 x 10^4/mm^3 for 0.102 ppm and 0.306 ppm concentrations respectively for 5, 10 and 15 days of exposure and the mean control was found to be 4.3 x10^4/mm^3. The levels of Haemoglobin in the fish exposed to 5, 10 and 15 days were found to be 75, 73 and 70 g/L and 71, 65 and 65 g/L for 0.012 ppm and 0.306 ppm treatments respectively and the control haemoglobin values was 84 g/L.Haematocrit values (%) obtained in the blood of treated fish were 26, 22 and 19 g/L and 22, 18 and 15 g/L for 5, 10 and 15 days of exposure in 0.012 ppm and 0.306 ppm sub-lethal concentrations of malathion. The recorded mean of control Haemoglobin was 29 g/L. The ESR values showed no changes at 0.102 ppm sub-lethal treatment at 5 days of exposure while increases in ESR (%) were found to be 17% and 19% during 10 days and 15 days of exposure at this same sublethal toxicity. Another sub-lethal concentration effected ESR values viz. 17%, 18% and 21% in fishes exposed to 0.306 ppm.   The mean ESR (%) value recorded was 15% for 15 days of exposure.

Both MCH and MCHC (g/L) values obtained in the blood of two sub-lethal treated fish were higher than the control. 509, 513 and 516 g/L were the MCH (g/L) for 0.012 ppm treated fishes while 561, 578 and 591 g/L were the MCH (g/L) for 0.036 ppm treated fishes. The mean control MCH (g/L) value recorded was 413 g/L for 15 days of exposure. MCHC (g/L) values recorded in the blood of 0.102 ppm treated fishes were 32, 37 and 42.1 g/L at 5, 10 and 15 days of exposure.   Likewise, increases in MCHC (g/L) were obtained in the blood of 0.306 ppm treated fishes and the values were 34.7, 42.0 and 50.0 g/L for 5, 10 and 15 days of exposures. The mean control MCHC (g/L) values was found to be 32.4 g/L.

DISCUSSION

In the present investigation, the fresh water fish (*Oreochromis mossambicus*) was subjected to preliminarily tests with varied concentrations of malathion. LC_{50} of malathion was 1.02 ppm at 96 hours exposure to the fish *O. mossambicus*. Hence the fishes were exposed to 2 sub-lethal concentrations of 0.102 ppm and 0.306 ppm of malathion for varying, periods of exposures 1, 2, 3, 4, 5, 10 and 15 days. The selection of concentration was 1/10 and 1/30 of the LC_{50} of malathion. The changes in both physiological (respiratory activity) and haematological parameters (blood characteristics) in selected body tissues were evaluated at different exposure period. These investigations fall in agreement with earlier studies (Shanker Murthy *et al.*, 2013).

Fishes are adapted for aquatic respiration, during which they take water in, through the mouth and passed through gill chambers covered by the operculum. The flow of water is continuous for almost the whole of the respiratory cycle. In its passage, the water gives up Oxygen to the blood and takes away the carbondioxide through diffusion. The process of Oxygen transported in the circulating fluid by haemoglobin present in the blood corpuscles. Oxygen uptake is widely used in physiology as a biological indicator that integrates the overall metabolic activity of an animal in response to specific environmental factors, because it reflects energy expenditure and ultimately, the food requirements. The metabolic rate of fish is usually measured by their rate of Oxygen uptake from water (Mo2); Mo2 is a criterion that has been suggested as an index of sub-lethal for fish and one that, if altered, may directly limit a fish aerobic performance (Shareena *et al.*, 2009). The comparative data on the whole animal Oxygen consumption of control and experimental fish, calculated per gram body weight per hour in sublethal concentrations of malathion for *Oreochromis mossambicus* was given in the Table: 4-7. The results of the experiments and control values are graphically represented in Figures: 3-6. By taking time on X axis and the amount of Oxygen consumed per gram body weight on Y axis.

In the present study, malathion sublethal concentration (1/10 and 1/30 of LC_{50}) were tested on the total Oxygen consumption and rate of Oxygen consumption in the freshwater fish, *O. mossambicus* when the fishes were exposed to sublethal concentrations of malathion, they were migrated to the bottom of the test chamber immediately. This is because of the toxic stress. Their Schooling behavior was totally disturbed and they were swimming independently and this was followed by irregular, erratic and dangling movements with the imbalanced swimming activity. The fishes were exhibited peculiar that is trying to leap out from the test chamber which can be viewed as escape phenomenon. Respiratory disruption was observed due to cough and yawing this is because of toxic stress. They often barrel rolled or spiraled at regular intervals and engulfed the air through mouth before respiration ceased. A change in color of the gill lamellae from reddish to light brown with coagulation of excess mucous on the gill lamellae was observed. The symptoms of malathion poisoning in the fish include loss of schooling behavior. Swimming near the upper surface, hyper activity, zig-zag movement, loss of buoyancy, elevated cough, increased gill mucous secretion, flaring of the gill arches, head shaking and restless before death.

The decrease in total Oxygen consumption observed at all exposure periods appears to be a protective measure to ensure that there is low intake of the toxic substance. When fishes are exposed to potential toxicants, the chemicals may directly affect metabolic reactions and attribute to respiratory distress as a consequence of impaired oxidative metabolism. Respiratory distress may arise as a result of either reduced oxygen diffusion over the gill membranes caused by an increase in the thickness of the mucous layer covering the secondary lamellae (Skidmore, 1964; Bradbury *et al.*, 1986; Venkata Ramana, 1987; Vijayalakshmi and Tilak, 1996; Sarkar, 1999; Deva Prakasa Raju, 2000; Mushigeri and David, 2003 and Amanulla Hameed *et al.*, 2005).

The rate of Oxygen consumed by the affected fish was very low. Once the respiration of the fish is affected, in turns all the biological activities of the fish will also be reduced. The decrease in whole animal Oxygen consumption might be due to the damage in the structural integrity of the cells of respiratory organs as reported by Barbieri *at al.*, (2002)

in the mullet, *Mugil platanus*; Pane *et al.* (2004) in rainbow trout, *Oncorhynchus mykiss*; Chebbi and David (2010) in the carp, *Cyprinus carpio*; Magar and Afsar Shaikh (2012) in *Channa punctatus*; Jothinarendran (2013) and Mariya Das *et al.* (2013) in *Labeo rohita*. Greater decrease in the rate of oxygen consumption would be due to internal action by toxicant altering the metabolic cycle at sub cellular level (Bradbury *et al.,* 1986) and also due to damage caused to RBC. Similar observation was made by Deva Prakasa Raju (2000) and Mushigeri and David (2003). The results observed in the present study were in agreement with Chebbi and David (2009a) who observed considerable variation in respiratory rate in freshwater, *Cyprinys carpio* exposed to quinalphos.

In the present study, the control fish behaved in natural manner with well-coordinated movement, but in the toxic environment, fish exhibited irregular, erratic and darting swimming movements and loss of equilibrium which might be due to inhibition of AChE activity leading to accumulation of acetylcholine in cholinergic synapses ending up with hyper stimulation (Mushigeri and David, 2005). Throughout the experimental period, the fish showed severe respiratory distress and rapid opercular movements leading to the higher amount of toxicant uptake. Increased mucus secretion, higher ventilation volume, labored breathing and engulfing of air through the mouth was also observed in fish exposed to both technical grade and 25% EC. However, the above said changes in the fish were more pronounced in 25% EC than in technical grade quinalphos. From the above discussions and the results obtained, it can be concluded that the decrease in Oxygen consumption of an organism as a responses to the toxic stress is the cumulative effect of several stages at which the toxicant acts.

All the studies mentioned above indicate a considerable effect of insecticides on Oxygen consumption in different species of fish in sublethal concentrations. The present study also reveals that $1/10^{th}$ of $LC_{50}/96$ concentration had more effect than $1/30^{th}$ of $LC_{50}/96$ hrs concentration of malathion due to the active ingredients present in the form of emulfiable concentration which might be the reason for more effect in alterations of the Oxygen consumption of *Oreochromis mossambicus* exposed to sublethal concentrations of malathion. The active ingredients contained in malathion might be causing cumulative or additive or Synergistic toxic action. Hence, it has a bearing on toxic stress as a result of more Oxygen consumption.

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CONFLICTS OF INTEREST

We declare that we have no conflict of interest

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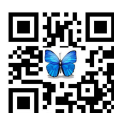
Table 1: Chemical characteristics of Test chemical

Common name	Malathion
Chemical formula	$C_{10}, H_{19}, O_6, PS_2$
Molecular weight	330.36
Color	Colorless liquid Deep brown to yellow
Physical state	Liquid
Melting point	2.9 EC
Boiling point	156-157 EC
Density	At 25 EC 1.23 g/cm3
Solubility: Water at 20 EC	145 mg/L

Table 2: Toxicity testing of malathion on test fish *Oreochromis mossambicus*

Conc.	No. of fishes alive				% alive at				% Mortality			
Control (0.0)	10				100				0.0			
(ppm)	24h	48h	72h	96h	24h	48h	72h	96h	24h	48h	72h	96h
0.2	9	9	8	7	90	90	80	70	10	10	20	30
0.4	8	8	7	6	80	80	70	60	20	20	30	40
0.6	8	6	6	6	80	60	60	60	20	40	40	40
0.8	7	6	6	6	70	60	60	60	30	40	40	40
1.0	7	6	6	6	70	60	60	60	30	40	40	40
1.2	6	6	6	5	60	60	60	50	40	40	40	50
1.4	6	6	6	4	60	60	60	40	40	40	40	60
1.6	6	6	6	4	60	60	60	40	40	40	40	60
1.8	5	5	4	3	50	50	40	30	50	50	60	70
2.0	4	3	3	3	40	30	30	30	60	70	70	70

**Table 3:   LC$_{50}$ determination of malathion toxicity in *Oreochromis mossambicus*
(Results obtained on experiments conducted in triplicates)**

Concentration (ppm)	Concentration difference	No. of alive fishes	No. of dead fishes	Mean death	Mean death X Conc difference
Control 0.0	0.0	10	0.0	0.0	0.0
0.2	0.2	7	3	3	0.6
0.4	0.2	6	4	4	0.8
0.6	0.2	6	4	4	0.8
0.8	0.2	6	4	4	0.8
1.0	0.2	6	4	4	0.8
1.2	0.2	6	4	4	0.8
1.4	0.2	4	6	6	1.2
1.6	0.2	4	6	6	1.2
1.8	0.2	3	7	7	1.4
2.0	0.2	3	7	7	1.4
				Total	9.8

Fig. 1. Total Oxygen consumption (ml of O_2/gm/hrs) and rate of Oxygen consumption (ml of O_2/gm/Wet wt.) by *Oreochromis mossambicus* exposed to sublethal concentration (0.012 ppm) of malathion at 24, 48, 72 and 96 hours

Fig. 2. Total Oxygen consumption (ml of O_2/gm/hrs) and rate of Oxygen consumption (ml of O_2/gm/Wet wt.) by *Oreochromis mossambicus* exposed to sublethal concentration (0.036 ppm) of malathion at 24, 48, 72 and 96 hours

Fig. 3. Total Oxygen consumption and rate of Oxygen consumption by *Oreochromis mossambicus* exposed to sublethal (0.102 ppm) concentrations of malathion

Fig. 4. Total Oxygen consumption and rate of Oxygen consumption by *Oreochromis mossambicus* exposed to sublethal (0.306ppm) concentration of malathion

(5a)

(5b)

Fig. 5a-c. Changes in the haematological characteristics (5a) RBC (10⁶ x mm³), (5b) WBC (104 x mm³) and (5c) Hb (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations (1/10 and 1/30 of LC₅₀) of malathion for 24, 48, 72 and 96 hours
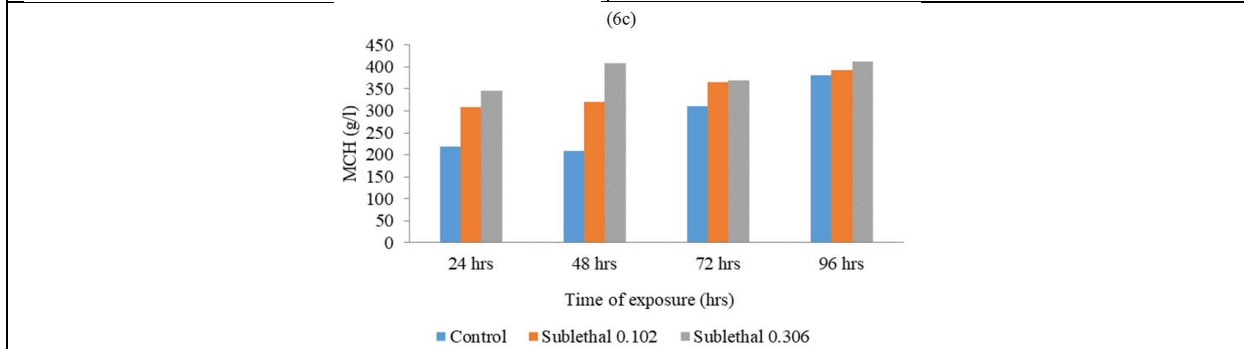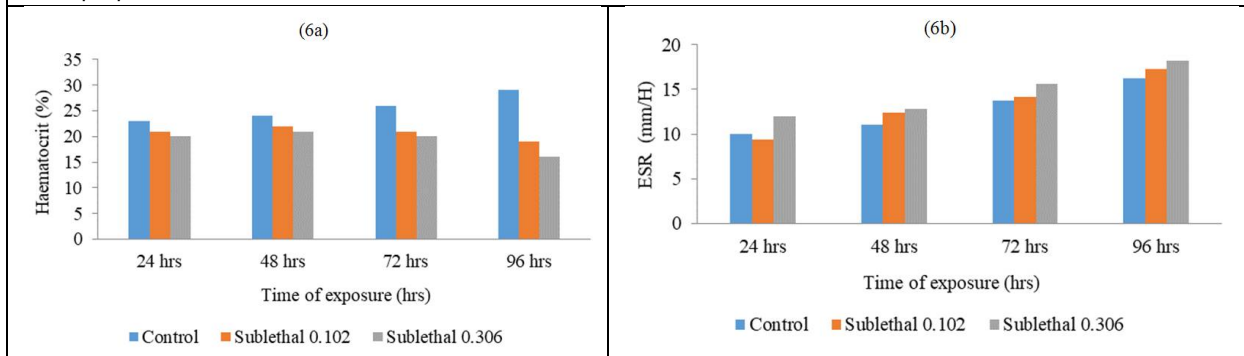
Fig. 6a-c. Changes in the haematological characteristics (6a) Haematocrit (%), (6b) ESR (mm/H) and (6c) MCH (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations (1/10 and 1/30 of LC₅₀) of malathion for 24, 48, 72 and 96 hours.
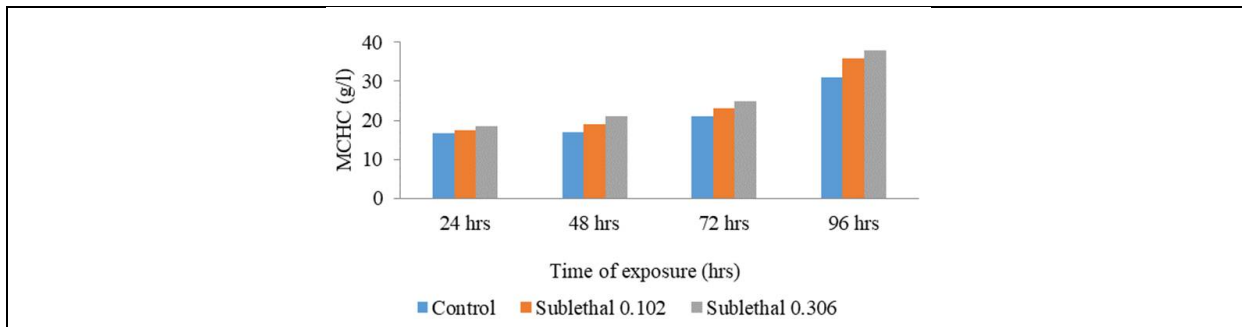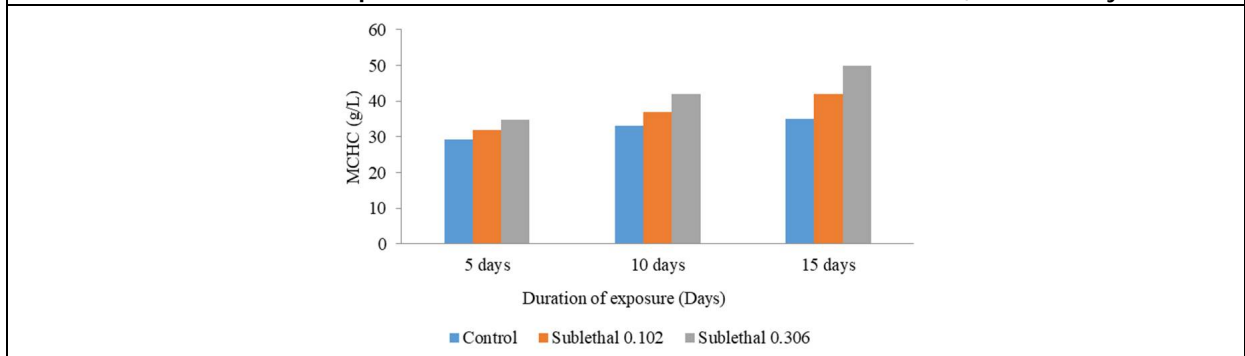
Fig. 7. Changes in the haematological characteristics MCHC (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations (1/10 and 1/30 of LC$_{50}$) of malathion for 24, 48, 72 and 96 hours
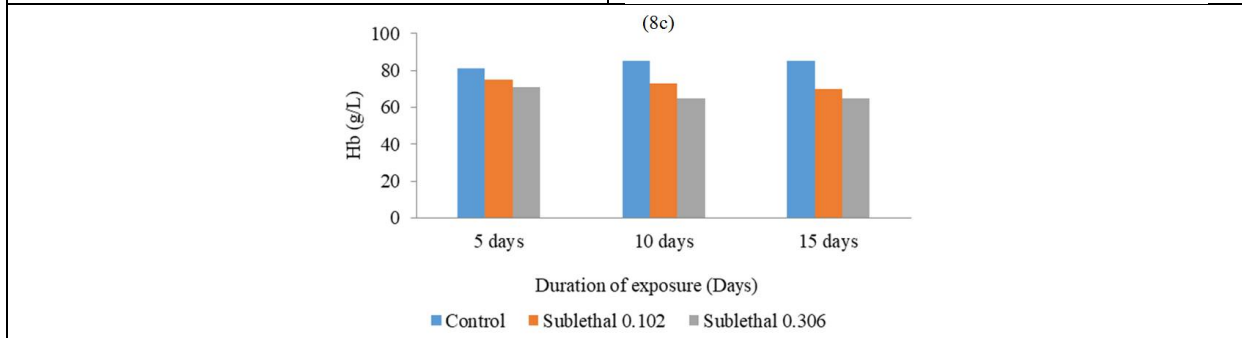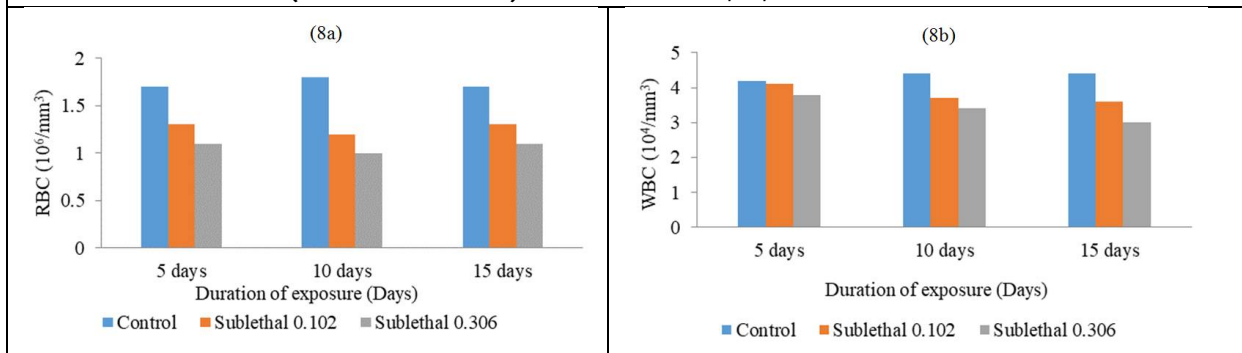
Fig. 8a-c Changes in the haematological characteristics (8a) RBC (10^6 x mm^3), (8b) WBC (10^4 x mm^3) and (8c) Hb (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations of malathion for 5, 10 and 15 days

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(9c)
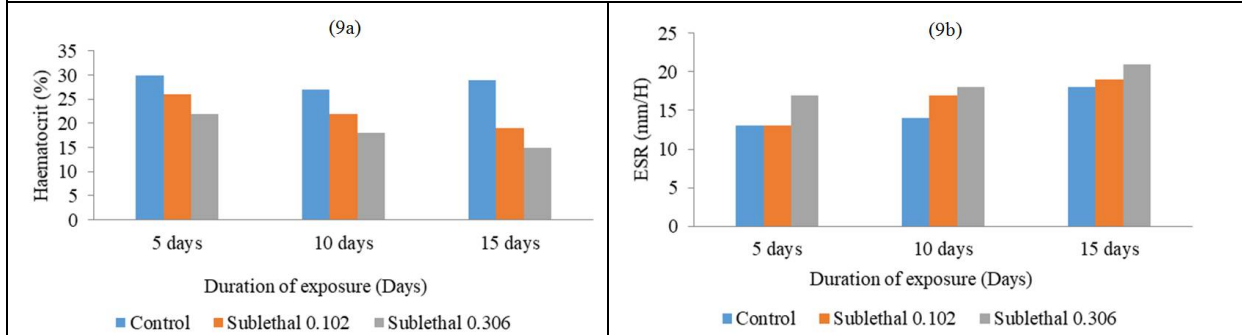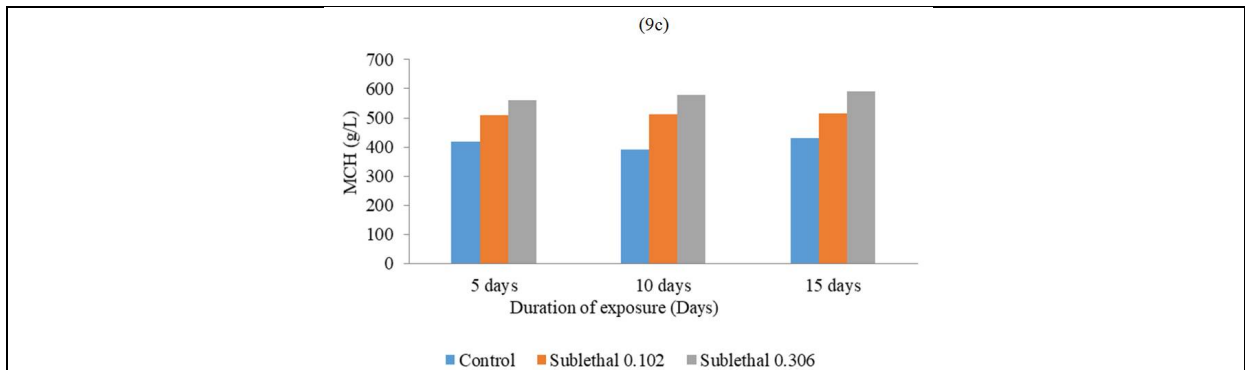
Fig. 9a-c. Changes in the haematological characteristics (9a) Haematocrit (%), (9b) ESR (mm/H) and (9c) MCH (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations of malathion for 5, 10 and 15 days.

Fig. 10. Changes in the haematological characteristics MCHC (g/L) of *Oreochromis mossambicus* exposed to two sublethal concentrations of malathion for 5, 10 and 15 days

RESEARCH ARTICLE

COVID-19: Utilizing Zoom App 100% Essentials

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ABSTRACT

The study showcases the significance of the Zoom app during this epidemic to the students and teachers in the Master of Education Department (MAED) utilizing a descriptive mixed-methods approach by observation and viva voice as instruments in data collection and assessment with Charts for data presentation. Based on the results write shop on writing a research proposal is essentials to enhance everyone interested in preparing a research proposal in fact out of 125 participants no one negates but responds with their symbolic thumbs-up reaction. Mainly, the write shop described favorable outcomes such that participants admitted that the webinar on writing a research proposal is significantly importance, the zoom app is extremely important, wherein the Experts demonstrated it well with an Excellence performance and agreed that they were satisfied and enhanced their capability in writing research proposal thus, utilizing zoom during this time of crisis is essentials. Therefore, it is proven by facts that during this epic where students and teachers struggled against this Covid-19 understood that one of the best online or distance learning tools to provide teachings and substantiated learning is the Zoom app and so everyone should be in safe and far from Coronavirus contamination.

Keywords: Zoom App; Covid-19; writing; research proposal

INTRODUCTION

The study described how effective is a write shop on Research Proposal Writing utilizing Zoom to the MAED students of Surigao State College of Technology (SSCT) as if this Zoom is an avenue for teachings, meetings, conferences, and webinars (Gray, et.al 2020). Specifically, the study addressed to enhance the capability of those students, teachers, and professionals of venturing research most probably in research proposal writing (Wong 2016; Arifani 2016; Cadman 2002; Klopper 2008; Madsen 1983). In the event, unexpected impressive results showed that the said event overwhelmed the 125 participants in attendance, participated in the write shop wherein during feed

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backing moreover, on the chat section or dialogue box nearly everyone had contributed their questions relative to enhance their capability in writing a research proposal. The write shop last for two hours starting at 10:00 am, ended at 12:00noon but no one leaf the room until the session ended, and all of them showed respect to the Lecturer by giving their reactions thru a "Thumbs-up" symbolizing their contentment. Ended, the write shop guided those students and teachers on differentiating the difference between thesis and research proposal, eventually, comprehended the purpose of research, the significance of it, the theories, the methodologies, and ascertained the appropriate instruments to be used based on the types of approach either it is qualitative, quantitative, or mixed methods.

METHODOLOGY

The study is descriptive using mixed methods (Williams, 2007) with Observation (Kawulich, 2005) and Viva voice (Pearce & Lee, 2009) as an approach to account for the participants' behavioral responses about the importance of the write shop, blended with an evidence-based assessment using symbolic signs of "Thumbs-up" and "Thumbs-down" to come up a decision either the said write shop on research proposal writing has succeeded or failed (Silverman &Ollendick, 2005; Ozonoff, Goodlin-Jones, & Solomon, 2005; Fletcher, Francis, Morris & Lyon, 2005). Moreover, Charts were used for data presentation and categorically the results and findings of the study [(Abu Doush, Pontelli, Simon, Son & Ma, 2009): (Lee &Mc Greevey, 2002); (Krishnaswamy, A. (2004)].

RESULTS AND DISCUSSION

It shows that out of 125 participants there were 95 female and 30 male with equivalent percentage of 76% and 24% respectively this is reflective that in this course females are more dominant than males in numbers compared to female, see Figure 1. No. of participants.

Other Charts presentations were able to describe the actual survey, observation and viva voice regarding the Expert's performance, the webinar significance, and the importance of Zoom app employing mathematical language-percentages.

Participants showed their 100% Thumbs up, reacted as satisfied on the webinar on writing research proposal (*Sentro Ng Wika at Kultura Ng SSCT, Surigao | Facebook*, n.d.)

CONCLUSIONS

Thematically, the study is reflective that the Zoom app or any online app can be the best means for teaching only if students were satisfied during the learning process. Therefore, it is clearly understood that the teacher's factor will be the first thing to consider, his ability, his skills, and his expertise in teaching can motivate students to be more active doing their activities and assignments. However, this study is insightful that a teacher's output depends on his students' performances this means replica strata, yet throughout this midst of a crisis, it is thought-provoking to everyone on how to substantiate students' learning if he or she has its difficulties in the teaching process.

Recommendation

1. It is much appreciated if all of the students should be provided first with the link and internet access or modem;
2. It is more privilege if teachers will be given financial assistance to their needs as to distance learning;
3. It is more significant if during-after this Covid-19, the Zoom app should be sustained hence, findings revealed that students and teachers were attentive and responsive using these tools in the learning process; and
4. It is advantageous if teachers should be well trained in all aspects of teaching most probably in their field of expertise.

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ACKNOWLEDGEMENT

Remarks
There were 41 participants in attendance inside the Board Room. While 84 participants were in the Zoom.

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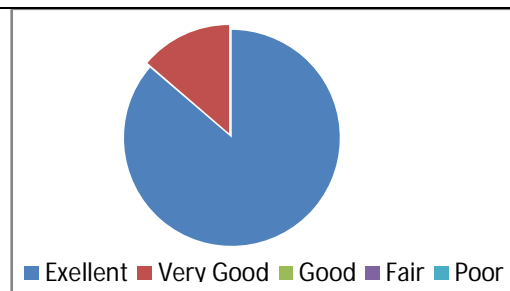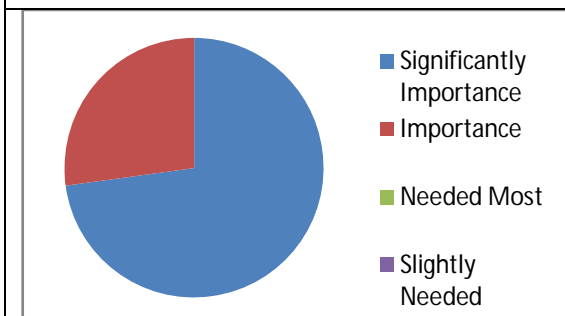
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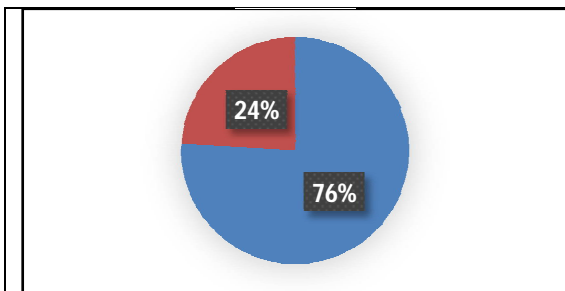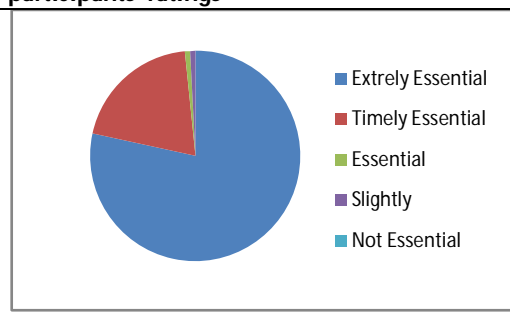
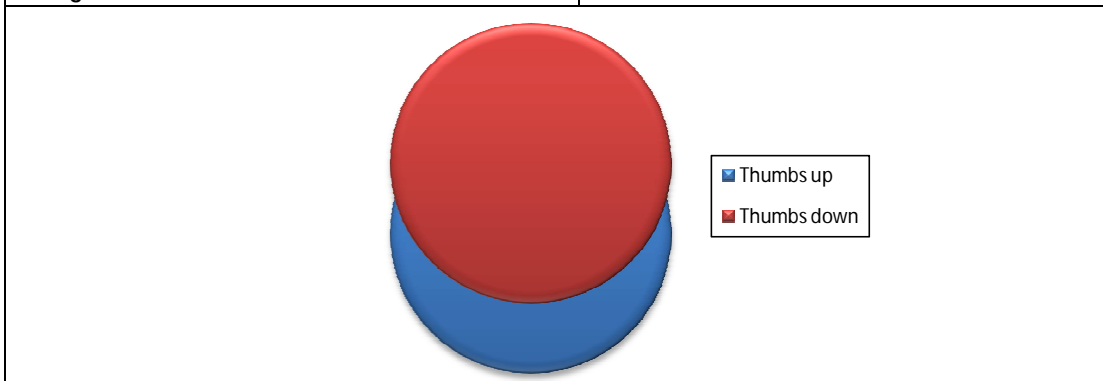**Figure 1. No. of participants, who attended the webinar**

Figure 2. Experts' performance based on participants' ratings

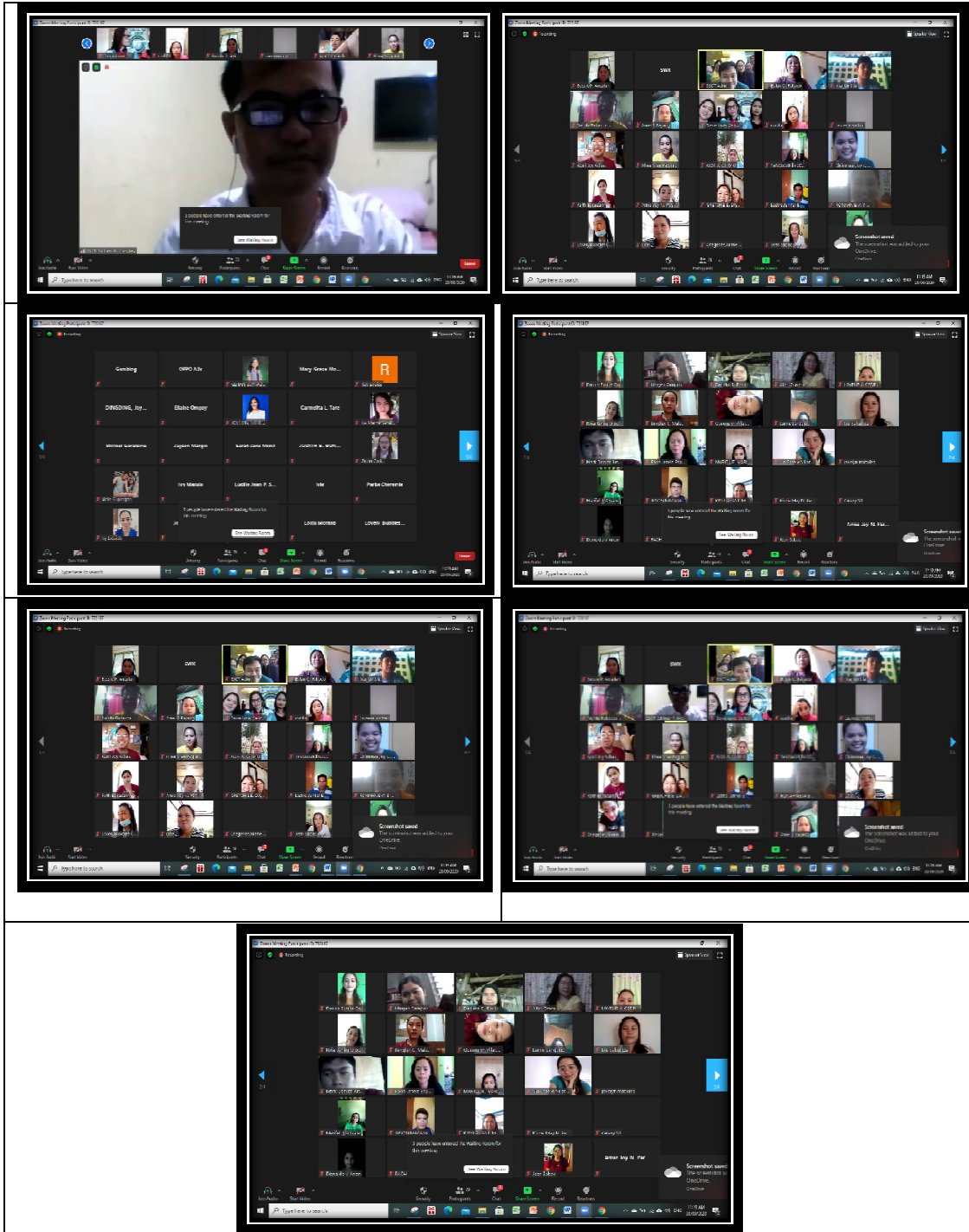
Figure 3. The Webinar based on participants' ratings

Figure 3. The Zoom App based on participants' ratings

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Appendix 1. Actual viewing during the write shop on writing publishable article, in attendance with 84 participating MAED students and teachers of SSCT-Surigao City Campus…

RESEARCH ARTICLE

Combined Effect of Constraint Induced Movement Therapy and Mental Imagery Training in Upper Extremity Function in Chronic Stroke

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ABSTRACT

Stroke survivors are often afflicted with temporary hemiparesis and experience weakened upper extremity function on one side of their body. Studies done on Constraint Induced Movement Therapy (CIMT) as well as Mental Imagery Training (MIT) individually have shown that they accelerate motor learning and thereby improve subsequent physical performance. Studies have also been done using combined physical therapy and mental imagery training in the improvement of balance and posture. The present study has been taken up to find out the combined effect of CIMT and MIT on improving upper extremity function in subjects with chronic stroke. Thirty individuals were assigned randomly into three treatment groups. Group I received CIMT and MIT. Group II received CIMT alone and Group III received MIT alone. All the subjects were assessed before and after the intervention by using Fugl-Meyer Score (upper limb section) to assess the impairment and Action Research Arm Test to assess the arm function on the subjects. Pre and post treatment score were measured in all the three groups and compared using student t-test. The study demonstrated significantly higher improvement in the subjects who participated in group I (CIMT and MIT) when compared with the other groups, p-value being significant in all the three groups (p<0.001). This study concludes that CIMT and MIT when combined have significant effect on improving upper extremity function in subjects with chronic stroke.

Keywords: chronic stroke, hemiparesis, CIMT, MIT.

INTRODUCTION

Stroke refers to neurological dysfunction which happens suddenly due to interruption or reduction of blood supply to the brain, affecting the focal areas of the brain accompanied by signs and symptoms that correspond. The

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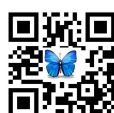
incidence of stroke increases dramatically with age, doubling every decade after 55 years of age [1]. Clinically a variety of deficits are possible including changes in the level of consciousness and impairments of sensory, motor, cognitive, perceptual, and language functions. Upper limb hemiparesis is one of the most debilitating effects of stroke and is the primary impairment underlying functional disability following stroke [2].When stroke survivors experience weakened upper extremity function on one side of their body, they compensate for this by using their unaffected side reinforcing the non-use of their hemiplegic limb. Such patients often fail to develop full potential use of their affected upper extremity perhaps due to a 'learned non-use phenomenon' [3].Finding effective and functionally based interventions to improve motor control in individuals with upper extremity hemiplegia becomes vital in the light of the changing health care environment.

Physical therapy is used to maintain and restore range of motion and strength in affected limbs, and to maximize mobility in walking, wheelchair use, and transferring (from wheelchair to toilet or from standing to sitting, for instance).Most of the treatment approaches used traditionally in neurological rehabilitation have focused on reducing motor impairment and minimizing physical disability, but very few approaches concentrate on learned non-use phenomenon. One such approach is Constraint Induced Movement therapy [2]. When hand function is impaired, this is aggravated by learned non-use which leads to a loss of cortical representation of the upper limb. Researchers claim that this can be reversed by two weeks of constraint of the unaffected limb combined with intensive practice in use of the paretic hand [4].The plasticity of the brain -- its ability to adapt and reorganize -- and a natural rewiring of the neural connections make it possible for one part of the brain to change functions and take up the work of the injured part. This rewiring of the brain and restoration of function can be helped with therapy, and this is the goal of CIMT [5].Wolf SL, Winstein CJ, Miller JP, Edward T, Uswatte G, Morris D et al (2006) did a study on the effect of CIMT on upper extremity function 3 to 9 months after stroke on 222 individuals with ischemic stroke and showed that CIMT produced statistically significant and clinically relevant improvements in arm motor function that persisted for at least 1 year [6]. Sarah Blanton, Steven L.Wolf (1999) did a case report to demonstrate the Constraint Induced Movement Therapy on 4 months post-stroke and they found that 2 weeks of Constraint Induced Movement Therapy may be an effective method for restoring motor function within a few months after cerebral insult [3].

Another new technique, which has the evidence to suggest that mental rehearsal of movement can produce effect normally attributed to practicing the actual movements, is Mental Imagery Training. Imagining hand movements could stimulate the redistribution of brain activity, which accompanies recovery of hand function, thus resulting in a reduced motor deficit [7]. Mental practice has also been suggested to be a viable tool for improving motor learning and performance in rehabilitative settings [2]. The general idea is that motor imagery is part of a broader phenomenon (the motor representation) related to intending and preparing movements [8].

A recent study compared motor imagery training and physical training and found enhanced performance in both [6]. In addition there is evidence for functional redistribution following motor imagery training in healthy volunteers, demonstrating that motor imagery training alone seems to be sufficient to promote the modulation of neural circuits leading to the same plastic changes in the motor system as those following repeated physical practice [6]. Two ways of dealing with motor images have been identified. One is the cognitive approach, using psychological methodology for disclosing the structure and content of the mental phenomenon. The other one is more physiological, based on observation of bodily correlates of motor imagery and eventually on inferring its structure and content from these observations [8]. The dual coding theory splits the cognitive apparatus into two different modes of generation, the verbal and the non-verbal modes. Imagery would pertain to the latter mode. Hence, images would be generated using modality-specific units (Paivio's images).Images correspond to natural objects or object parts, or groupings of objects, which can become simultaneously available to build a mental image (of somebody's face, of my living room, of an action, etc) [8]. This theory implies that the verbal and the non-verbal (imagery) systems are functionally independent, in the sense that they can be activated separately: yet they remain interconnected, so that it is possible to generate mental images through verbal instructions, and to describe mental images verbally [8]. Physiological

correlates have been recorded in many experiments involving mental motor imagery. Electromyographic activity (EMG) was frequently found to increase with respect to rest during motor simulation. Jacobson found micro movements and increased EMG in those limbs involved in imaginal movements, but not in the contralateral ones. These discharges were related to the requirements of the imagined task (e.g. rhythm) [8].   The issue of physiological correlates of motor simulation has been investigated using brain activity mapping techniques. Roland *et al.* asked normal subjects to imagine a rapid and skilled sequence of digit movements. They found a significant and localized regional cerebral blood flow (rCBF) change mainly in the supplementary motor area (SMA) [7]. Decety *et al.* studied normal subjects imagining a graphic movement (writing 'one, two, three, etc). The subjects were instructed to imagine the movement at the 'first person perspective' and to try to 'feel their writing hand'. Regions corresponding to the prefrontal cortex, SMA, and also the cerebellum were activated significantly, as well as in the basal ganglia [8]. Finally this was reinvestigated again by Decety *et al.* using PET. Three- dimensional graspable objects (cylinders and spheres of different sizes, colors and orientations) were presented to subjects who were instructed to 'imagine themselves grasping the objects with their right hand' [8]. A significant regional cerebral blood flow (rCBF) increase was observed in areas concerned with motor behavior. At the cortical level, area 6 in the inferior part of the frontal gyrus was strongly activated on both sides. Activity also increased in left prefrontal areas, extending to the dorsolateral frontal cortex (areas 9 and 46), and in the parietal lobule (area 40). Finally, the anterior cingulate cortex (areas 24 and 32) was bilaterally activated. At the sub cortical level, the caudate nucleus was found to be strongly activated on both sides and the cerebellum was involved only on the left side [8].   Schaechter JD, Kraft E, Hilliand TS, Kijkhnizen RM, Benner T, Finklestein SP et al (2002) did a preliminary study to assess the motor recovery and cortical reorganization after Constraint Induced Movement Therapy in stroke subjects., They found significant improvement in the functional use of the patients' upper extremity affected by stroke. They also found that the motor impairment of the patients reduced significantly immediately after CIMT and even after 6-month follow-up [9]. Liu KP, Chan CC, Lee TM, Hui-Chan CW (2004)did a randomized controlled trial to study the efficacy of Mental Imagery Training at promoting relearning for people after a stroke. Results showed better relearning of both trained and untrained tasks in mental imagery based intervention compared with the control group and they concluded that mental imagery can be used as a training strategy to promote the relearning of daily tasks for people after an acute stroke [10]. The above studies that have been done on Constraint Induced Movement Therapy as well as Mental Imagery Training individually have shown that they accelerate motor learning and thereby improve subsequent physical performance. Randomized controlled studies, demonstrated greater improvements on the balance tasks e.g. one legged standing among elderly women who combined mental practice with physical practice than those who participated only in physical practice [2].Three week mental practice program, when combined with physical practice, improved posture of individuals with abnormal curvature of the spine [2].These studies have been done using combined physical therapy and mental imagery training in the improvement of balance and posture. This study is focused to find out the combined effect of Constraint Induced Movement Therapy and Mental Practice in upper extremity function in chronic stroke.

METHODOLOGY

Source of Data
Various Neuro -Hospitals in and around Bangalore

Method of Collection of Data
30 subjects diagnosed with chronic unilateral stroke, between age group of 40 to 65 years, with duration of stroke of more than 6 months, not participating in any experimental drug field study, and with Mini Mental State Examination (MMSE) score of more than 24 [3], were included in the study. Their passive range of motion was at least 90 degrees of shoulder flexion and abduction, 45 degrees of shoulder lateral rotation, no more than -30 degrees of elbow extension, 45 degrees of forearm supination and pronation, wrist extension to neutral, and finger extension (all digits) with no greater than 30 degrees of flexion contracture at the MCP and IP joints [3]. They had the ability to

independently and safely transfer to and from the toilet, stand from a sitting position, and maintain standing balance for 2 minutes with upper extremity support [3]. Subjects who cannot image as evidenced by a score of 25 or less on Movement Imagery Questionnaire-Revised [11], or with visual perceptual problem, or having cognitive impairments, aphasia, or other neurological dysfunctions, or musculoskeletal abnormalities were excluded.

Materials Used

➢ Physical evaluation proforma.
➢ Mini-mental State Examination to evaluate the cognitive status of the subjects. Materials used in this are pen, watch, paper.
➢ Movement Imagery Questionnaire to evaluate the imaginary capacity of the subjects.
➢ Materials used for ARAT: Wooden blocks (10cm, 7cm, 5cm, 2.5cm), stone (10X2.5X1cm), 2 glasses, tube (2.25cm), washer, marble, cricket ball.
➢ Materials used in FMA: Knee hammer, pencil, paper and cylinder shaped object. CD or audiotape with instructions for mental practice interventions.
➢ Padded treatment table for mental imagery training intervention.
➢ Sling to constraint the uninvolved upper extremity in CIMT intervention.

Procedure

Subjects diagnosed by the Neuro-physician as stroke and who fulfilled the inclusion/exclusion criteria were included for the study. Informed consent was taken from each subject. Subjects were then assigned to one of the three groups on the basis of simple random sampling.
Demographic data of all the subjects were also assessed i.e. age and gender. Subjects were made to understand about the study and the purpose of the study in their own language.
There were 10 subjects in each of the 3 groups.
Group-I received constraint induced movement therapy and mental imagery training,
Group-II received Constraint Induced Movement Therapy (CIMT) alone and
Group-III received Mental Imagery Training (MIT) alone.
Fugl-Meyer Score- upper limb section was used to assess the impairment, and Action Research Arm Test was used to assess the arm function on the subjects before starting the interventions to collect the baseline data.

CIMT Intervention for Both Group-I and Group-II Subjects

The intervention consisted of the patients wearing a sling/mitt on their uninvolved hand during all waking hours, except for water based activities such as washing hands, toileting and bathing, for the entire 15 days of treatment period. Initially the patients were asked about what activities they had participated, prior to the stroke, and they were made to carry out those activities like grooming, dressing, writing etc. Each task was sub divided into a hierarchy of component movements that progressed in complexity to minimize the failure or frustration. The procedure was explained properly to the patients and they were made to understand the purpose of the intervention. The day started with practicing a simple task related to their activities of daily living and making them repeat those activities followed by a rest period for every 1-2 hours to prevent fatigue. The patients were also monitored during meals. They were encouraged to keep an activity log that described all tasks performed with the paretic limb and each morning the activity log was reviewed with the patient, discussing performance and use of the sling/mitt during the previous evening.

MIT Intervention for Group-I and Group-III Subjects

In this, the subjects were made to listen to a tape-recorded mental practice intervention lasting approximately for 10 minutes in a quiet examination room. The patient was in supine position on a padded treatment table. The intervention consisted of an initial relaxation period lasting 2-3 minutes in which the subjects were asked to imagine themselves in a warm, relaxing place. They were instructed to sequentially contract and relax their muscles starting

from their feet and moving upwards. The same procedure was followed for their hands and arms. This portion of the audio-tape was followed by 5-7 minutes of suggestions for internal cognitive visual images related to using the affected arm in functional tasks as follows:

- During the first 5 days, the audiotape functional task practiced was that of reaching for and grasping a cup.

In this, subjects were made to imagine a cup in front of them within their reaching level and then made to imagine as though they were lifting their affected upper extremity up and extending it forward towards the cup, then followed by opening of the fingers one by one to grasp the cup and the task ended by holding a cup. At the end of the task subjects were made to imagine of releasing the fingers one by one and placing the upper extremity back to the normal position.

- During the next 5 days, the task practiced was that of turning pages in a large reference book.

In this, subjects were made to imagine a large reference book in front of them within their reaching level and then made them to imagine as though they were lifting their affected upper extremity up and extending it forward towards the book. Then the subjects were instructed to imagine about opening of the fingers one by one to reach the book and start turning the pages and the task ended by turning the pages one by one. At the end of the task subjects were made to imagine releasing the fingers one by one and taking them back and placing them down in the normal position.

- In the last 5 days the functional task practiced was that of reaching for and grasping an item on a high shelf and bringing it towards themselves.

In this, subjects were made to imagine a cup on the shelf in front of them within their reaching level and then made to imagine lifting their affected hand up and extending it forward, towards the cup, then opening of the fingers one by one to grasp the cup and bringing the cup towards them. The task ended by bringing the cup towards them. At the end of the task subjects were made to imagine keeping the cup back and releasing the fingers one by one and taking the hand back and placing it down in the normal position.

This period of cognitive visual imaging was followed by 2 minutes of refocusing into the room. During refocusing, the subjects were reoriented to their surroundings and to their body. They were then asked to re-concentrate on their surroundings (e.g. the buzz of the lights, becoming aware of voices or other noises that he may be able to hear outside). Count down was given from 10 to 1. At the last count, the subjects were asked to open their eyes.

After 15 days of intervention the post-test measures of Fugl-Meyer Score was used to assess impairment, and Action Research Arm Test was used to assess the arm function. Readings were taken and compared with the pre-test measures to see the effects.

As this study involves human subjects, the ethical clearance has been obtained from the ethical committee of Padmashree Institute of Physiotherapy, Nagarabhavi, Bangalore, as per ethical guidelines research from biomedical research on human subjects, 2000, ICMR, New Delhi.

RESULTS AND DISCUSSION

The mean age in Group I is 56.00 with the standard deviation of 4.42, Group II is 54.40 with a standard deviation of 7.43 and Group III is 55 with a standard deviation of 6.83.

Group I has 70% males and 30% females, Group II has 40% males and 60% females and in Group III there are 50% males and 50% females.

In Group I, 50% of the subjects have stroke duration of less than one year and the remaining 50% have duration of stroke between 1-2 years. In Group II, 20% of subjects have stroke duration of less than a year, 60% of subjects come under the stroke duration of 1-2 years and the remaining 20% have stroke duration of more than 2 years. In Group III, 40% of the subjects have stroke duration of less than a year, 40% of subjects have duration of stroke between 1-2 years and the remaining 20% have stroke duration of more than 2 years.

In Group I 30% of the subjects are of left sided involvement and the remaining 70% of the subjects are of right sided involvement. In Group II 60% of the subjects are of left sided involvement and the remaining 40% of the subjects are of right sided involvement. In Group III 50% of the subjects are of left sided involvement and the remaining 50% of the subjects are of right sided involvement.

Figure 1 shows that the mean of MMSE score of Group I is 25.70 with a standard deviation of 1.70. The mean of MMSE score of Group II has scored 24.60 with a standard deviation of 0.96 and Group III has 25.20 with a standard deviation of 1.87 with the p value of 0.326.

Figure 2 shows that the mean of MIQ-R score of Group I is 40.40 with a standard deviation of 2.91 and Group III has scored 38.30 with a standard deviation of 3.80 with the p value of 0.183.

Analysis of variance (ANOVA) has been used to find the significance of study parameters between three groups of subjects, Student t test (two tailed, dependent) has been used to find the significance of study parameters on continuous scale within each group.

The pre-intervention assessment score of FMS in Group I is 28.20 with a standard deviation of 2.20, ranging from 25-32. The post-intervention assessment score has increased to 33.30 with a standard deviation of 2.26, ranging from 30-37.The percentage change is 18.1%. p value is < 0.001 which is strongly significant.

In Group II the pre-intervention assessment score of FMS is 25.50 with a standard deviation of 3.66, ranging from 19-31. The post-intervention assessment score has increased to 30.10 with a standard deviation of 4.75, ranging from 22-37. Percentage change is 18.1%. p value is < 0.001 which is strongly significant.

In Group III pre-intervention assessment score of FMS is 22.70 with a standard deviation of 5.85, ranging from 10-31. The post-intervention assessment score has increased to 25.40 with a standard deviation of 6.04, ranging from 14-36. Percentage change is 11.9%. p value is < 0.001 which is strongly significant.

Before the intervention (pre-assessment) the score of ARAT in Group I was 33.80 with a standard deviation of 9.44, ranging from 15-48. After the intervention (post-assessment) the score has increased to 41.30 with a standard deviation of 7.97, ranging from 27-54. Further statistical analysis, percentage is found out i.e 22.2%.   p value is < 0.001 which is strongly significant.

In Group II the score of ARAT before the intervention was 30.60 with a standard deviation of 7.18, ranging from 21-42. After the intervention (post-assessment) the score has increased to 37.20 with a standard deviation of 7.51, ranging from 27-51. Further statistical analysis, percentage is found out i.e. 21.6%. p value is < 0.001 which is strongly significant.

In Group III the score of ARAT before the intervention was 27.90 with a standard deviation of 9.06, ranging from 15-42. After the intervention (post-assessment) the score has increased to 37.70 with a standard deviation of 8.30, ranging from 18-45. Further statistical analysis, percentage is found out i.e. 17.2%. p value is < 0.001 which is strongly significant.

This figure shows that the outcome (difference of pre and post) of FMS for Group I is 5.10, with a standard deviation of 0.88, for Group II shows 4.60 with a standard deviation of 1.77 and group III is 2.70 with a standard deviation of 1.16. p-value is 0.001 which is strongly significant.

The Outcome (difference of pre and post) of ARAT for Group I is 7.50 with a standard deviation of 2.84 for Group II is 6.60 with a standard deviation of 1.89, and Group III is 4.80 with a standard deviation of 3.23. p-value is 0.059 which is moderately significant.

The study was intended to find out the combined effect of constraint induced movement therapy and mental imagery training in upper extremity function in chronic stroke.

In this study subjects of all the three groups showed a significant improvement in the post-assessment scores of Fugl-meyer and Action Research Arm Test. The results are well supported in a study to assess the motor recovery and cortical reorganization after Constraint Induced Movement Therapy in stroke patients, which showed that patients made significant gains in functional use of the stroke affected upper extremity and significant reductions in motor impairment immediately after CIMT and even after 6-month follow-up [9]. Liu KP,Chan CC, Lee TM, Hui-ChanCW (2004)did a randomized controlled trial to study the efficacy of Mental Imagery Training at promoting relearning for people after a stroke. Results showed better relearning of both trained and untrained tasks in mental imagery based intervention compared with the control group and they concluded that mental imagery can be used as a training strategy to promote the relearning of daily tasks for people after an acute stroke [10]. Randomized controlled studies, demonstrated greater improvements on the balance tasks e.g. one legged standing among elderly women who combined mental practice with physical practice than those who participated only in physical practice [2].Three week mental practice program, when combined with physical practice, improved posture of individuals with abnormal curvature of the spine [2].

In this present study it is believed that weakness, neglect, fear, in-ability, hyper-tonicity are the factors which could have decreased the ROM and also decreased the functional capacity of the affected side. Hence the compensation is through the unaffected side.

The subjects trained with combined CIMT and MIT(Group I) had a more significant improvement from pre to post scores of Fugl-meyer and ARAT when compared with CIMT and MIT given alone.
In combined CIMT and MIT, subjects were trained by using both the methods i.e. MIT and CIMT.
Through MIT the subjects were able to imagine actual movements with improved attention and were able to perform the movements more easily by breaking the complex movements into simpler ones.

Then through CIMT, subjects were trained to come out of the neglect and to use the affected side repeatedly which results in decrease in the impairment level and increase in the functional capacity of the affected side.

The subjects' improvements between the pretest and posttest occurred because the subjects through mental practice, were provided with additional practice of functional tasks using the affected arm along with the CIMT. On a physiological level, this practice causes the priming of the motor cortex and appropriate activation of the neuromotor pathways, which resulted in the subjects' improvements.

The cortical changes observed after undergoing task-specific, repetitive physical practice coupled with mental practice with motor imagery leads to a greater amount of cortical reorganization and improved functional outcome [11].

The subjects trained with CIMT (Group II) also had a significant improvement from pre to post scores of Fugl-meyer and ARAT.

In CIMT, the unaffected side is restrained with the sling or a mitt, with an intention that the subjects are forced to use their affected side, or they are left with no other option other than using their affected side to carryout various activities/ADL.

Physiological background says that, when cells in an area of the brain responsible for a particular function die after a stroke, the patient becomes unable to perform that function. The plasticity of the brain-its ability to adapt and reorganize—and a natural rewiring of the neural connections make it possible for one part of the brain to change functions and take up the work of the injured part. This rewiring of the brain and restoration of function can be helped with CIMT [5].

This is the possible reason why there is increase in the ROM and decrease in the impairment level on Fugl-meyer and increase in arm function on action research arm test scores used as an outcome measures.

Compared to the first group, the second group subjects showed difficulty in breaking the complex task into a simpler one, had lack of attention towards the affected side and also found difficulty in imagining the actual movements. Hence the second group's level of improvement was less when compared to the first group.

The subjects trained with MIT (Group III) also had a significant improvement from pre to post scores of Fugl-meyer and ARAT. It is supported by Magill who suggested that mental practice is effective because it augments existing motor schema [11].

Before the intervention the subjects had limited ability to use the affected fingers but had enough ability to perform gross movements with the affected arm. After participating in a mental practice intervention targeting grasping, reaching, and turning pages, subjects have improved both in the gross motor scores and the fine motor components.

The specificity of the changes in the areas targeted suggests enhancement of the existing motor plan as a possible mechanism [11].

In this MIT intervention, patients have to listen to the audio tape which improves their attention level which is one of the factors that decreases the functional capacity following a stroke.
Physiological correlates have been recorded in many experiments involving mental motor imagery. Electromyographic activity (EMG) was frequently found to increase with respect to rest during motor simulation. Jacobson found micro movements and increased EMG in those limbs involved in imaginary movements, but not in the contra lateral ones. These discharges were related to the requirements of the imagined task (e.g. rhythm) [8].

Hale, Bakker et al have observed minute activations in the targeted musculature as if the activity were being physically performed [11]. Moreover, not only are the appropriate muscles fired during mental practice, but electromyographic amplitude during mental practice is proportional to that observed during actual task performance [11]. During mental practice, not only are the appropriate muscle patterns and neural events exhibited as if the activity was actually being performed, but these activations are also exhibited in the appropriate proportions (i.e. A more strenuous imagined task elicits greater physiologic reactions than a less strenuous task) [11]. These are the possible reasons why this training is also effective.

But the duration of this MIT intervention was only 10 minutes and the most of the remaining time they were using their unaffected limb instead of the affected one in carrying out their Activities of daily living. So there was a lack of repetitive movements to the affected side. This may be a reason why the result had shown less improvement in this group than other two groups.

The above data suggest that stroke subjects participating in a 15 days of combined CIMT and MIT can improve motor function of the affected upper extremity which is associated with massed practice cortical reorganization of the damaged hemisphere during affected hand movements

Limitations of the Study

Only a small sample size is taken in this study. If a larger sample is selected, the findings may be further substantiated. Dominant upper extremity involvement, which could have affected the overall outcome of the study was not considered. Severity of spasticity was not considered. The duration of chronic stroke in this study was more than 6 months. Upper limit of the duration of chronic stroke was not considered. This could have also affected the overall outcome.

CONCLUSION

It is evident from the results that combined CIMT and MIT have shown significant level of improvement in function when compared with CIMT and MIT alone in the stroke subjects as quantified using FMS and ARAT. Although all the three groups showed significant improvement, the subjects in group I (CIMT and MIT) showed greater improvement when their outcome (difference of pre and post) was compared with the other two groups. The experimental hypothesis is accepted which states that *"Combined effect of Constraint Induced Movement therapy and Mental Imagery Training will show a significant improvement in upper extremity function in chronic stroke"*. Hence this study can be concluded as combined CIMT and MIT has more effect than that of giving alone on improving upper extremity function in subjects with chronic stroke and hence can be used in clinical set ups.
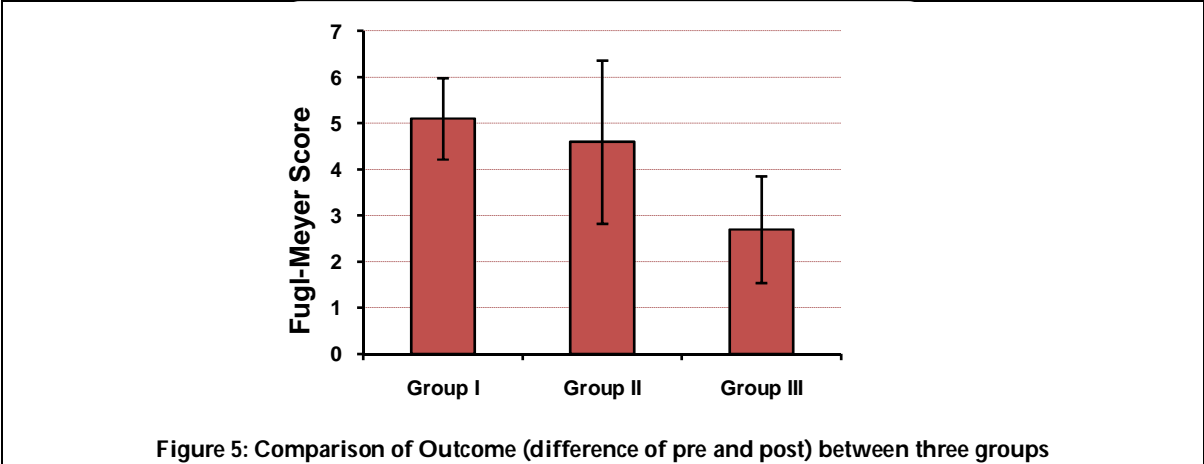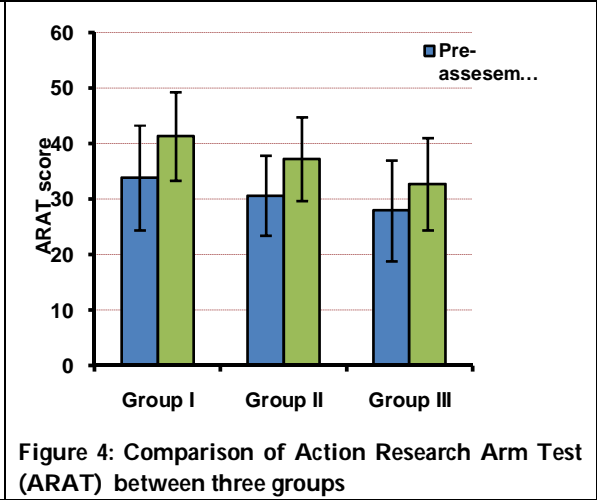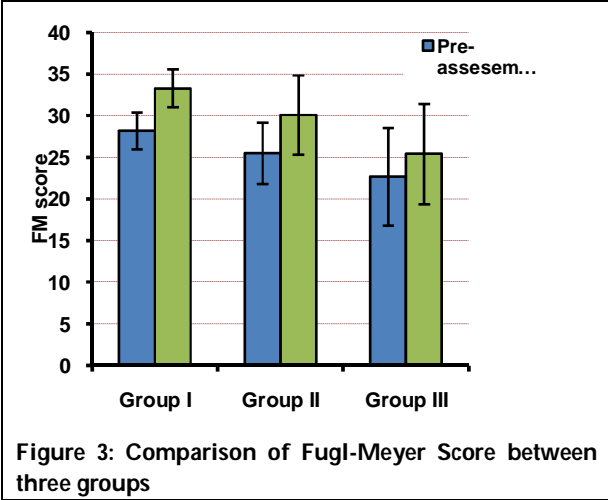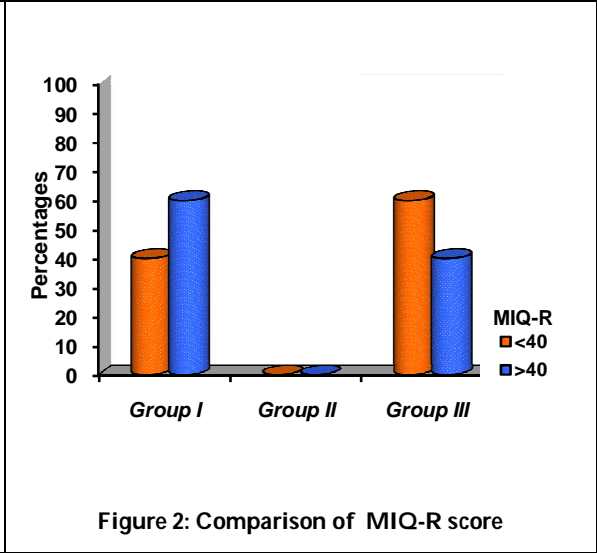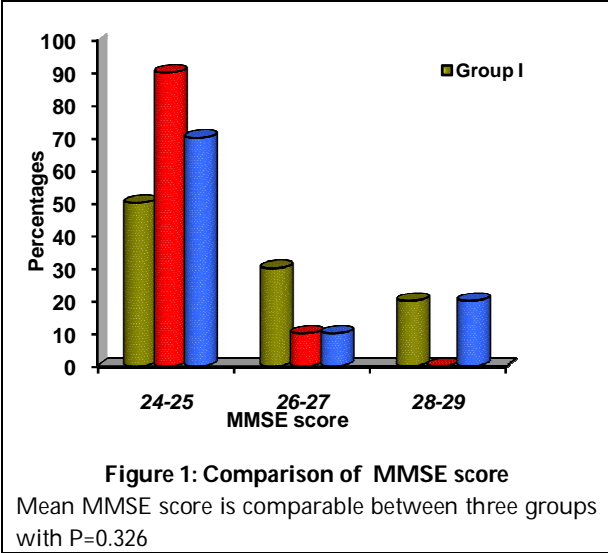
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Figure 1: Comparison of MMSE score

Mean MMSE score is comparable between three groups with P=0.326

Figure 2: Comparison of MIQ-R score

Figure 3: Comparison of Fugl-Meyer Score between three groups

Figure 4: Comparison of Action Research Arm Test (ARAT) between three groups

Figure 5: Comparison of Outcome (difference of pre and post) between three groups

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RESEARCH ARTICLE

Large Sets of Wrapped Hamilton Cycle Decompositions of Blown up Cycles $C_m[\overline{K}_n]$

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ABSTRACT

In this paper, we prove the existence of large sets of Wrapped Hamilton cycle decompositions of the blown-up cycles $C_m[\overline{K}_n]$ for $m, n \geq 3, n \neq 6$.

INTRODUCTION

A subgraph C_k of the form $(x_1 x_2 x_3 \ldots x_{k-1} x_k)$ of K_v with k vertices $x_1, x_2, x_3, \ldots, x_k$ and k edges $x_1 x_2, x_2 x_3, \ldots, x_{k-1} x_k, x_k x_1$ is said to be a k-cycle. When $k = n$, a k-cycle is called a Hamilton cycle. A decomposition of a graph G is a collection $\{H_1, H_2, \ldots, H_k\}$ of edge-disjoint subgraphs whose union is $E(G)$. Decomposition of G into Hamilton cycles is called a Hamilton cycle decomposition. We call a Hamilton cycle decomposition $\mathfrak{C}$ is simple, if no Hamilton cycle in $\mathfrak{C}$ is repeated. The problem of finding a Hamilton cycle decomposition is not new, see [4].

The graph $G[H]$ denotes the composition (also known as wreath product or lexicographic product) of graphs G and H, obtained by replacing every vertex of G by a copy of H and every edge of G by the complete bipartite graph $K_{|H|,|H|}$. We say that $G[H]$ arose from G by blowing up by H. In this paper, we consider the blown-up cycle $C_m[\overline{K}_n]$, where $\overline{K}_n$ denotes the complement of the complete graph K_n. Let $V(C_m[\overline{K}_n]) = \cup_{i=0}^{m-1} X_i$, where $X_i = \{x_{i,0}, x_{i,1}, \ldots, x_{i,n-1}\}, 0 \leq i \leq m - 1$, be the partite sets of the graph $C_m[\overline{K}_n]$.

Lindner and Rodger [5] introduced a tricycle system, which is defined as follows: a $3m$-tricycle of a complete tripartite graph $K_{n,n,n}$ is a cycle $(x_{0,0}, x_{1,0}, x_{2,0}, x_{0,1}, x_{1,1}, x_{2,1}, \ldots, x_{0,m-1}, x_{1,m-1}, x_{2,m-1})$ of length $3m$ of the form denoted by any cyclic shift of itself or $(x_{1,0}, x_{0,0}, x_{2,m-1}, x_{1,m-1}, x_{0,m-1}, x_{2,m-2}, \ldots, x_{2,1}, x_{1,1}, x_{0,1}, x_{2,0})$. We say that a $3n$-tricycle of $K_{n,n,n}$, as a wrapped Hamilton cycle in $K_{n,n,n}$. A wrapped Hamilton cycle decomposition of $K_{n,n,n}$ is denoted by $WHD(3:n)$. In general, a wrapped Hamilton cycle in $C_m[\overline{K}_n]$ is of the form

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$(x_{0,0}, x_{1,0}, \dots, x_{m-1,0}, x_{0,1}, x_{1,1}, \dots, x_{m-1,1}, \dots, x_{0,n-1}, x_{1,n-1}, \dots, x_{m-1,n-1})$ or any of its cyclic shift $(x_{1,0}, x_{0,0}, x_{m-1,n-1}, \dots, x_{0,n-1}, \dots, x_{m-1,1}, x_{m-2,1}, \dots, x_{0,1}, \dots, x_{m-1,0}, x_{m-2,0}, \dots, x_{2,0})$. A wrapped Hamilton cycle decomposition of $C_m[\bar{K}_n]$ is a decomposition of the edges of $C_m[\bar{K}_n]$ into wrapped Hamilton cycles and is denoted by $WHD(m:n)$. A large set of wrapped Hamilton cycle decompositions of $C_m[\bar{K}_n]$, denoted by $LWHD(m:n)$, is a partition of the set of all wrapped Hamilton cycles of $C_m[\bar{K}_n]$ into $WHD(m:n)'s$. In [3], Zhao and Rodger, proved the existence of a $LWHD(3:n)$ for $n \equiv 0,1,3(mod\ 4)$. In this paper, we prove the existence of $LWHD(m:n)$ for all $m, n \geq 3, n \neq 6$.

A latin square L of order n, see [6], is an $n \times n$ array in which each cell contains a single element from Z_n, such that each element occurs exactly once in each row and exactly once in each column. A quasigroup of order n is a pair $(Q, \circ)$, where Q is a set of size n and $"\circ"$ is a binary operation on Q such that for every pair of elements $a, b \in Q$, the equations $a \circ x = b$ and $y \circ a = b$ have unique solutions. A latin square of order n is equivalent to a quasigroup of order n, consequently, each cell can be expressed as a triple $(r_i, c_j, r_{ij} = r_i \circ c_j)$, that is the symbol of the cell (r_i, c_j) is r_{ij}. A transversal T of a latin square of order n on the symbols $\{1,2,\dots,n\}$ is a set of n cells, exactly one cell from each row and each column, such that each of the symbols in $\{1,2,\dots,n\}$ occurs in a cell of T. If the quasigroup $(Z_n, \circ)$ has n disjoint transversals, then for each $i \in Z_n$ the ith transversal T_i can be written as $T = \{(r, c_{ir}, r \circ c_{ir}) | r \in Z_n\}$, where c_{ir} is the column containing the cell in row r of T_i.

We follow the terminologies of [3] for the sake of completeness. Let $Sym(Z_n)$ denote the symmetric group on Z_n. For permutations $\sigma_0, \sigma_1, \dots, \sigma_{m-1}$ and for a wrapped Hamilton cycle $H = (x_{0,0}, x_{1,0}, \dots, x_{m-1,0}, x_{0,1}, x_{1,1}, \dots, x_{m-1,1}, \dots, x_{0,n-1}, x_{1,n-1}, \dots, x_{m-1,n-1})$ let

$\sigma_0 H = (\sigma_0(x_{0,0}), x_{1,0}, \dots, x_{m-1,0}, \sigma_0(x_{0,1}), x_{1,1}, \dots, x_{m-1,1}, \dots, \sigma_0(x_{0,n-1}), x_{1,n-1}, \dots, x_{m-1,n-1})$

$\sigma_1 H = (x_{0,0}, \sigma_1(x_{1,0}), \dots, x_{m-1,0}, x_{0,1}, \sigma_1(x_{1,1}), \dots, x_{m-1,1}, \dots, x_{0,n-1}, \sigma_1(x_{1,n-1}), \dots, x_{m-1,n-1})$

$$\vdots \qquad\qquad \vdots$$

$\sigma_{m-1} H = (x_{0,0}, x_{1,0}, \dots, \sigma_{m-1}(x_{m-1,0}), x_{0,1}, x_{1,1}, \dots, \sigma_{m-1}(x_{m-1,1}), \dots, x_{0,n-1}, x_{1,n-1}, \dots, \sigma_{m-1}(x_{m-1,n-1})),$

where $\sigma_j(x_{i,k}), 0 \leq j \leq m-1$, denotes the image of the element $x_{i,k}$ under the permutation σ_j.

As in [3], if G is a subgroup of $Sym(Z_n)$, then $Sym_G(Z_n)$ denotes a set of right coset representatives of G in $Sym(Z_n)$. For $X \subseteq Z_n$, $Sym(X)$ denote the subgroup of $Sym(Z_n)$ which fixes the elements that are not in X. Let $\wp$ be a family of distinct permutations on Z_n. A subgroup G of $Sym(Z_n)$ is called a complete automorphism group of order $|G|$, see [3], over Z_n of $\wp$ if the following two conditions are satisfied:

(a) $\sigma P \in \wp$ for any $\sigma \in G$ and $P \in \wp$; and

(b) For each $P, P^{'} \in \wp$, if $\sigma \in Sym(Z_n)$ is the unique permutation satisfying $\sigma P = P^{'}$, then $\sigma \in G$.

It is easy to see that, not every subgroup of $Sym(Z_n)$ has a complete automorphism group.

Lemma 1.1. If $\wp$ is a family of n distinct permutations on Z_n and has a complete automorphism group G of order n, then all permutations in $\{\sigma\wp | \sigma \in Sym_G(Z_n)\}$ are pairwise distinct, where $\sigma\wp = \{\sigma P | P \in \wp\}$. Thus, $\cup_{\sigma \in Sym_G(Z_n)} \sigma\wp$ is a partition of the set of all permutations on Z_n.

Proof. Suppose there exist $\sigma_1 \neq \sigma_2 \in Sym_G(Z_n)$ such that $\sigma_1 \wp \cap \sigma_2 \wp \neq \emptyset$. That is to say, there exist $P, P^{'} \in \wp$ such that $\sigma_1 P = \sigma_2 P'$. Then $(\sigma_1 \sigma_2^{-1})P = P'$ and $\sigma_1 \sigma_2^{-1} \in G$ by the definition of complete automorphism group G over Z_n. This implies $G\sigma_1 = G\sigma_2$, so σ_1 and σ_2 belong to the same right coset of G. This is a contradiction.

Let $\wp = \{P_0, P_1, \dots, P_{n-1}\}$ be a given family of n distinct permutations with a complete automorphism group G of order n. Below, by the shift-equivalence of wrapped Hamilton cycles, each wrapped Hamilton cycle in $C_m[\bar{K}_n]$ will be denoted by a fixed form which is decided as follows. Using $\wp$, we partition the set of all wrapped Hamilton cycles in $C_m[\bar{K}_n]$ into the following n orbits:

$O(P_i) = \begin{cases} \sigma_0 \sigma_1 \sigma_2 \dots \sigma_{m-1} H = (\sigma_0(x_{0,0}), \sigma_1(x_{1,0}), \sigma_2(x_{2,0}), \dots, \sigma_{m-1}(x_{m-1,0}), \sigma_0(x_{0,1}), \sigma_1(x_{1,1}), \dots, \sigma_{m-1}(x_{m-1,1}), \dots, \\ \sigma_0(x_{0,n-1}), \sigma_1(x_{1,n-1}), \dots, \sigma_{m-1}(x_{m-1,n-1})) \mid \sigma_0 \in Sym(Z_n^*), \sigma_1 \in Sym_G(Z_n), \sigma_2, \sigma_3, \dots, \sigma_{m-1} \in Sym(Z_n) \end{cases}, i \in Z_n.$

For each $i \in Z_n$, notice that $|O(P_i)| = |Sym(Z_n^*)|. |Sym_G(Z_n)|. |Sym(Z_n)|. \dots. |Sym(Z_n)| = (n-1)!\ (n-1)!\ n!\ n! \dots n!$. So $n. |O(P_i)| = (n-1)!\ n!\ n!\ n! \dots n!$, which equals the total number of distinct wrapped Hamilton cycles in $C_m[\bar{K}_n]$. It is

easy to see that these $(n-1)n!n!n!\ldots n!$ wrapped Hamilton cycles are pairwise distinct. Let $I = (0)$ denote the identity permutation in $Sym(Z_n)$.

The following lemma guarantees the existence of a $WHD(m:n)$, $m, n \geq 3, n \neq 6$, which has been already given in [7].

Lemma 1.2 [7] There exists a $WHD(m:n), m, n \geq 3, n \neq 6$.

Proof. The existence of a latin square with n disjoint transversals is equivalent to the existence of a pair of orthogonal latin squares of order n. A pair of orthogonal latin squares exists for $n \neq 2,6$, see [6], and so there exists latin square of order n with n disjoint transversals $T_i = \{(r, c_{ir}, r \circ c_{ir}) | r \in Z_n\}, 0 \leq i \leq n-1$ on symbols Z_n. To each T_i, we construct a Wrapped Hamilton cycle H_i of $C_m[\overline{K}_n]$ based on the vertex set $\cup_{i=0}^{m-1} X_i$ as follows:

$$H_i = \begin{cases} \cup_{r=0}^{n-1} \{x_{0,r}, x_{1,c_{ir}}, x_{2,r}, x_{3,c_{ir}}, \ldots, x_{m-3,r}, x_{m-2,c_{ir}}, x_{m-1,r \circ c_{ir}}\}, & \text{if } m \text{ is odd} \\ \cup_{r=0}^{n-1} \{x_{0,r}, x_{1,c_{ir}}, x_{2,r}, x_{3,c_{ir}}, \ldots, x_{m-3,c_{ir}}, x_{m-2,r}, x_{m-1,c_{ir}}\}, & \text{if } m \text{ is even} \end{cases}$$

From the construction of each cycle, it is clear to see that, the above n cycles partition the edges of $C_m[\overline{K}_n]$.

We call a latin square A of order n has a complete automorphism group of order n, if the rows of A form a complete automorphism group of order n. If the latin square A of order n has a complete automorphism group of order n, then we denote it by $CL(n)$. One can verify that, not every latin square has a complete automorphism group.

Lemma 1.3 For $m, n \geq 3, n \neq 6$, if there exists a $CL(n)$ and a $WHD(m:n)$, then there exists an $LWHD(m:n)$.

Proof. Let $A = CL(n)$ be a latin square on Z_n, where the rows of A form a complete automorphism group. By lemma 1.2, there exists a $WHD(m:n)$, say $\mathcal{H} = \{H_0, H_1, \ldots, H_{n-1}\}$. Now, let $(\wp(A)) = \{A_0, A_1, \ldots, A_{n-1}\}$, where $A_r, 0 \leq r \leq n-1$, is the rth row of A. That is, $\wp(A)$ contains n distinct permutations with a complete automorphism group G of order n. From the orbit classification of the set of all wrapped Hamilton cycles in $C_m[\overline{K_n}]$ above using $\wp(A), H_i \in O(A_i)$ for $i \in Z_n$.

Now, $\{\sigma_0\sigma_1\sigma_2 \ldots \sigma_{m-1}\mathcal{H} | \sigma_0 \in Sym(Z_n^*), \sigma_1 \in Sym_G(Z_n), \sigma_2, \sigma_3, \ldots, \sigma_{m-1} \in Sym(Z_n)\}$ is an $LWHD(m:n)$ by the orbit classification defined above Lemma 1.2, where $\sigma_0\sigma_1\sigma_2 \ldots \sigma_{m-1}\mathcal{H} = \{\sigma_0\sigma_1\sigma_2 \ldots \sigma_{m-1}H_i | H_i \in \mathcal{H}\}$.

Theorem 1.1 There exists an $CL(n), n \neq 2$.

Proof.

Case 1. n is odd.

In this case $CL(n)$ is simply the addition table of Z_n, which has a complete automorphism group $G = \langle\sigma\rangle$ of order n, where σ is a permutation given by $\sigma = (0\ 1\ 2 \ldots n-1)$.

Case 2. n is even.

In this case we define the latin square $CL(n) = (r_{ij}), 0 \leq i, j \leq n-1$, as follows:

$r_{ij} = \begin{cases} i + j, & i \text{ or } j \text{ is even} \\ i + j - 2, & i \text{ and } j \text{ are odd} \end{cases}$

Let $\wp(CL(n))$ be the set of permutations of $Sym(Z_n)$ that contains the rows of $CL(n)$. Now it is easy to see that $G = Aut(\wp(CL(n)))$ and hence is a complete automorphism group of order n.

Theorem 1.2 There exists a $LWHD(m:n)$ for any $m, n \geq 3, n \neq 6$.

Proof. The result follows from Lemmas 1.2, 1.3 and Theorem 1.1.

ACKNOWLEDGMENTS

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REVIEW ARTICLE

A Combined Phytopharmacological Study on Sweet and Bitter Orange *Citrus sinensis* and *Citrus aurantium* (Rutaceae)

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ABSTRACT

The discovery of a novel chemical component from a medicinal plant may form the basis of development of various therapeutic agents with better activity. More than 500 medicinal plants have been reported to possess medicinal properties. In Citrus genus *Citrus sinensis* and *Citrus aurantium* belongs to the family *Rutaceae* and is native to tropical and subtropical areas in Southeast Asia. The whole plant possess high medicinal value and traditionally used in treating various ailments for human beings. The plant is used internally as well as externally. The extracted oils have been recognized as safe for their wide uses as antibacterial, antifungal, antioxidant, anti-inflammatory and anxiolytic effects, and have analgesic activity. Limonene was determined as the main component of bitter orange peel essential oil followed by β-myrcene, linalool and α-pinene. The major compound in Tunisian neroli essential oil extracted from C. *aurantium* blossoms is 25.7% linalool. *C. sinensis* is consumed all over the world as an excellent source of vitamin C, which is a powerful natural antioxidant that builds the body's immune system. It has been used traditionally to treat ailments like constipation, cramps, colic, diarrhoea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, depression and stress.

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Phytochemical investigation of the plant shows high quantity of phenolic compounds including several flavonoid compounds. The citrus extracts and essential oils are known to exhibit various biological activities such as antimicrobial and antioxidant activities. The plant has been demonstrated to possess multiple pharmacological activities such as anti-inflammatory, antidiabetic, antibacterial, analgesic, anti-infertility, antidiarrhoeal, antinociceptive and cardioprotective. This review highlights on the existing information particularly on the phytochemistry and various pharmacological properties of *Citrus sinensis* and *Citrus aurantium* which may provide incentive for proper evaluation of the plant as a medicinal agent.

Keywords: *Citrus sinensis*, *Citrus aurantium*, Phytochemical investigation, powerful natural antioxidant.

INTRODUCTION

The foundations of typical traditional systems of medicine for thousands of years that have been in existence have formed from plants. The plants remain to offer mankind with new medicines. Some of the beneficial properties ascribed to plants have recognized to be flawed and medicinal plant treatment is based on the experimental findings of hundreds to thousands of years. Medicinal plants have proved their sole role in coping with a number of deadly diseases including cancer and the diseases associated with viral onslaught viz. Hepatitis, AIDS etc. Even today, plants are not only indispensable in health care, but form the best hope of source for safe future medicines[1].The WHO endorses and promotes the addition of herbal drugs in national health care programs because they are easily accessible at a price within the reach of a common man and are time tested and thus considered to be much safer than the modern synthetic drugs[2].Thus, the research of pharmacologically/ biologically active agents obtained by screening natural sources such as plant extracts had led to the detection of many pharmaceutically valuable drugs that play a key role in the treatment of human diseases[3]. The phytochemical-pharmacological research work has recently yielded effective solutions to certain diseases which synthetic drug industry has failed to afford. The most important among them are the research work on *Citrus sinensis* and *Citrus aurantium*. Such plants have been found to contain molecules of high drug values and are considered as medicinal herbs of great significance.

Phytochemical Studies

The genus *Citrus* belongs to the family *Rutaceae* and is native to tropical and subtropical areas in Southeast Asia. The citrus plants are grown worldwide and ranks top in world production and trade among the fruit trees. Citrus fruits are richer sources of bioactive compounds having beneficial effect on human health such as vitamin C, carotenoids, flavonoids, limonoids, essential oils, acridone alkaloids, minerals and vitamin B complex. Majority of citrus fruits are eaten fresh (such as sweet orange, mandarins, grapefruits etc). Many citrus fruits are used to prepare juices, pickles and other recipes as flavoring agents. *Citrus aurantium* L. (Rutaceae), known as sour or bitter orange, is extensively consumed in Mediterranean countries as marmalade and a flavoring agent [4]. The extracted oils have been recognized as safe for their wide uses as antibacterial, antifungal, antioxidant, anti-inflammatory and anxiolytic effects [5-8], and have analgesic activity[9].Limonene was determined as the main component of bitter orange peel Essential Oils, followed by β-myrcene, linalool,-pinene, and α-pinene [10]. The major compound in Tunisian neroli Essential Oil extracted from C. *Aurantium* blossoms is 25.7% linalool [11]. The (R)-(-)-linalool was 59–64% in*Citrus*(south andsouth-central Brazil), whereas the hydrolate (orange water) of *C. Aurantium* has not katone (17%),α-terpineol (10%), linalool (10%), and limonene (0.8%) [12]. The biological function of the chemical components of the *Citrus aurantium* L zest essential oil is not limited to their antioxidant and antimicrobial activity. Some of them also have antitumor (linalool, borneol), anti-inflammatory (sabinene, pinene), and analgesic function (citral). C. *sinensis* represents the largest citrus cultivar groups grown around the world, accounting for about 70% of the total annual production of Citrus species [13].C. *sinensis* is native to Asia and is now widespread throughout the Pacific

and warm areas of the world. *C. sinensis* is consumed all over the world as an excellent source of vitamin C, which is a powerful natural antioxidant that builds the body's immune system [14]. It has been used traditionally to treat ailments like constipation, cramps, colic, diarrhoea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, depression and stress [15]. Chemical Composition *C. sinensis* is a rich source of secondary metabolites which contribute to the pharmacological activities attributed to this plant. Several types of chemical compounds have been identified in fruits, peel, leaves, juice and roots of *C. sinensis*, which include the following groups: flavonoids 1–54[16]. Steroids 55,56, hydroxyamides, alkanes and fatty acids 57–60, coumarins 61–67, peptides 68–70 [17], carbohydrates 71–74 [18], carbamates and alkylamines 75–78 [19], carotenoids 79–82[20],volatile compounds 83–148 [21], and nutritional elements such as potassium, magnesium, calcium and sodium .

Ethanomedical Uses

Citrus aurantium and *C. sinensis* is consumed all over the world as an excellent source of vitamin C, which is a powerful natural antioxidant that builds the body's immune system [22]. It has been used traditionally to treat ailments like constipation, cramps, colic, diarrhea, bronchitis, tuberculosis, cough, cold, obesity, menstrual disorder, angina, hypertension, anxiety, depression and stress [23].

Pharmacological Studies

Pharmacological studies have confirmed that *Citrus sinensis* and *Citrus aurantium* exhibit a broad range of biological effects. However, the crude extract of the plant have been used as a traditional medicine for the treatment of various diseases, some of which are very interesting for possible future development.

Antibacterial and Antifungal Activity

Antibacterial efficacy of peel extract of *C. sinensis* and *C. aurantium* against 3 bacteria by Agar well diffusion assay. *K. Pneumoniae* exhibited higher susceptibility to peel extracts while *B. Cereus* was least affected. *C. sinensis* peel extract had high inhibitory potential than peel extract of *C. aurantium*. It has been experimentally shown that extracts and essential oil from peels of Citrus fruits exhibit inhibitory activity against microorganisms. In a study, Kirbaşlaret al[24]evaluated antibacterial activity of peel oil from Citrus fruits. It was observed that peel oil of *C. sinensis* and *C. aurantium* exhibited more or less similar inhibition of Gram positive and Gram negative bacteria. The study of Siddique et al [25]showed the efficacy of essential oil from peel of *C. aurantium* to inhibit Gram positive bacteria but not Gram negative bacteria. The ethanolic extract from peel of *C. sinensis* was shown to inhibit Gram positive to higher extent than Gram negative bacteria10.Tumane et al. [26] observed marked inhibitory effect of ethanolic and methanolic extract of *C. aurantium* against a panel of bacteria. In the present study, we determined inhibitory effect of peel extract of *C. aurantium* and *C. sinensis* against *C. capsicum* isolated previously from chilli anthracnose by poisoned food technique. The peel extracts inhibited mycelial growth of the fungus to >50%. Among peel extracts, *C. aurantium* had high antifungal effect than that of *C. sinensis*. The essential oil and extracts from peels of citrus fruits are shown to exhibit antifungal activity. It has been found thatthe peel extract of *C. sinensis* significantly inhibited the growth of *Fusarium oxysporum*to higher extent when compared to leaf extract[27]. The essential oil from fully ripe fruit peel of *C. reticulate*was shown to exhibit inhibitory activity against *Alternaria alternata, Rhizoctonia solani, Curvularialunata, Fusariumoxysporum and Helminthosporium oryza* [*e*28]. It has been found that essential oil from peel of *C. sinensis* exhibit inhibitory activity against growth of *Aspergillus flavus* [29,30].

Cosmetic Uses

Citrus aurantium Amara (Bitter Orange) Flower Oil has the most reported uses of the cosmetic ingredients in this report, with a total of 99; the majority of the uses are in leave-on skin care preparations. *Citrus aurantium* Dulcis (Orange) Flower Extract has the second greatest number of overall uses reported, with a total of 70; a majority of the uses are in rinse-off and leave-on skin care preparations. The results of the concentration of use survey conducted by the Council indicate that *Citrus aurantium* Dulcis (Orange) Flower Extract has the highest reported maximum

concentration of use; it is used at up to 6% in eye shadow. *Citrus aurantium* Dulcis (Orange) Flower Oil had the second highest reported maximum concentration of use; it is used at up to 0.66% in a depilatory [31].

Antioxidant Activity

Essential oils rich in monoterpenes are recognized as food preservatives and monoterpenic essential oils are natural antioxidants that are active against certain cancer [32]. Indeed, a number of monoterpenes have antitumoral activity that can prevent the formation or progress of cancer and cause tumor regression. Limonene and perillyl alcohol have a well-established protective activity against many types of cancer [33]. The citrus essential oils by DPPH assay, four essential oils from *Citrus aurantium* L zest showed scavenging effects in the range from 17.7% to 34.1% [34]. These results are different from us, in our study. While in our study, the essential oil from *Citrus aurantium* L showed the highest antioxidant activities valued 88.1% in DPPH assay. The biological function of the chemical components of the *Citrus aurantium* L zest essential oil is not limited to their antioxidant and antimicrobial activity. Some of them also have antitumor (linalool, borneol), anti-inflammatory (sabinene, pinene), and analgesic function (citral) [35].

Antidiabetic Activity

The orange fruit peels were extracted using cold maceration with a mixture solvents of ethanol: acetone (4:1). Induction of diabetes in rats, in this study, was conducted based on the method of destruction of the pancreas by giving diabetonic alloxan. Dose of 150 mg/kg alloxan monohydrate was given by intraperitoneal route, which was able to induce the diabetic condition in rats. Induction of alloxan caused an increment in blood glucose levels to ± 200 mg/dL, which was considered as diabetic rats. The negative control rats were treated with CMC-Na administration of 0.5%, while the rats administered with glibenclamide dose of 0.45 mg/kg were taken as positive control. All the treatments were given the orange peel extract with dosage of 125 mg/kg, 250 mg/kg, and 500 mg/kg for 10 days [36,37].

Antihypercholesterolemic Activitiy

The cholesterol-lowering effect test, which is used to determine the effect of citrus fruit peel extract in lowering cholesterol levels in the blood, was conducted in dosages with three different ratings. The positive control was treated with cholesteramine form of 0.8 g/kg BW daily. The study stated that cholestyramine, which was given 800 mg/kg BW, reduced cholesterol by 52.97% ± 1.12% when administered for 30 days. In this study, cholesteramine given for 14 days (2 weeks) reduced the blood cholesterol by 34.20% ± 10.42%. The CMC-Na 0.5% was used as a negative control, and it does not affect the blood cholesterol levels [38].

Anticancer Activity

D-Limonene, the most abundant constituent of navel orange essential oil, has been shown to have anti-proliferative and apoptosis-inducing effects [39,40], thus it has been used as a chemo preventive and chemotherapeutic agent against multiple types of tumors [41,42]. Other components of the essential oil, such as α-pinene, have been shown to inhibit growth of non-small-cell lung carcinoma cells [43]. Citral could reduce the proliferation of MDA-MB-231 cells by inhibiting the cancer stem cell marker ALDH1A3 (Aldehyde dehydrogenase 1 family, member A3) [44]. This evidence suggested that essential oil might have good anticancer activity as well.

Anti-Parasitic Activity

Parasitic diseases are serious worldwide public health problems, and *C. sinensis* is an alternative in the treatment and control of these diseases. The hexane (IC5042.13µg/mL), chloroform(IC5088.03µg/mL), ethyl acetate (IC5026.67µg/mL), acetone (IC50> 100µg/mL), and methanol(IC50> 100µg/mL) extracts of *C. Sinensis* peel, displayed moderate antimalarial activity against chloroquine (CQ)-sensitive (3D7) strain of *Plasmodium falciparum*. In this study, various standard drugs were used: artemisin in (3D7 strain IC500.0045µg/mL), chloroquine (3D7 strain IC500.021µg/mL), CQ diphosphate (D6 strain IC500.00311µg/mL), mefloquine (D6 strain IC500.01608µg/mL) andquinine (3D7 strain IC500.02µg/mL). The petroleum ether and methanol extracts of *C. sinensis* showed moderate

antimalarial activity against *P. Falciparum* FCK 2 strain having IC50values of 51.06and 53.61μg/ mL, respectively[45,46].

Analgesic, Antipyretic and Wound Healing Activity

In the analgesic study of *C. sinensis* of petroleum ether extract (150 and 300 mg/kg doses) was carried out by writhing reflux method. Antipyretic activity was evaluated on yeast-induced pyrexia in rats and wound healing activity was performed by using Incision and Excision wound model. Phytochemical study showed the presence of flavonoids and free phenolic acid. The acute toxicity was observed at 3 g/kg. The CSPE produced significant analgesic and antipyretic effect in the dose dependent manner and appreciable wound healing activity was exhibited at the dose level of 300 mg/kg on the models. CSPE possess moderate and dose dependent analgesic and antipyretic activity. Wound healing study demonstrates the potent healing activity [47].

Anti-Inflammatory Activity

Ethanolic extract of *C. sinensis* showed dose dependent anti-inflammatory activity in carrageenan induced paw edema in rats. Low and medium doses i.e., 200 and 300 mg kg-1 of the ethyl acetate extract had shown significant difference in the anti-inflammatory activity as compared to the normal control (p<0.05) group as well as diclofenac (p<0.05) treated group whereas, insignificant difference when compared with disease control group. High dose (400 mg kg-1, p.o.) treated group showed insignificant difference in the anti-inflammatory activity when compared to the normal control and standard groups but significant difference (p<0.05) when compared with disease group. Hence, it was observed that the higher dose significantly attenuated carrageenan induced ipsilateral right hind paw edema in rats[48].

CONCLUSION

From the above study, it can be concluded that plants have a very versatile life-style. Every part of the plant is serving as a boon for all living ones all over the universe. In order to evaluate the overall implications of *C. aurantium* and *C. sinensis* as having different activities such as antidiabetic activity, analgesic activity, wound healing activity, antipyretic activity, anti-inflammatory activity, antiparasicitic activity, anti hypercholesterolemic activity and anticancer activity due to having different phytoconstituents. As this plant has no contraindication and no toxic effect, therefore it used as a valuable medicinal plant. Thus, it is believed that detailed information presented in this review would help the researchers to get aware of this plant and extensive research should be undertaken on *C. aurantium* and *C. sinensis* for establishing new therapeutic drugs for mankind.

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RESEARCH ARTICLE

Effect of Ultracavitation on Body Fat in Middle Aged Obese Females

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ABSTRACT

This study aims to analyse the effect of Ultracavitation to reduce the body fat in middle aged obese females. Thirty obese females between 30 to 40 yrs of age were randomly selected as subjects for the study. Pretest measurements of Waist Hip ratio and Waist circumference measurement were recorded with the help of inch tapes. After the pretest measurement, the subjects received ultra cavitations for three weeks and on 21st day post test measurements were taken for all the subjects. The results of the study indicate that Waist Hip ratio and Waist measurement and in turn body fat reduced in middle aged obese females following ultra cavitation for three weeks.

Keywords: Ultracavitation, Body fat, Obesity, Waist Hip Ratio, Waist measurement

INTRODUCTION

Obesity is a condition where excess body fat accumulates in the body to the extent of having negative impact on health. People are generally considered obese when their body mass index (BMI), a measurement obtained by dividing a weight of a person in kilogram by the height of a person in meters, is over 30 kg/m², with the range 25–30 kg/m² defined as overweight. In the last 3 decades, the worldwide prevalence of obesity has increased 27.5% for adults and 47.1% for children. Obesity results due to combination of excess intake of food, decreased or absence of physical exercise and susceptible genetic background. It had been identified worldwide as the leading preventable cause of death. In 2015, 600 million adults (12%) and 100 million children were obese. In the last 3 decades, the worldwide prevalence of obesity has increased 27.5% for adults and 47.1% for children [1].Obesity is more common in women than men. It had emerged as one of the most threatening health problems of this century. The main treatment for obesity consists of dieting and physical exercise. Drugs and Surgical procedures are also tried to control obesity. The results are inconsistent and hence there is a need to identify alternate methods for safe reduction

of body fat. Ultracavitation is one of the recent methods tried to control obesity. Ultrasonic cavitation simply delivers sound waves to wash out fat from the body. It is a non invasive procedure which delivers low frequency waves to specific body parts. The layer of fat below the site of treatment undergoes vibration and gets heated. The pressure produced gradually liquefies fat cells and releases the same into the bloodstream. It is believed that these fatty acids is processed by the body through the lymphatic system. This study aims to analyze the usefulness of Ultracavitation in reducing the body fat of middle aged obese females. The results of the study will help the obese females and physiotherapists to incorporate new techniques in body fat management.

MATERIALS AND METHODS

This study is a pre post experimental trial. Thirty middle aged obese females between the age group of 30 and 40 attending to obesity clinics in Salem without major associated conditions were randomly selected. Purpose of the study was detailed to the subjects and a written consent was obtained before inclusion into the study. Pretest assessment of Waist circumference measurement was estimated using inch tape. The midpoint between the lowest ribs and the iliac crest was identified and circumference measurement was taken at that level using inch tape in centimeters [2]. Subjects were asked to stand with little clothing, keeping feet close to each other and bearing even weight on both side and arms at the side. The subjects were asked to relax during the measurement. Measurement was done at the end of the expiratory phase of a normal respiration. Measurement was taken two times and if there is a difference of less than1 cm between the measurements then average of the same was recorded. If the difference is more than 1 cm then both the measurements are done again. After the pre – test assessment of waist circumference was over, ultra cavitation treatment was given once a week with an intensity of 30 watts and an emission of hundred percent. The total duration of the procedure lasted for 120 minutes i.e 60 minutes on either side. Feasibility of the study was analyzed by a pilot study with six subjects. Statistical analysis was performed on the collected data. Normality of the data was estimated using Shapiro-Wilk test and was found to be normally distributed. Paired t test was used as a statistical test to analyze the influence of Ultra cavitation on Waist circumference.

RESULTS

Statistical analysis was carried out using a paired t-test. The outcome of the test revealed that Waist circumference in the subjects who received Ultracavitation had significantly reduced. The numerical values of the statistical analysis are listed at the end of the article for reference. The result of the study state that there is a statistically significant reduction of Waist circumference and in turn the body fat in middle aged obese females who received Ultracavitation for a period of 3 weeks.

DISCUSSION

The results of the study are in favor of Ultracavitation as a tool to reduce Waist circumference and in turn the body fat in middle aged obese females between the age group of 30 and 40. Energy is delivered across the skin surface by the techniqueis in a low intensity, but this energy is brought to a sharp focus in the fat present in the subcutaneous level. No damage occurs at the skin level as the intensity of ultrasound energy is low in that level. Adipose tissue disruption results when focus of the ultrasound beam is done along with proprietary application techniques at specific depths beneath the epidermis. Body's inflammatory response mechanisms get activated by the chemo tactic signals after adipocytes have been disrupted. The lipids and cell debris are engulfed by the Macrophage cells are attracted to the area. Local adipose tissue volume reduces as the result of the process [3]. Mohammad zadeh M (2016) established significant reductions in anthropometric indices following treatment with Radiofrequency and Ultra cavitation [4]. Angélica R Araújo *et al* (2018) demonstrated that Ultracavitation satisfactorily reduced waist circumference and skin folds and hypodermic layer thickness [5]. Fatemi used Liposonix device to verify whether

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Ultracavitation resulted in the reduction of localized fat and observed an average reduction of 4.7cm in the exposed area [3].

CONCLUSION

We conclude from the results of the study that Ultracavitation effectively reduces the Waist circumference and in turn the body fat in middle aged obese females.

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Table 1. Waist circumference data subjected to paired 't' test

Pre test Mean	Post test Mean	Standard deviation of the Difference	Standard error of the difference	t calculated value	t table value for 19 degrees of freedom at 5% level of Significance	Inference
113.85	105.625	2.567	0.574	14.329	2.26	t calculated value > t table value. Hence statistically significant at 5% level of significance

RESEARCH ARTICLE

An Experimental Study on Effect of Small Sided Games on Agility of Junior Soccer Players

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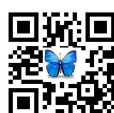Manipal University, Jaipur, India.
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ABSTRACT

The purpose of the study was to investigate the effect of small sided games (SSG) on agility of junior soccer players. Pre test - Post test Random Design was used and total 40 (n=40) junior soccer players were selected as subjects from KV, Churachandpur, Manipur, India and equally divided into two groups and four weeks small-sided games were provided to the experimental group, whereas control group did not receive any training. The dependent variable: Agility was assessed by 4 x 10 mts Shuttle Run. For normality of the data Shapiro - Wilk Test was applied. As Descriptive Statistics mean, standard deviation and standard error of means were calculated. Further, One-Way Analysis of Co-Variance (ANCOVA) was applied and the significance level was set at 0.05. All statistical analyses were computed on IBM SPSS software; Version: 25. After statistical calculations it is found that there is significant effect ($p<0.05$) of small-sided soccer games on agility ($p<0.05$) of junior soccer players.

Keywords: Small-sided games, Agility, Soccer players

INTRODUCTION

The game, football is considered to be the world's most popular sport also known as soccer. In this game there are two teams of eleven players. Soccer is played on a large grass field with a goal at each end. The object of the game is to get the soccer ball into the opposing team's goal. The key to soccer is that, with the exception of the goalie, players cannot touch the ball with their hands, they can only kick, knee, or head the ball to advance it or score a goal. Soccer is played at all levels throughout the world from small kids leagues to professional and international teams. Perhaps

the most famous soccer tournament is the World Cup (Soccer: Learn all about the sport of Soccer n.d.), football | History, Rules, & Significant Players | Britannica n.d.). Soccer is such a game which demands almost all physical fitness components like speed, strength, agility, coordination, endurance, balance, perception, reaction time, power, aerobic and anaerobic capacity too. Small sided games (SSGs) referred as skill-based conditioning games or game-based training, are modified games played on reduced pitch areas, often using adapted rules and involving a smaller number of players than traditional football games. Small-sided games allow the integrated training of the technical and tactical aspects fundamental development of young soccer players. The variability in movement during SSGs contributes to the development of a more generalized motor program to cope with a variety of similar but different situations. In addition, their structure and organization allow them to be used freely by any group of young people on any surface or space of play (e.g., street football), favouring the development of skills essential for long-term development. However, above all, they allow for engagement in activities deliberately designed to improve football performance. Evidence suggests that deliberate practice is more likely to lead to elite status when compared with more generic football-related activities and SSGs contribute to this specific area of talent development (Sarmento et al. 2018, Ford, Hodges, and Williams 2009). After reviewing the literature, the investigator thus has design the present study as: An experimental study on effect of small sided soccer games (SSG) on agility of junior soccer players.

Purpose of the Study

The purpose of the study was to investigate the effect of small sided soccer games (SSG) on agility of junior soccer players.

Design of the Study

Selection of Subjects: For the present study Pre test - Post test Random Design was used by which total 40 (n=40) junior soccer players were selected as subjects from KV, Churachandpur, Manipur, India and equally divided into two groups, such as Experimental Group (EG) Control Group (CG). Four weeks small sided games (SSG) were given to the experimental group for five days per week.

Training Protocol: There were three parts of the training protocol: Warm-Up Part, Main Part and Cooling down Part. The control group was not given any training; this group was involved with only warming-up part. Details of training protocol has been given in table no. 01

Assessments: For the collection of data the selected dependent variable: agility was assessed by 4 x 10 mts Shuttle Run Test before and after the completion of four weeks Small sided soccer games. The unit of agility was second.

Statistical Analysis: For normality of the data Shapiro-Wilk Test was applied. In Shapiro-Wilk Test P-value of Pre-Test and Post-Test Data of agility were 0.133 and 0.069 (p>0.05), that's mean the data are approximately normal distribution. As Descriptive Statistics mean, standard deviation and standard error of means were calculated. Further, One-Way Analysis of Co-Variance (ANCOVA) was applied (Verma and Ghufran 2012); (Verma 2009). The significance level was set at 0.05. Lastly, all statistical analyses were computed on IBM SPSS software; Version: 25 (IBM SPSS Statistics 25 Free Download 2017).

DISCUSSION OF FINDINGS

Observing the findings it has been evident that there is a statistically significant difference between experimental group and control group. In other way we can more clearly say that there is significant effect of small sided soccer games on agility of junior soccer players. The reason behind significant effect is nothing else, but it is the proper application of small sided games for four weeks on experimental group. The purpose of the study was to experiment

the effect of small sided soccer games on agility of junior soccer players during various SSG formats i.e.1 vs 1, 2 vs 2, 3 vs 3, 4 vs 4, 5 vs 5 and 7 vs 7 and different small-sided dimensions of the field i.e.5 x 5 mts, 5 x 8 mts, 6 x 6 mts, 8 x 8 mts, 15 x 15 mts, 16 x 16 mts, 15 x 10 mts, 25 x 20 mts, 30 x 25 mts, 40 x 40 mts etc. The investigator increased the players in the present study with the progression of small-sided games (Training). Not only that with the increased players, dimensions of the play-field also had been altered. For that purpose the investigator altered the dimensions of the field only by increasing and decreasing the dimensions like 5 x 5 mts, 5 x 8 mts, 6 x 6 mts, 8 x 8 mts, 15 x 15 mts, 16 x 16 mts etc. and sometimes many circles of different radius were made for the purpose of passing, attacking and defending the ball within the stipulated circles or rectangles or squares. Due to small-sided games, with altering the numbers of players one has to move quickly and take quick directions deliberately for changing the directions. For this reason a habit of taking and changing the direction quickly is developed within players. That's why in the present study the agility of junior soccer players has been significantly developed. Many studies which directly or indirectly support the present studies like Katis and Kellis 2009, Los Arcos et al. 2015, G. Rajasekar et al. 2014, Dellal et al. 2011, Owen et al. 2011 etc.

CONCLUSIONS

It can be concluded that there is a significant positive effect of four weeks small-sided soccer games (SSG) on agility of junior soccer players.

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Table 1

Training Protocol on Small Sided Games, Total Duration: 72 Minutes						
Warm-Up Part	**Main Part, Duration: 50 Min.**					**Cooling Down Part**
	Week-1					
	Day-1	**Day-2**	**Day-3**	**Day-4**	**Day-5**	
10 Min.	1 v/s 1 (With Goalpost), Area: 5 x 5 Mts.	2 v/s 2 (With Goalpost), Passing & Supporting Area: 5 x 8 Mts.	3 v/s 3 (With Goalpost), Passing, Supporting & Defending Area: 8 x 8 Mts.	4 +1GK v/s 4 +1GK Passing, Supporting, Defending & Depth	Match Day (Small Sided)	12 Min.
Warm-Up Part	**Main Part, Duration: 50 Min.**					**Cooling Down Part**
	Week-2					
	Day-1	**Day-2**	**Day-3**	**Day-4**	**Day-5**	
10 Min	4 v/s 4 + 4 Supporter Outside the Square Area: 15 x 15 Mts.	4 v/s 4+1 Common (Common will play / Join the team who having the ball), Area: 16 x 16 Mts. .	3 v/s 1 (4 Markers will be placed in a square formation 3 players will play against 1 defender and attackers will help to each other in four markers), Area: 6 x 6 Mts.	4 v/s 4+2 Common (Box play = Ground will be divided into 6 boxes: 2+2+2, Middle 2 boxes for common players and only 2 defenders can go to the box), Area: 15 x 10 Mts.	Match Day (Small Sided)	12 Min.
Warm-Up Part	**Main Part, Duration: 50 Min.**					**Cooling Down Part**
	Week-3					
	Day-1	**Day-2**	**Day-3**	**Day-4**	**Day-5**	
	5 v/s 5 (4	7 v/s 7	2+2+1	5 Defenders	Match Day	

10 Min	Goal Posts = 2 in each end line) Area: 25 x 20 Mts.	(Double rondo) Two circles will be made in which 5 players will stand outside the circle with two opponents inside the circle, Area: 5 mts radius 2 circles in 10 mts distance.	common v/s 2 (In a circle), Area: 5 mts radius circle	v/s 3+1 common (Common will only support attackers during counter attack in attacking third), Hi pressing by attackers Area: 30 x 25 Mts.	(Small Sided)	12 Min.
Warm-Up Part	**Main Part, Duration: 50 Min.**					**Cooling Down Part**

	**Week-4**					
	Day-1	**Day-2**	**Day-3**	**Day-4**	**Day-5**	
10 Min	4 v/s 4 (Passing & Supporting = 2 squares will be made and 4,4 players will be divided into 2 squares, ball will be played in one square and only 2 v/s players can go to other square area) Area: 15 x 7 Mts.	7 v/s 7 (Player can't pass back to the same player with whom he got the ball), Area: 30 x 25	1 v/s 1 (Use only weak foot), Area: 5 x 5 mts.	4 = 1GK V/S 5 (Ball supply by coach to free player, quick marking by defenders if not than shooting by attackers) Area: 40 x 40 Mts.	Match Day (Small Sided)	12 Min.

Table 2

**Normality Test of Data of Soccer Players**			
**Shapiro-Wilk Test**			
		Df	P-value (Sig.)
Agility	Pre-Test	40	0.133*
	Post-Test	40	0.069*

*Not Significant at p≤0.05

Table 3 - Descriptive Statistics

| Mean, SD and Standard Error of Experimental and Control Group in Pre-Post Test on Agility of Soccer Players						
			Pre-Test		Post-Test	
Variable	Group	N	Mean ± SD	Std. Error	Mean ± SD	Std. Error
Agility (Unit: Second)	Experimental	20	11.64 ± 0.70	0.17	10.38 ± 0.75	0.16
	Control	20	11.79 ± 0.79	0.17	11.57 ± 0.72	0.16

In the above table : 03 it has been found that means ± standard deviations of experimental group and control group in pre test are 11.64 ± 0.70 and 11.79 ± 0.79 seconds on agility, whereas in case of post test it is 10.38 ± 0.75 and 11.57 ± 0.72 seconds respectively. On the other hand standard error in pre test is 0.17 second in both groups respectively and in post test it is 0.16 seconds on agility in both groups.

Table 4

One Way Analysis of Co-Variance (ANCOVA) Between Experimental Group & Control Group in Pre - Post Test on Agility of Soccer Players							
Variable			Sum of Squares	df	Mean Sum of Square	F- ratio	p-value (Sig.)
Agility Unit: Second	Pre-Test	B	.222	1	.222	0.397	0.532
		W	21.264	38	.560		
	Post-Test	B	14.341	1	14.341	**26.644***	**0.000***
		W	20.454	38	.538		
	Adjusted Post-Test	B	12.186	1	12.186	**34.530***	**0.000***
		W	13.058	37	.353		
*Significant at 0.05 level, F$_{0.05}$ (1, 38) = 4.10, F$_{0.05}$ (1, 37) = 4.11 or p≤0.05							

In the above table : 04 (ANCOVA) it is evident that 'F' value and 'P' value in pre-test are 0.397 and 0.532 respectively, where 'P' value (0.532) is greater than 0.05 level of significance (p>0.05). That's mean there is no significant difference between experimental group and control group on agility in pre-test phase. On the other hand 'F' values and 'P' values in post-test and adjusted post-test are 26.644, 34.530 and 0.000, 0.000 respectively, where 'P' values (0.000 & 0.000) are less than 0.05 level of significance (p<0.05). That's mean there is significant difference between experimental group and control group on agility in post-test and adjusted post-test phase respectively.

	Pre-Mean	Post-Mean	Pre-SD	Post-SD
Experimental	16.73	17.21	1.67	1.62
Control	16.76	16.75	1.72	1.72
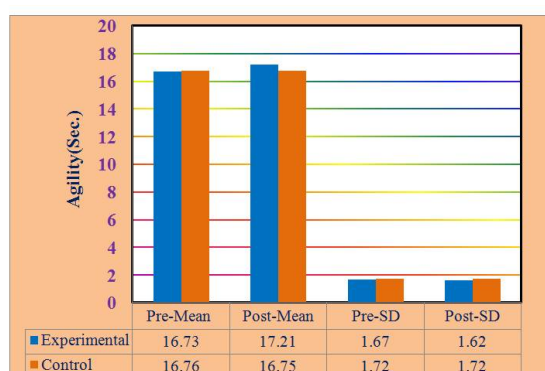
Figure: 01 Comparing Means & SD in Pre-Post Test of Experimental Group & Control Group in Respect of Agility of Soccer Players

RESEARCH ARTICLE

Connected Domination Integrity in graphs

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ABSTRACT

Let $H = (V, E)$ be a simple graph. A dominating set T of a graph H is a connected dominating set if the induced subgraph $< T >$ is connected. The number of vertices in a minimum connected dominating set is defined as the connected domination number of a graph H and it is denoted by $\gamma_c(H)$. This concept was introduced by E.Sampathkumar. The Connected domination integrity of graph H is defined by $CDI(H) = min\{|T| + m(H - T) : T$ is a connected dominating set of $H\}\}$ where $m(H - T)$ denotes the order of the largest component in $H - T$. In this paper, we introduce a new vulnerability parameter, connected dominating integrity in a graph and derive CDI of some families of graphs. Also derive few bounds of CDI.

Keywords: Connected Dominating Sets, Connected Domination Integrity Sets

INTRODUCTION

Graph theory is a very eminent tool in the communication networks. A simple graph H is the collection of vertex set V and edge set E. Let $v \in$ (H). Then the degree of v is the number of adjacent vertices with v, denoted by $deg(v)$. The maximum (minimum) degree of v are $\Delta(H)(\delta(H))$. For the basic definitions and bounds in graphs, we refer the book written by Frank Harary [8]. A Communication network or social network, nodes and links are represented as vertices and edges of the graph. The study of domination problem [7,8] was studied from 1950s onwards.

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Dominating sets are used to find shortest or efficient paths within ad-hoc mobile networks. A subset S of $V(H)$ is called dominating set DS if for every $v \in V - S$, there exist a $u \in S$ such that v is adjacent to u. The minimum cardinality of a minimal dominating set in G is called the domination number of G denoted as $\gamma(H)$ and the corresponding minimal dominating set is called a γ-set of H. An independent set I is a subset of $V(H)$ in which no two vertices or nodes are not adjacent. The independent domination number $i(H)$ of a graph H is the size of the smallest independent dominating set. A maximal independent set in a graph is also a minimal dominating set. A dominating set of H is said to be a connected dominating set if subgraph induced by S$(i.e. < S >)$ is connected. It creates a virtual network backbone for packet routing and control. Through connected dominating set, messages can be routed from the source to a neighbour closest to the destination node, and then finally reach to the destination [12]. Kazuya Sakai et al. [10] have studied about new networking protocols based on Connected Dominating Sets.

The stability of such modes are measured by the vulnerability parameters like connectivity, toughness, scattering number, tenacity, integrity and rupture degree, etc. Among all these measures, integrity (C.A, Barefoot, [2]) measure is a combination of the set of failure nodes and the biggest subnetwork within which still the communication is going. The integrity of a graph H is defined as $I(H) = min\{|X| + m(H - X): X \subset H\}$ where $m(H - X)$ is the order of a component of $H - X$. Sundareswaran and Swaminathan introduced another measure, Domination Integrity (DI) of a graph H is defined as $DI(H) = min\{|X| + m(H - X)\}$, where X is a dominating set of H and studied many bounds and results on $DI(H)$ [3,13-17]. In general, few communication networks may give same value for both $I(H)$ and $DI(H)$. To distinguish the stability of two different networks with same number of nodes and links, we should identify a new measure which is motivated us to introduce connected domination integrity (CDI). This new parameter can be helpful to increase the scalability of such networks.

Definition 2.1. The connected domination integrity of a connected graph H is denoted by $CDI(H)$ and defined as $CDI(H) = min\{|X| + m(H - X): X$ is a connected dominating set$\}$ where $m(H - X)$ is the order of a component of $H - X$.

Definition 2.2. A subset X of $V(H)$ is said to be a connected domination integrity set $CDI - set$ of H is denoted if$CDI(H) = \{|X| + m(H - X): X$ is a connected dominating set$\}$ where $m(H - X)$ is the order of a component of $H - X$.

Example 2.3. Consider the graph $H = P_{10}$ with vertices $v_1, v_2, v_3, \ldots, v_{10}$. Let $S = \{v_2, v_3, \ldots, v_9\}$ then, $(H-S) = 1$ so CDI $(P_{10}) = 9$ and $DI(P_{10}) = 6$; $I(P_{10}) = 5$

Fig.1 P_{10}

$\{v_4, v_8\}$ is a I-set, $\{v_2, v_5, v_8, v_{10}\}$ is a $DI - set$ and $\{v_2, v_3, v_4, v_5, v_6, v_7 v_8, v_9\}$ is the $CDI - set$. Clearly, $I(P_{10}) < DI(P_{10}) < CDI(P_{10})$.

Observations 2.4
For any connected graph $H, I(H) \leq DI(H) \leq CDI(H)$.

Proposition 2.5
- For complete graph $K_n, CDI(K_n) = n$
- For Path $P_n, CDI(P_n) = n - 1$
- For Cycle C_n then $CDI(C_n) = n$
- For star $K_{1,n-1}$ then $CDI(K_{1,n}) = 2$
- For Double star $D_{n,m}$ then $CDI(D_{r,s}) = 3$

- For Wheel W_n then $CDI(W_n) = \left\lfloor \frac{n}{3} \right\rfloor + 3$
- For Petersen graph P, then $CDI(P) = 7$
- For complete bipartite $K_{n,m}$ then $CDI(K_{m,n}) = min(m,n) + 1$

Observations 2.6

Let H be a connected graph with n vertices

- $2 \leq CDI(H) \leq n$.
- $DI(H) \leq CDI(H)$
- $\gamma(H) \leq \gamma_c(H) + 1 \leq CDI(H) \leq \alpha_o(H) + 1$.
- $\delta(H) + 1 \leq CDI(H)$.
- Let H be a connected graph with n vertices and a maximum degree $\Delta(H)$, then $\left\lceil \frac{n}{\Delta(H)+1} \right\rceil \leq CDI(H)$.
- For any tree T with n vertices and maximum vertex degree $CDI(T) = n - \Delta(T) + 2$, since $\gamma_c(T) \leq n - \Delta(T)$ and $T - S$ contains only $K_2's$ and $K_1's$ after the removal of any γ_c-set S of T.

Proposition 2.7

For any graph H, $CDI(H) = 2$ if and only if $H \cong K_{1,n}$.

Proof:

Let $H = K_{1,n}$. $V(H) = \{u, v_1, v_2, \ldots v_n\}$, where u is the center vertex of $K_{1,n}$ and $v_1, v_2, \ldots v_n$ are the pendent vertices of u. Clearly, $\{u\}$ is a connected domination integrity set of H. Therefore, $CDI(K_{1,n}) = 2$. Suppose $CDI(K_{1,n}) = 2$. Let X be a CDI- set of H. Then $|X| + m(H - X) = 2$. Since X is connected dominating set, $1 \leq |X| \leq 2$. If $|X| = 2$, then $m(H - X) = 0$, it is not possible. Therefore, $1 \leq |X| < 2$. So, $|X| = 1$. Then $m(H - X) = 1$. The components in $H - X$ are totally independent and all are adjacent with a single vertex (since H is connected). Thus, $H = K_{1,n}$.

Proposition 2.8

For any graph H, $CDI(H) = n$ if and only if $H \cong C_n$ $(or)K_n$.

Proof:

Let $H = C_n$ (or) K_n. It was proved that $\gamma_c(K_n) = 1$ and $\gamma_c(C_n) = n - 1$. Therefore, $CDI(H) = n$. Suppose $CDI(H) = n$. Let X be a CDI- set of H. Then $|X| + m(H - X) = n$. Therefore $|X| = k$ and $m(H - X) = n - k$. Since $n = DI(K_n) \leq CDI(K_n) \leq n$. Hence $H \cong K_n$. It is known that, $n - 1 = \gamma_c(C_n) \leq CDI(C_n)$. Therefore $H \cong C_n$.

Proposition 2.9

For any positive integer $k > 0$. There exist a connected graph H such that $CDI(H) - DI(H) = k + 1$.
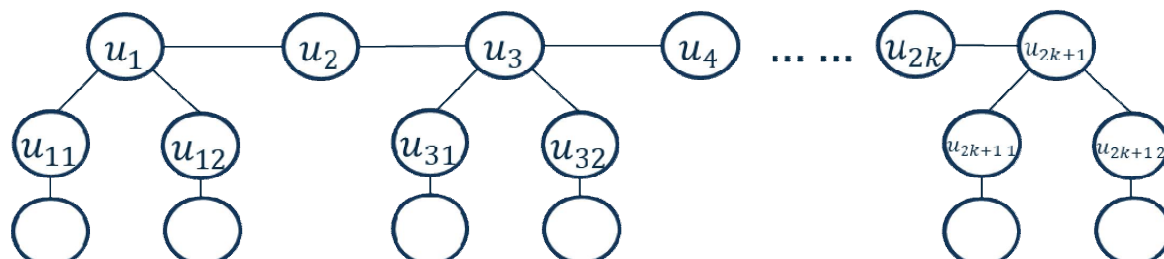
Proof.

Fig 2. a connected graph H with $CDI(H) - DI(H) = k + 1$

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Finding $DI(H)$ for the above graph:

$S = \left\{u_{11}, u_{12}\dots, u_{(2k+1)1}, u_{(2k+1)2}\right\} \cup \left\{u_2, u_4, \dots, u_{2k}\right\}$ is a DI- set of H. Therefore, $m(H - S) = k + 1$.

Thus, $DI(H) = (2k + 2) + (k + 1)$

$\qquad\qquad = (3k + 3)$

$\qquad \therefore DI(H) = 3k + 3 \rightarrow (i)$

Finding $CDI(H)$

$S = \left\{u_{11}, u_{12}\dots, u_{(2k+1)1}, u_{(2k+1)2}\right\} \cup \left\{u_1, u_2, u_3\dots, u_{2k}\right\}$ is a CDI- set of H. Therefore $m(H - S) = 1$.

Thus, $CDI(H) = (2k + 2) + (2k + 1) + 1$

$\qquad\qquad\qquad = (4k + 4)$

$\qquad \therefore CDI(H) = 4k + 4 \rightarrow (ii)$

$(ii) - (i)$

Hence $\quad CDI(H) - DI(H) = k + 1$

Proposition 2.10

For any connected graph H, $CDI(H)$ is NP-complete.

Input: A graph $H = (V, E)$ and an integer l.

Is $CDI(H) \leq l$?.

It can be easily verify that, in polynomial time whether S is a dominating set and there is a polynomial time algorithm to compute $m(H - S)$. Hence the decision problem $CDI(H)$ is in NP.

For any graph G, it can be converted to H by adding a node y to G by connecting any node x in G. Then, connect y to any node z in a K_n graph.
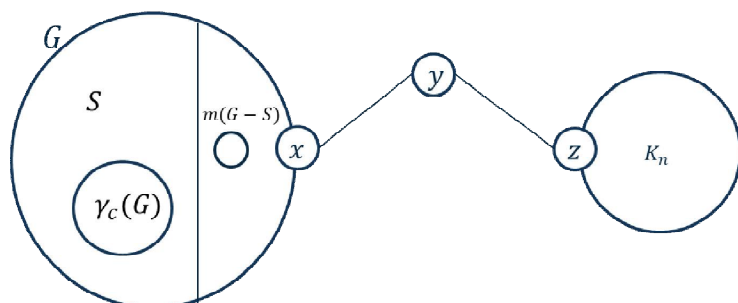

Fig. 3A connected graph H for proving $CDI(H)$ is NP-complete

Claim: $CDI(H) = \gamma_c(G) + n$

Proof. *Let $S \subseteq V(G)$ be $\gamma_c(G) - $ set m of G. that $CDI(G)$ is minimum.*

$\qquad\qquad$ *Since, $CDI(G) = \min\{|S| + m(G - S)$, S is connected dominating set of $G\}$*

Case (i):Suppose $x \in S$. Then,

$\qquad\qquad\qquad\qquad$ *$S \cup \{y, z\}$ is a gamma set of H*

$\qquad\qquad\qquad\qquad CDI(H) = \gamma_c(G) + 1 + n - 1$

$= \gamma_c(G) + n$

$\qquad\qquad\qquad\qquad\qquad CDI(H) = \gamma_c(G) + n$

Case (ii):Suppose $x \in m(G - S)$. Then,

$\qquad\quad$ *$S \cup \left\{y, z\right\}$ is a gamma set of H*

$\qquad\qquad\qquad\qquad CDI(H) = \gamma_c(G) + 3 + n - 1$

$\qquad\quad = \gamma_c(G) + n + 2$

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$CDI(H) = \gamma_c(G) + n + 2$
$We\ know\ that\ \gamma_c(G)\ is\ NP-Complete \rightarrow (iii)$
$\therefore so\ using\ (i),\ (ii),\ (iii)\ it\ can\ be\ concluded\ that\ CDI\ is\ also\ NP-Complete.$

Proposition 2.11
If a graph T is a tree, then $CDI(T) = n - l + 1$, where l represents number of leaves.

Proof:

Since any tree T on n vertices $\gamma_c(T) = n - l$ where l represents number of leaves, the number of vertices in any maximum order component is 1. Therefore, $CDI(T) = n - l + 1$.

Proposition 2.12
For a tree T of order $p > 3$, $DI(T) = CDI(T)$ if and only if every internal vertex of T is a support.

Proof:

Let S be the collection of all internal vertices of T which includes all the support vertices of T. Then, clearly S is a γ_c − set as well as γ –set of T. Therefore, $m(T - S) = 1$. Hence $DI(T) = CDI(T)$.

Proposition 2.13
If H is a cycle then $DI(H) = CDI(H)$ if and only if $H \cong C_3$.

Proof:

Given H is a cycle. Suppose $H \cong C_3$. Then $DI(H) = CDI(H)$. Conversely, let $DI(H) = CDI(H)$. S. Arumugam et. al. proved in [1], if H is a cycle then $\gamma(H) = \gamma_c(H)$ if and only if $H \cong C_3\ or\ C_4$. Since $DI(C_4) = 3\ and\ CDI(C_4) = 4$, $H \cong C_3$.

Proposition 2.14
Let H be any connected graph and S be a minimum connected domination integrity set (CDI-set) . If $DI(H) = CDI(H)$ then $\Delta(< S >) < \Delta(H)$.

Proof:

Let S be a DI-set of H. That is, $DI(H) = |S| + m(H - S)$. Suppose $\Delta(< S >) = \Delta(H)$. Then $S - \{x\}$ is a domination integrity set of cardinality less than $DI(H)$, where $x \in S$ with $\Delta(H) = \deg_{<S>} u$. This is a contradiction.

Remark 2.16
Let H be a connected graph of order n. Then if $DI(H) = CDI(H)$ then $\gamma(H)$ need not be equal to $\gamma_c(H)$.

P_5: u_5 u_4 u_3 u_2 u_1; $\gamma(P_5) = 2 < \gamma_c(P_5) = 3$; $DI(P_5) = CDI(P_5) = 4$
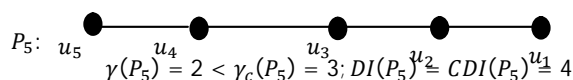
Observation 2.16
Wyatt J. Desormeauxa et. al. defined the connected order-sum number of H [18] as follows:

Let H be a connected graph of order n and let $s: d_1, d_2, \ldots, d_n$ denote the degreesequence of H written in non-increasing order. We define the connected order-sum number of H, denoted $ord_c(H)$, to be thesmallest integer k such that the sum of the first k terms of s is at least as large as $n + k - 2$; that is, k is the smallest integersuch that

$$\sum_{i=1}^{k} d_i \geq n + k - 2$$

They also proved the following results [17].

- If H is a connected graph with no dominating vertex(vertex adjacent to all other vertices in the graph is called a dominating vertex), then $\gamma_c(H) \geq ord_c(H)$.
- If T is a tree of order at least 3, then $\gamma_c(T) = ord_c(T)$.

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From the above results, it is obvious to prove the following:

- If H is a connected graph with no full degree vertex, then $ord_c(H) \leq \gamma_c(H) \leq CDI(H)$.
- If T is a tree of order at least 3, then $CDI(T) \geq ord_c(T)$.

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RESEARCH ARTICLE

Effect of Yogic Practices to Improve Pulmonary Status in Asthma Patients

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ABSTRACT

The study is to find the effect of yogic practices to improve peak expiratory flow rate and exercise tolerance (pulmonary status) in asthma patients. 20 asthma patients between 40-55 years of age were selected randomly using simple random sampling method. The pretest measurement of peak expiratory flow rate and exercise tolerance was measured using the peak flow meter and six minutes walk test for all the subjects. After the pretest assessment the subjects received yogic practices for a period of 8 weeks and on the end of 8th week posttest measurement of peak expiratory flow rate and exercise tolerance was done using the peak flowmeter and six minutes walk test for the group in a similar fashion as that of pretest measurement. The results of the study concluded that yogic practices improved peak expiratory flow rate and exercise tolerance in asthma patients.

Keywords: Asthma, Yogic practices, Peak expiratory flow rate, Exercise tolerance test.

INTRODUCTION

Asthma is a common chronic disease of the respiratory tract involving airway inflammation, bronchial hyper reactivity and changeable airflow limitation. The prevalence of asthma which affects approximately 300 million people worldwide. Asthma includes reduced peak expiratory flow rate and exercise tolerance. Asthma is a disease that occurs in all genders. This prevalence is expected to increase gradually as life shifts from village to city and people prefer a more modern life .It is one of the most common chest diseases with an increasing morbidity and mortality .It is estimated that four hundred million people will have asthma by 2025. Objectives of asthma treatment must be control of disease and ensuring a better quality of life. Asthma continues with repeated attacks even with the administration of optimal drugs, so non pharmacological strategies that help patients to cope better with asthma in disease management. Therefore currently many patients with asthma resort to complementary and integrative

applications such as breathing techniques, yoga, herbal products, acupuncture, and homeopathy for asthma treatment (1). Yoga is an ancient art, based on a harmonizing system of development for the body, mind and spirit. It is a best curative and preventive medicine for most of the problems of man resulting from the so called modern living. Yoga is recognized as one of the important and valuable gifts of our culture. Yoga is a system inclusive of physical and mental training that can benefit people of all ages. It involves asanas (body postures) and Pranayama (art of breath control), among which of its physical uses are to reduce stress-related conditions help with circulatory and respiratory disorders such as asthma and bronchitis, and improve over-all health. The yogic practices improves pulmonary status in asthma patients (2 ,3) .Warmup exercises are done before the practice of asanas. Warm-up exercises ensure mobility of joints and flexibility of muscles. Asanas involve articulation and bending Asanas open the energy channels, chakras and psychic centers of the body while increasing flexibility of the spine, strengthening bones and stimulating the circulatory and immune systems. Along with proper breathing or pranayama, asana also calm the mind and reduce stress. With regular practice one can ensure overall physical and mental health and the possible prevention of diseases. In time, performing the poses slowly and consciously becomes a mental exercise in concentration and meditation. Pranayama is generally defined as breath control .Regular practice increases and enhances the quantity and quality of pranayama, clears blocked nadis and chakras, and results in the practitioner feeling energetic, enthusiastic and positive. Practiced correctly under the right supervision pranayama brings harmony between the body, mind and spirit, making one physically, mentally and spiritually strong. Meditation is a practice in which an individual trains the mind or induces a mode of consciousness, either to realize some benefit or for the mind to simply acknowledge its content without becoming identified with that content or as an end in itself. Meditation has a calming effect and directs awareness inward until pure awareness is achieved, described as "being awake inside without being aware of anything except awareness itself". Broncho dilatation, gaseous exchange improves peak expiratory flow rate and exercise tolerance. The aim of the study is to find the effectiveness of yogic practices to improve pulmonary status in asthma patients. The study is essential as most of the general population suffers from this problem and also to make physiotherapists to realize the benefits received from the alternative approaches like yogic practices to improve peak expiratory flow rate and exercise tolerance in asthma patients.

MATERIALS AND METHODOLOGY

 20 asthma   patients attending Vinayaka Missions Kirupananda Variar Medical College, Salem between 40-55 years of age were selected randomly using simple random sampling method and included for the study. Patients having cardiac and psychiatric problems are excluded from study. The yogic practices consisted of warm-up exercises, asanas, pranayama and meditation which improves pulmonary status in asthma patients (2, 3). The group underwent a pretest assessment of exercise tolerance   and peak expiratory flow rate with the help of   six minute walk test and peak flow meter . Exercise tolerance test was measured   in asthma patients (4) using six minutes walk test. Materials like chalk powder, stop watch, whistle, meter tape were used to collect the data for this test. A hard and flat 200 meters walkway was marked for six minute walk test. Subjects were asked to stand in the start line and were instructed to walk in their self pace and rest as needed back and forth along the marked walkway for a period of six minutes following a whistle blow. Stop watch & whistle were used to start and stop the test. The distance walked was measured by multiplying the number of times of full completion of marked walkway with 200 meters and the excess using a meter tape. The distance walked in six minutes was recorded with a help of a meter tape in meters. Peak expiratory flow rate of the subjects was measured in asthma patients (5,6,7,) using peak flow meter. The sliding pointer was set to zero before the start of the procedure. The subjects were asked to stand straight and hold the handle of the peak flow meter. They were further asked to take a deep breath and put the mouth piece in their mouth and seal their lips and teeth tightly around the mouthpiece, following which they were asked to blow out as hard and as fast as they can. The number on the scale corresponding to the sliding pointer was noted. The pointer was rest to zero every time and the best of three recording was taken into account. The numerical value corresponding to the slide pointer was taken as the score. After the pretest measurement was over, the subjects received   yogic practices for 8 weeks and then posttest measurement was taken. The yogic practices consisted of

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warm-up exercises, asanas, pranayama and meditation for a period of eight weeks once a day in the morning session between 8.30 am to 9.30 am. The training was given for six days in a week. Training was carefully monitored under the supervision throughout the study. Training period of eight weeks was divided into two halves each for a period of four weeks. In the first four weeks the training intensity was lesser and the training duration was for 50 minutes. The selected asanas, pranayama and meditation were imparted as programmed in the training schedule Table I. In the next four weeks the training intensity was increased with a training duration of 60 minutes. The selected asanas, pranayama and meditation were imparted as programmed in the training schedule Table II .At the end of each session, relaxative asana i.e savasana was practiced as a cool down segment. All the subjects underwent a warmup exercise protocol before asanas. It included forward bending, backward bending, side bending, and twisting, jogging and mukha dhouti for a period of 5 minutes. These exercises served to loosen the joints and thereby prepared the individual for yogic practices.

Posttest measurements were done at the end of the 8th week using six minutes walk test and peak flow meter in the same way as that of pretest measurement. After yogic practices the collected data was subjected to statistical analysis using paired't' test.

RESULTS AND DISCUSSION

The results of the study were derived from the statistical analysis using the paired t-test . The results of the statistical analysis using a paired t-test revealed that there is a significant improvement in peak expiratory flow rate (5,6,7) and exercise tolerance(4) in patients with sub-acute asthma patients. The results of the study showed that difference between the pretest and posttest mean values were statistically significant thereby indicating that peak expiratory flow rate and exercise tolerance had significantly improved following yogic practice for a period of eight weeks .The improvement in peak flow rate and exercise tolerance may be because the asanas, pranayama and meditation would have resulted in bronchodialation of the airways thereby improving the expiratory capacity of the lung.4. Jain SC (1991): studied the effect of yoga training on exercise tolerance in adolescents with childhood asthma and concluded that yoga training resulted in a significant increase in pulmonary function and exercise capacity. 5. Saxena T et al (2009): proved that FEV_1 % and Peak expiratory flow rate had significant improvement following yoga when compared to the group treated with medications.

CONCLUSION

The results of the study make us to conclude that   yogic practices improved Peak expiratory flow rate and Exercise tolerance in sub-acute asthma patients.

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Table No. I: Training Schedule (1-4 Weeks)

S no	Yogic practice	Repetition	Rest between asana	Rest between repetition			Duration of exercise	Total duration
1	Warmup exercise						5 min	
2	Asanas Trikonasana Veerabhadrasana Paschimotanasana Bhujangasana Dhanurasana	2	15 sec	1.30 min			20 min	50 min
3	Pranayama Nadisuddhi Surya bhedhana		Inhale 5 sec 5 sec	Hold - -	Exhale 10 sec 10 sec		10 min	
4	Meditation						10 min	
5	Relaxation Savasana						5 min	

Table No. II: Training Schedule (5-8 Weeks)

S No	Yogic practice	Repetition	Rest between asana		Rest between repetition	Duration of exercise	Total duration
1	Warmup exercise					5 min	
2	Asanas Padahastasana Janusirasana Gomukasana Bhujangasana Dhanurasana	3	10 sec		1 min	25 min	60 min
3	Pranayama Nadisuddhi Bhramri		Inhale 5 sec 5 sec	Hold 5 sec 5 sec	Exhale 10 sec 10 sec	15 min	
4	Meditation					10 min	
5	Relaxation Savasana					5 min	

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Table No. III. The collected data were analyzed using paired't' test

Sno	Variables	Pre test Mean	Post test Mean	Mean difference	Standard deviation	't' calculated value	't' tab value
1.	Peak flow rate scores (liters/minute)	375.5	393.5	18	6.95	11.61	2.09
2.	Exercise tolerance test scores (meters)	373.3	395.85	22.55	5.826	17.346	2.09

Significant at 0.05 level. t calculated value > t table value.

RESEARCH ARTICLE

Biochemical Composition of *Siganus lineatus* (Valenciennes, 1835) from Parangipettai, South East Coast of Tamil Nadu, India

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ABSTRACT

In *Siganus lineatus*, proteins, lipids and carbohydrates were studied in muscle and liver tissue with regard to different tissue samples (gill, muscle and liver). *S. lineatus*, South East Coastal of Tamil Nadu, collected from Parangipettai. Various biochemical parameters such as total protein, lipid and carbohydrate have been analyzed. 8.27mg/g (±0.58) lipids are elevated in infected liver tissues. Whereas protein declines were observed in 73.60 mg/g of infected muscle tissue (±2.61) compared to 70.29 mg/g of normal fish, ±1.86. Some fluctuations in the total content of carbohydrates in infected fish have been observed.

Keywords: *Siganus lineatus*, protein, carbohydrate, lipid, biochemical parameters.

INTRODUCTION

Fish, not only in human food diets but also in animal and poultry rations, plays an important role. It is a palatable food that is rich in vitamins, calcium, phosphorus and iodine and is readily digested. In India, when compared to other foods of animal origin, fish is regarded as an inexpensive food item. A very healthy food item is considered to be marine fish. They are an excellent source of protein that also provides different minerals and vitamins needed for good health (Imad Patrick Saoud et al., 2008). Fish provides an important amount of dietary protein and lipid diet in many countries as a major source of food for human nutrition. Since it contains long muscle fibers, fish flesh is easily digestible (Nawzet Bouriga et al., 2010). Healthy fish flesh is regarded as a marker for the natural aquatic environment (Mona et al., 2011). Nutrients nourish the body, foster growth, preserve and repair parts of the body. In human nutrition, fish plays a major role. The importance of fish is now well understood as a source of high-quality, balanced and easily digestible protein. In addition, it is also a famous source of several other nutrients and is

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accepted as a healthy food. Nevertheless, diet-borne pollutants transferred along the food chain are at risk (Agrawal and Srivastava, 2003; Jat and Kothari, 2006; Gupta and Srivastava, 2006; Ayas et al., 2007). All the essential components, such as carbohydrates, proteins, fats and minerals, and vitamins, should be included in a healthy food. In addition, health benefits such as the prevention of cardiovascular diseases and certain types of cancer, including colon, breast and prostate, have been linked to this. An increasing amount of evidence suggests that fish flesh and fish oil are helpful in reducing serum cholesterol because of their high content of polyunsaturated fatty acids (Stansby, 1985). In addition, omega-3 polyunsaturated fatty acid, a special type of fatty acid, is recognized as an important drug for preventing a number of coronary heart diseases (Edirisinghe, 1998). Cardiologists are advised to use generous amounts of fish in food to obtain adequate protein without ingesting excess fatty acids and lipids (Dyerberg, 1986). Information on the biochemical composition of the diet is also required from nutrition experts and individuals who are interested in the caloric content of food for weight control. In order to study the proximate composition of *Siganus lineatus* by estimating its major biochemical components, such as total protein, carbohydrate, lipid, head and body parts, the present work was planned.

MATERIALS AND METHODS

Collection of rabbit fish
S. linaetus was collected from Parangipettai, Chidambaram, South East Coast of Tamil Nadu, India, from fresh marine rabbit fishes. The samples collected were stored in sterile bottles and examined for tissue damage and disease infection in fishes (eye, gill and skins). (Fig. 1).

Collection of tissues
The gills, liver and muscle tissues were collected and freeze-dried after the fish had been decapitated. Tissues were washed three times in fresh water and rinsed briefly in double-deionised water; the water left on the surface of the tissue was dried with filter paper. For further studies, tissue (gill, liver, muscle and spleen) was collected and stored in a sterile bottle.

Biochemical parameters
Proteins: By following Lowry's method (1951), which involves two steps, the protein content of the muscle tissue was estimated. In the first step, the carbamyl groups of protein molecules react with the reagent's copper and potassium, resulting in the formation of a potassium biuret complex of blue copper. Together with tyrosine and phenol compounds present in the protein, this complex reduces the foline reagent's phosphomolybdate to intensify the solution's color. The concentration of color (optical density) was measured using U.V. Spectrophotometer (BioAuarius CECIL CE7250 7000 series) to find out the protein content at a wave length of 620 nm.

Lipids: By following the method of Folsch et al., the total lipids were extracted from dry tissues (1957). 50 mg of dry tissue was weighted and homogenized with a 5 ml mixture of Chloroform Methanol (Chloroform and Methanol mixed at the ratio of 2:1). Using a laboratory centrifuge, the contents were centrifuged for 15 minutes at 2500 rpm (REMI R8C Model). The supernatant was taken in an aluminum boat (pre-weighed) and dried for about 45 minutes in a hot-air oven at 600 C. The final weight was recorded after drying had been completed. The total lipid content of the tissue is given by the distinction between the initial and final weights. The concentrations were administered as a proportion of the dry weight of the tissue.

Carbohydrates: For colorimetric determination of sugars, methylated sugars and polysaccharides, anthrone in sulphuric acid can be used (Dubois et al (1956). The test is very straightforward, quick, inexpensive and highly sensitive. The colour produced is very stable and the presence of proteins is largely unaffected by the analysis. 10 mg of a weighted amount of fine dried powder was taken in a test tube to estimate the amount of carbohydrate. To which 5 ml of 1N sodium hydroxide was added, the content was homogenized and centrifuged at 2500 rpm for

about 15 minutes. The supernatant was separately taken and retained for further analysis. 0.5 ml of solution was taken into the test tube to estimate the quantity of carbohydrates and 0.5 ml of distilled water was added to this to get 1 ml of solution. To this was added 5 ml of anthrone in sulphuric acid (50 mg of anthrone in 100 ml of sulphuric acid concentrate). Then shake the contents well and hold them for about 15 minutes for incubation. The test tubes were boiled in a water bath for approximately 15 minutes after completion of the incubation process. When the samples cooled to normal room temperature, colours were created. The optical density of the samples was calculated using U.V. Spectrophotometer (BioAuarius CECIL CE7250 7000 series) and noted the absorption readings at 600 n m wave length. They took all the samples in triplicates.

Statistical analysis

The mean ± SEM of all data is represented. The single-way ANOVA and Tukey's test compared data that showed homogeneous variances. The analysis was performed using Statistic a version 7.0 (Stat Soft, Tulsa, OK) software and the minimum level of significance was set at $P < 0.05$.

RESULTS

Proteins

The maximum protein content of the *S. lineatus* muscle was 73.56 mg/g (±1.39) in the muscle and the minimum protein content was observed in the *S. lineatus* gill (70.44 mg/g (±1.20). In comparison to tissue, the muscle protein increased statistically and was highly significant (p<0.05). (Table 1 and Fig 2). The maximum was found in muscle region in the present study and the minimum was noted in the gill region of rabbit fish.

Lipids

The maximum lipid content in the normal *S. lineatus* muscle during the monsoon period was 8.14 mg/g (±0.59) and the minimum lipid content was 4.01 mg/g (±0.54) in the muscle, while the lipid level increased. Lipid levels decreased in the gill when compared to all the tissues during the study period. The total lipid increase was statistically significant (p<0.05). (Fig.3, Table1).

Carbohydrates

Increased carbohydrate levels of 4.69 mg/g (±1.05) are found in the *S. lineatus* muscle in the liver, with minimum values of 4.06 mg/g (±0.14) in the muscle compared with other tissues. The maximum was observed in liver tissue and the minimum lipid in muscle tissue was observed to be suitably significant in comparison with other treatment groups (Fig 4, Table 1).

DISCUSSION

Fish is usually regarded as a very good source of animal protein. In addition to the percentage of protein it contains, the importance of fish in the diet is due to the amino acid composition of the protein and its accessibility to the system. Recent knowledge shows that the biological value of protein in food depends on the composition of amino acids. Biochemical constituents are affected by the mobility of fish metabolism and the geographical area. An integral part of a nutritious human diet is supposed to be marine fish. However, fish from different species do not provide their consumers with the same nutrient profile (Takama et al., 1999), and the nutritional value of fish varies with the season (Imad Patrick Saoud et al., 2008). The current study contemplates the estimation of increased protein, lipid and carbohydrate levels of *Siganus lineatus* in the muscles and liver. In the body, proteins occur in the form of amino acids and other metabolites that serve as the body's building blocks. The protein content of the cell is therefore considered to be an important instrument for assessing the physiological standards of Chezhian et al (2005). In the present study, the maximum value of total muscle protein content reported during the study was reported in the

Siganus lineatus muscle. Obviously, the fat content increased due to heavy feeding by Huss (2000). The lipid content in the infected muscle was elevated when compared to all two-year seasons. Such lipid distribution in the infected muscle indicates that fish, like other animals, store fat in their muscles during starvation, reproductive phases and infestation periods for energy supply. Increases in carbohydrate levels in the muscles and liver of ordinary fish occurred during the pre-monsoon season from 2010 to 2012, with low levels occurring during the post-monsoon season. The very low carbohydrate values recorded in the current study could be due to the fact that glycogen does not contribute much to the body's reserves in many marine animals. Similar findings in 11 species of clupeids were reported by Ramaiyan et al., (2014). Carbohydrates are considered the first to be degraded under the animal stress condition of Chezhian et al (1990). According to Dhavale and Masurekar (1998), as a result of pollutant stress, decreased levels of carbohydrate constituents in tissues of toxicant-exposed animals may be due to the prevalence of hypoxic conditions in tissues. The content of carbohydrates and minerals in garfish was almost constant (Gökhan Boran and Hikemt Karaçam (2004). Although the highest quantity of carbohydrates was found in *L calcarifer* Ravichandran et al (2005). The muscle and liver carbohydrate levels of infected fish occurred during the pre-monsoon season and decreased during the post-monsoon season in the years 2011-2012. The next composition is generally known as the percentage composition of basic components such as water, protein, lipids, carbohydrates and minerals. Fishes are known to be one of the cheapest sources of animal protein and other essential nutrients needed in human diets (Sadiku and Oladimeji, 1991). In recent years, due to their protective role against the development of cardiovascular disease and rheumatoid arthritis, fish lipids have also assumed great nutritional significance (Shahidiand Botta, 1994). Although determining the proximate composition and mineral elements of *Siganus lineatus* Rabbit fish has enormous values due to the good quality effect on human health.

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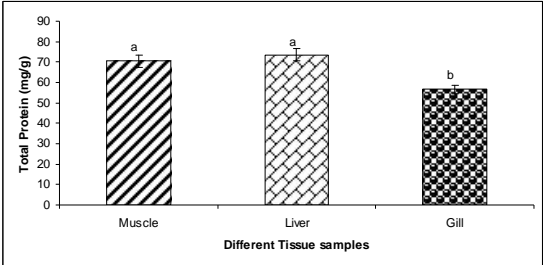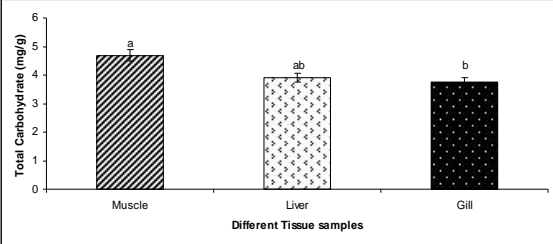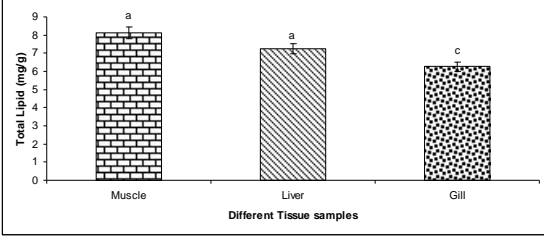
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Table 1: biochemical parameters (protein, lipid and carbohydrate) content in *S. lineatus.*

Tissues	Total Protein (mg/g)	Total Carbohydrate (mg/g)	Total Lipid (mg/g)
Muscle	73.40±0.12[a]	4.69±1.55[b]	8.14±1.09[c]
Liver	70.54±0.11[a]	3.90±1.44[a]	7.24±1.53[a]
Gill	56.54±0.35a	3.75±1.02[a]	6.26±1.04[a]

Values are presented as mean ± SEM. Different letters indicate significant differences between treatments for the same tissue (one-way ANOVA; $P<0.05$).

Figure 1: *Siganus lineatus* (Valenciennes, 1835) fish collected from Parangipettai, South East Coast of Tamil Nadu, India.

Values are presented as mean ± SEM. Different letters indicate significant differences between treatments for the same tissue (one-way ANOVA; $P<0.05$).

Fig 2: Total protein content in the liver of *S. lineatus*, Parangipettai, South east coast of Tamilnadu

Values are presented as mean ± SEM. Different letters indicate significant differences between treatments for the same tissue (one-way ANOVA; $P<0.05$).

Fig 3: Total carbohydrate content in the liver of *S. lineatus*, Parangipettai, South east coast of Tamilnadu

Values are presented as mean ± SEM. Different letters indicate significant differences between treatments for the same tissue (one-way ANOVA; $P<0.05$).

Fig 4: Total carbohydrate content in the liver of *S. lineatus*, Parangipettai, South east coast of Tamilnadu

RESEARCH ARTICLE

Effect of Plasticizer on the PVC/PEMA Polymer Based Gel Polymer Electrolytes for all Li ion Battery Applications

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ABSTRACT

A gel polymer electrolyte (GPE) based on poly vinyl chloride/Poly ethyl methacrylate (PVC/PEMA) polymer membrane is prepared and its performance for lithium ion batteries are investigated. The polymer and salt interaction has been analyzed by Fourier Transport Infrared Spectroscopy. The results from electrochemical studies show that GPE has highly ionic conductivity and low apparent activation energy of 0.011eV. The ionic conductivity of the GPE at room temperature is 0.3764×10^{-2} S/cm. The temperature dependent conductivity follows the Arrhenius relation. The GPE is stable at the potential higher than 4.5V. Differential scanning Calorimetry (DSC) study has indicated enhancement in the amorphous phase of polymer due to the addition of plasticizer. Thermal stability and the surface morphology of the polymer electrolyte are examined by using Thermo gravimetric /Differential Thermal analysis and scanning electron microscope respectively. Finally the electrode/ electrolyte interfacial resistance is measured by monitoring the impedance response at different time intervals.

Keywords: PVC; PEMA; Gel polymer electrolytes; ionic conductivity; thermal stability; electrochemical stability window.

INTRODUCTION

The polymer electrolyte development has made attention of many researchers to find an application not only in lithium batteries but also in other electrochemical devices such as electro chromic devices and super capacitors [1-5].

The polymer electrolytes are generally used in Li ion battery due to the properties such as easy packaging properties and high energy density [6, 7]. The most commonly studied polymer is poly (ethylene oxide)(PEO) complex with alkali metal salts. It shows ambient temperatures conductivity is of the order of $10^{-7}Scm^{-1}$. In order to increase the ionic conductivity of the polymer electrolyte at room temperature, numerous methods have been developed which include doping inorganic fillers, incorporation of organic solvents, preparing various organic hybrids and synthesizing new polymers. The most familiar method is to mix liquid plasticizers such as EC, PC and DMF with the polymer matrix. These plasticizers will raise the flexibility of the polymer chain as well as the ionic conductivity. Polymer electrolytes prepared with plasticizer is the promising electrolytes for the lithium batteries owing to their high ionic conductivity [8]. However plasticized electrolytes also have drawbacks, such as poor mechanical properties at high degree of plastization, solvent volatility and reactivity of poor solvents with lithium electrode [9]. In the present study, we have chosen polyvinyl chloride (PVC) as a host polymer as it is commercially available, inexpensive and is compatible with a large number of plasticizers such as ethylene carbonate(EC), propylene carbonate (PC), dibutyl phthalate (DBP) and dioctyl adipate (DOA). The second host polymer polyethyl methacrylate (PEMA) also has executed chemical resistance, high surface resistivity and mechanical strength. Its high elongation strength makes it an important candidate for the formation of blends with other polymers [10, 11]. The present article gives the results of a study on plasticized PVC/PEMA blends which contain lithium per chlorate (LiClO₄) as electrolyte salt and DMC, EC as plasticizers.

EXPERIMENTAL

The PVC and PEMA with average molecular weights of 14×10^4 and 5×10^5 (Aldrich) respectively, were used as received. LiClO₄ anhydride was purchased from Aldrich, USA, and it was dried in vacuum at 100°C for 24h before use. The plasticizers and the solvent THF used in this study were purchased from Aldrich, USA and E-merck, Germany respectively and they were used as received.

Preparation of solid polymer electrolytes
The appropriate weights of PVC, PEMA and LiClO₄ (table 1) were dissolved in tetra hydrofuran (THF). The plasticizer containing polymer electrolytes were prepared by adding 67 wt% of (EC: DMC) into polymer salt composition. The solution was then stirred continuously until the mixture took a homogeneous viscous liquid appearance. The solutions of different composition were cast on glass plates and dried in an oven at 45°C for several hours until dry transparent films were obtained. The films were stored in desiccators to remove traces of solvent and avoid moisture contamination before characterizations.

Characterization techniques

Fourier Transform Infrared (FTIR) spectroscopy: Thin films were subjected to Fourier transform infrared (FTIR) study to investigate the complexation behavior. FTIR spectroscopy studies were carried out using the thermo scientific Nicolet iS5 iD3 ATR in the transmittance mode over the wave number region 4000-400cm⁻¹.

Ionic conductivity: The ionic conductivity of the polymer electrolyte was determined using electrochemical spectroscopy (EIS). The instrument used was Biologic science instrument sp-150. The measurement was done in the frequency range from 1Hz to 1 MHz at different temperatures between ambient and 353K. The impedance study was carried out by sandwiching the polymer electrolyte film between two stainless steel (SS) electrodes under spring pressure. The thickness of the film was determined using a micrometer screw gauge. The ionic conductivity, σ of each sample was calculated using the equation

$$\sigma = \frac{t}{R_b A}$$

Here t is the thickness of the sample, R_b is the bulk resistance and A is the area of electrolyte. The real impedance (Z′) was plotted against imaginary impedance (Z″) and the bulk resistance was obtained from the intercept with the real-axis.

SEM Analysis: The surface morphology of the prepared sample was obtained using JEOL JSM 840A scanning electron microscope (SEM).

Differential Scanning Calorimetry: The melting temperature and glass transition temperature of the blends of the composite polymer salt complex were obtained by using a differential scanning calorimeter (Pyris 6 DSC). Each sample was scanned at a heating rate of 10°C min^{-1} with a temperature (0 to 400°C) range under a nitrogen atmosphere (20.0 ml/min).

TG-DTA analysis: TG-DTA thermal analysis of polymer electrolytes samples were performed using a Perki – elmer pyris 6TG-DTA with the san rate of 10°C min^{-1}. The thermal stability of the polymer electrolyte has been studied for its use in practical applications because thermal stability is an important parameter for guaranteeing acceptable performance during high-temperature operation, which is related to safety concern. In this work, the thermal stability of the polymer electrolytes was observed using thermo gravimetric analysis (TGA).

RESULTS AND DISCUSSION

FT-IR analysis

Fourier transform infrared spectroscopy is an excellent technique which is used to analyze the interactions among the atom or ions in the electrolyte system. These interactions can induce changes in the vibrational modes of the molecules in the polymer electrolyte. The infrared region (4000-400cm^{-1}) is of prime importance for the study of an organic compound. The FTIR spectra of the starting materials PVC, PEMA, LiClO$_4$, DMC, and EC are shown in fig 1. These bands are of complex origin and depend on the spatial position of the atoms surrounding the C-Cl bonds [12]. The rest of the bands of the spectrum correspond to different C-C and C-H vibrations, in some cases increased by the vicinity of chlorine atoms. FTIR Spectrum of as-synthesized GPE is shown in fig 2. The vibrational peaks at 2920, 1422, 1331, 1245, 959, 836 and 621cm^{-1} of PVC are shifted to 2925, 1422, 1392, 1235, 969, 861 and 623 cm^{-1}. The peaks at 2925 and 1392 cm^{-1} are assigned to C-H asymmetric stretching methylene group vibration and in plane CH$_2$ deformation of PVC respectively. The vibrational peak at 899 cm^{-1} is assigned to c-cl stretching vibrations of PVC [13]. The peaks at 969, 623 cm^{-1} are assigned to the weak trans C-H wagging and cis C-H wagging vibration of PVC respectively. If a vinyl group is conjugated to a trans CH=CH group, the CH wag vibrations of the two groups can interact also [14]. PEMA has lone pair electrons at the oxygen (O) atoms located at the carbonyl (C=O) and C-O-C groups. The vibrational peaks at 2965, 1722, 1448, 1145, 861 and 757 cm^{-1} of pure PEMA are shifted to 2988, 1720, 1479, 1151, 862, 774 cm^{-1}. The FTIR spectrum of PEMA shows vibrational peaks at 2988 and 2925cm^{-1}, which are ascribed to the asymmetric and symmetric C-H stretching of the methylene group of PEMA respectively. The absorption peak of PEMA at 1720 is assigned to the C=O carbonyl group. The vibrational peaks at 1479, 1024, 774 cm^{-1} are assigned to -CH2 scissoring, -CH$_2$ wagging and -CH$_2$ rocking of PEMA respectively. Some other carboxylic acid ester groups were observed as broad features at 1151cm^{-1} [15].

The vibrational peaks at 1069, 1151, 1392, 1479 and 1771 of EC are assigned to stretching vibration band of C-H, symmetrical stretching vibration band of C-H, scissoring vibration band of CH$_2$, bending vibration band of CH$_2$ and stretching vibration band of C=O respectively[16]. The vibrational peaks at 1450 and 1267 of DMC are ascribed to asymmetric stretching of -δCH3 and -vCH$_3$-O respectively. The peak of DMC at 969 is assigned to –vCH$_3$-O mode of vibration [17].The frequency of C=O at 1801cm^{-1} indicates the interaction of the plasticizer with LiClO$_4$ [18]. The vibration of spectroscopically free ClO$_4^-$ ion of LiClO$_4$ is found at 623cm^{-1}. It is suggested that the 623 mode probably constitutes the solvent-separated ion pairs Li$^+$(S)ClO$_4^-$ (where S stands for the solvent) and solvent separated dimers

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[19]. It is also found that some peaks appearing in the pure polymer and salts disappeared in the complexes such as 3569, 2325 and 1392 and some new peaks are observed at 1558, 1108 and 717cm^{-1} in polymer complexes. The shifting of peaks and formation of new peaks suggests that the polymer–salt interaction in blend based composite polymer electrolytes.

Conductivity analysis

Electrochemical impedance spectroscopy (EIS) is an excellent tool for studying electrical properties of materials and their interfaces with electronically conducting electrodes. The ionic conductivity of PVC/PEMA-based polymer electrolytes containing different ratio of EC/DMC plasticizer were calculated from the equation $\sigma = l/R_b A$, where '*l*' and '*A*' represent the thickness and area of the film, respectively, and R_b is the bulk resistance of the electrolyte obtained from impedance measurements [20, 21].An electrochemical cell was assembled by sandwiching PVC–PEMA–LiClO$_4$-EC-DMC electrolytes between two stainless steel (SS) blocking electrodes. Ionic conductivities were measured by recording the ac impedance spectra of SS/ PVC–PEMA–LiClO$_4$-EC-DMC /SS in the temperature between 30 and 80 ∘C using Biologic science instrument, sp-150. Figure shows that the impedance spectra of PVC (5)–PEMA (20)–LiClO$_4$ (20)-EC (40)-DMC (60) polymer electrolyte system. Watanabe and Ogata [22] explained that the impedance spectrum for a symmetrical cell consists of two semicircles. In which one at low frequencies corresponding the interfacial impedance and other at high frequencies related to bulk electrolyte impedance. It has been reported [23–25] that the high-frequency semicircle does not appear in practical impedance plots for plasticized polymer electrolyte membranes. This feature indicates that the conductivity is mainly due to ions. The variation of ionic conductivity values for different ratio of plasticizers is shown in figure 3 It can be observed that ionic conductivities are changes when the content of DMC and EC changes from 0 to 100 wt. %. From the table 1, conductivities are increases gradually with DMC content from 0 to 60wt% then decreases. And also conductivities are increases from 0 to 40 wt % of EC and then decreases. Hence the maximum conductivity is obtained with the film ration of 40 and 60 wt % of E and DMC respectively with a value of 0.3764x10^{-2}S/cm. The conductivity rapidly decreases between 0 wt. % percent to 40 wt. % of DMC.

The temperature dependence of ionic conductivity

The ionic conductivity of the prepared membranes was increasing with increasing temperature. This is due to a hopping mechanism between coordinating sites, local structural relaxations, and segmental motions of the polymers [26]. From the obtained figure 4 that the temperature dependent conductivity plot obeys VTF relation. It describes the movement of ions through the plasticizers rich-phase which involves the plasticizers, the host polymers, and the salts i.e. viscous matrix. And also, the plasticizers influence the degree of mixing, the polymer plasticizer interaction by providing more charge carriers and more mobile medium for the ions [27], which results in enhancing the conductivity of the plasticized- electrolyte system.

Figure shows that the conductivity versus temperature behavior of the system is linear, i.e. Arrhenius relationship

$$\sigma = \sigma_0 \exp(-E_a/kT) \quad \text{----------------> (1)}$$

Where σ_0 is the pre exponential factor, E_a is the activation energy and k is the Boltzmann constant. Using the equation (1) and slope, E_a values are calculated which is in the range between 0.01 and 0.04eV. The low activation energy for lithium ion transport is due to the completely amorphous nature of the polymer electrolytes that facilitate the fast Li$^+$ ion motion in the polymer network. The complete amorphous nature also provides a bigger free volume in the polymer electrolyte system upon increasing the temperature [28]. For devices operating over a wide range of temperature range, it is desirable to have a uniform conductivity. Thus, systems with low activation energies are desirable [29]. It can be observed that sample AJ3 has higher ionic conductivity (0.3764x10^{-2} Scm^{-1} a 303K) and lower activation energy (0.011eV) compared with other compositions.

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DSC Studies

DSC analysis is mainly used to measure the melting temperature, heat of fusion, latent heat of melting, reaction energy and temperature, glass transition temperature, crystallinity of the sample. DSC curves of PVC/PEMA/LiClO₄/EC/DMC-based Polymer Electrolyte systems are shown in Fig. 5 (a, b, c).Figure 5a shows the DSC curve of the pure (PVC, PEMA) and blending polymers (PVE/PEMA) in the temperature range of 0 to 150℃. It gives the glass transition temperature (Tg) of PVC, PEMA and PVC/PEMA blend as 102.3, 56 and 80.02℃. The miscibility of the polymers would exhibit a single glass transition temperature due to the formation of single homogeneous phase in the polymers [30, 31]. From the thermogram, single Tg value was found to be 80.02℃. It gives the miscibility and homogeneity of the polymers. It is also reported by Zakaria [32] for the PEMA/PVC polymer blending. All the systems exhibited endothermic peaks around 50 to 60℃ which is attributed to the glass transition temperature of the membranes and the plots corresponding to the samples AJ1, AJ2, AJ3, AJ4, AJ5 and AJ6 show the transitions at 57, 56, 55, 58, 59 and 61℃ respectively. From the figure (5b), the glass transition temperature value of the samples are initially decreases and thereafter increases with decreasing concentration of DMCin the polymer matrix. The Tg values are listed in the table 2. The Tg value of the sample for 40:60 ration of EC: DMC is 58℃. From the table 2, Initially Tg Value decreases. It reveals that decrement of glass transition temperature cause the higher amorphous nature of the sample. The glass transition temperature value decreases due to the improvement of the segmental motion of the polymer chain in the polymer matrix. Hence polymer matrix gets softening [33]. The crystallinity of the composite micro-porous polymer electrolyte membranes were calculated based on the following

$$X_c = \frac{\Delta H_m}{\Delta H_m^0} \times 100\%$$

Assuming that PEMA as 100% crystalline. ΔH_m^0 -is the standard enthalpy of the fusion of pure PEMA 112 J/g. ΔH_m is the enthalpy of the fusion electrolyte. The glass transition temperature (Tg), melting temperature (Tm), enthalpy of the fusionΔH_m and degree of crystallinity percentage have been listed in the Table. Figure 5c shows that thermogram of the prepared composite polymer in the temperature range of 0 to 400℃. The melting temperature of the composite polymer is determined and is listed in the table. It is evident from the table that glass transition temperature (Tg), Melting temperature(Tm), enthalpy of the sample (ΔH_m) and percentage of crystallinity X_c (%) decreases initially. Therefore degree of the crystallinity of the polymer electrolyte decreases with decreasing EC content in the polymer matrix. Thereafter increase of crystallinity leads to segmental mobility decreases cause the reduction in the conductivity.

TG/DTA analysis

TG/DTA is one of the techniques to study weight loss of the prepared membrane in which one can measure the moisture uptake and diffusion characteristics. And also this technique is used to analysis the phase transitions, thermal degradation and crystallization of the polymers. The figure shows the TG/DTA curves of the membrane containing PVC-PEMA-LiClO₄-DMC (60%)-EC (40%). From the figure 6, decomposition starts at 34℃ with a weight loss of 4- 10wt% of GPE. It represents the presence of moisture in the membrane at the time of loading samples. The next decompositions observed in the curve at 240 ℃ with a weight loss of 66.53% of EC (60 wt. %) and DMC (40 wt. %) plasticizers ratio. Hence, the membrane is stable up to 273℃.The endothermic peak at 290℃ shows the DTA traces. This temperature also indicates the melting temperature of the PEMA.

SEM analysis

The morphological effects are responsible for the increment of conductivity. The surface morphology of the polymer PVC-PEMA blend based electrolytes prepared by the solution casting technique is affected by the immiscibility of the plasticizer with PVC. Tetra Hydro Furan evaporates very slowly after casting and following drying. Phase separation takes place due to the immiscibility of PVC with plasticizer. Also due to its segregation, PVC rich phase is formed. The appearance of plasticizer rich phase shows uniform pore structure which cause ion mobility resulting in higher conductivity. Hence the obtained film consists of two different phases, such as PVC-rich phase and a

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plasticizer-rich phase. From the image (fig. 7) of the obtained films containing both PVC and PEMA have large pores which cause the highest ionic conductivities. The formation of the porous structure is a complex process that depends on the interaction of the solvent with the polymers and the relative rates of the evaporation of compounds [34]

The interfacial stability

In order to ascertain the interfacial stability of polymer electrolyte with lithium metal electrodes the symmetric cell composed of Li/PE/Li was assembled and its interfacial resistance values were measured as a function of time by using Electrochemical Impedance Spectroscopy. It was monitored for 30 days at open circuit voltage and ambient temperature as well. The Nyquist plots exhibits wide depressed semicircle in the real axis intercepts at high frequency corresponding to bulk resistance and low frequency to the interfacial resistance. The interfacial resistance associated to the surface film layers combined with charge transfer kinetics at the interface [35, 36]. GPE display 318Ω on the first day and changes to 750 Ω after 4weeks. These results clearly reveal that GPE is more stable in contact with metallic Li electrode. In the present work, the impedance spectrum of BJ3 sample reveals semicircle it may be linked with formation of interfacial layer on the inner porous walls. The bulk resistance value varies with time because of the change of porous structure which alters the size of pore and disturbs growth passive layer [37]. Figure 8 shows the interfacial stability of the GPE.

Lithium transference number

The lithium transference number of PVC-PEMA based polymer electrolyte of high ionic conductivity system was found out by means of chronoamperometric technique as shown in Fig. and its impedance measurements were obtained before and after DC-polarization measurements with an applied potential difference of 10 mV/s.

From the figure 9, the initial current I_o is2.85 µA and steady state current value I_{ss} is 0.86 µA in the time range of 2h. Using ac impedance meter, initial (Ro) and steady state (Rss) resistance 263Ω and 477Ω respectively were measured. The small variation in R_0 and R_{ss} confirms the better stability against the Li electrode with our as prepared gel polymer electrolyte.

Lithium transference number can be easily obtained from the formula

$$t_{Li} = \frac{I_{ss}(V - I_0 R_0)}{I_0(V - I_{ss}R_{ss})} \qquad \text{--------> 2}$$

Where, t_{Li} –lithium transference number
          V   -applied potential
          R_0 -internal resistance of the passivation layer
          R_{ss} -resistance of the passivation layer (steady state)
According to the equation (2), the Li t^+ for the prepared GPE is calculated as 0.29

Electrochemical stability

The measurement of electrochemical stability analysis is significant for Ii ion battery applications. Figure 10 shows that the current vs voltage behavior of the membrane was obtained for high ionic conductivity systems. The voltammograms result reveals that the decomposition limit of the polymer electrolyte can be considered as voltage at which the current flows through the cell. The observed anodic limit of the membrane is about 4.6V. If electrochemical stability of the membrane is high then it is more suitable for lithium battery application.

CONCLUSION

In the present study, PVC/PEMA polymer electrolytes with LiClO₄ lithium salt have been prepared and their electrochemical studies have been analyzed. The addition of plasticizer reduces the crystallinity of the polymer host and enhances the transport properties. The membrane containing 40:60% (EC: DMC) showed better ionic

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conductivity 0.3764x10^{-2}S/cm at room temperature. The temperature dependence of conductivity of the GPE follows the Arrhenius equation and has low activation energy (0.011eV). The GPE had an electrochemical stability up to 4.6 V versus LI/Li$^+$. The transport number reveals the charge transport in the composite polymer electrolyte due to ions only. The addition of plasticizers increases the amorphous nature and charge carrier dissolution in the polymer matrix. The complex formation in PVC-PEMA-LiClO$_4$-DMC-EC system is confirmed by FTIR study. Good thermal stability of the composite polymer electrolyte membrane up to 260℃. The DSC measurement show single Tg for all blend polymer electrolyte which indicates compatibility of the PVC-PEMA blend and also reveals a decrease in T$_g$ and T$_m$. The porous nature of the membrane is found from Scanning electron microscopy images. Thus prepared gel polymer electrolyte showed enough rated performance and proves to be suitable for lithium batteries.

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Table 1: Conductivity for Various DMC/EC ratio

Sample Name	PEMA-PVC-LiClO4-EC(x)-DMC(y)		Conductivity values at different temperatures (x10^{-2} S/cm)					
	x(wt.%)	y(wt.%)	303K	313K	323K	333K	343K	353K
AJ1	0	100	0.2676	0.2997	0.3125	0.3328	0.3597	0.3794
AJ2	20	80	0.3349	0.3405	0.3505	0.366	0.3971	0.4457
AJ3	**40**	**60**	**0.3764**	**0.3967**	**0.3968**	**0.4091**	**0.4147**	**0.4332**
AJ4	60	40	0.3437	0.3527	0.3744	0.3936	0.4107	0.4308
AJ5	80	20	0.2359	0.2363	0.2459	0.258	0.2738	0.3069
AJ6	100	0	6.3E-04	9.17E-04	1.02E-03	1.07E-03	1.09E-03	1.24E-03

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Table 2: Thermal properties and crystallinity of the prepared electrolytes.

Sample name	T_g°C	T_m°C	ΔH_m (J/g)	X_c (%)
AJ1	57.00	285	83.60	74.64
AJ2	56.00	272	68.95	61.56
AJ3	55.00	267	53.10	47.41
AJ4	58.00	277	62.90	56.16
AJ5	59.00	290	71.41	63.75
AJ6	61.00	299	78.42	70.01

Fig 1. FTIR spectrum of pure PVC, PEMA, LiClO4, EC, DMC

Fig 2. FTIR Spectrum of as-synthesized GPE

Fig 3. Temperature dependent complex impedance plot PVC (5wt%)-PEMA (20wt%)-EC/DMC (67wt%)-LiClO₄ (8 wt. %)

Fig 4. Temperature dependent conductivity plots of the prepared electrolytes.

Fig 5a. DSC thermograms of pure PVC, pure PEMA and blend PVC/PEMA

Fig 5b. DSC thermograms of PVC-PEMA-LiClO₄-EC-DMCcomplexed films in the temperature of 0 to 140℃ indicates Tg values

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Fig 5c. DSC thermograms of PVC-PEMA-LiClO₄-EC-DMC complexed films in the temperature of 0 to 400℃ indicates Tm values
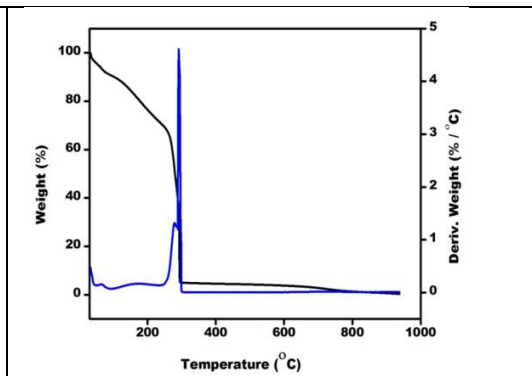

Fig 6. TG/DTA analysis of PVC-PEMA-LiClO₄-EC-DMCcomplexed electrolyte
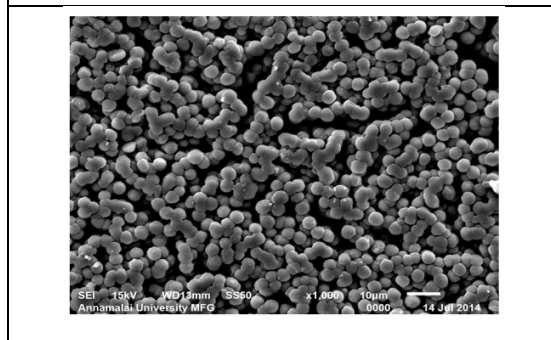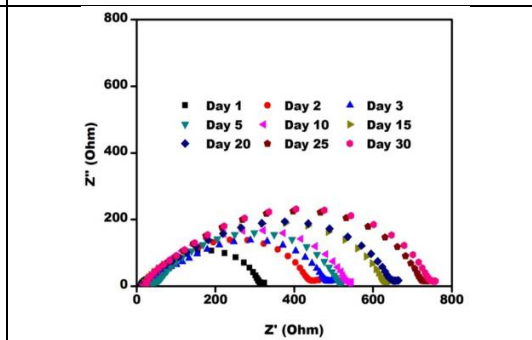

Fig 7. surface morphology of GPE

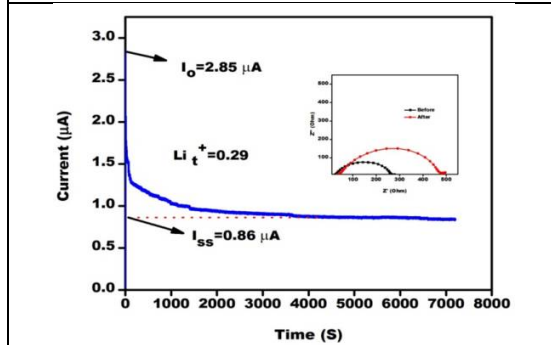**Fig 8. Interfacial stability of the GPE**

Fig 9. The chronoamperommetric profile for GPE Insets: Nyquist plot of symmetric cell before and after perturbation.
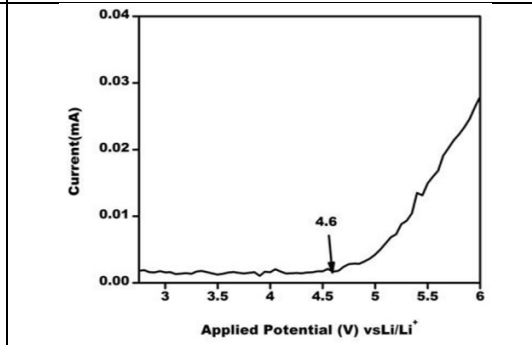

Fig 10. LSV curve of GPE

Generalized Fractal Dimension of Normal and Abnormal EEG Signals of VEP

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ABSTRACT

Fractal approach is one of methods to analyze the chaotic nature of the biomedical signals. Since these signals possess the multi scaling, the multifractal technique namely the generalized fractal dimension is used to analyze the irregularity and complexity of the signals. The generalized fractal dimension of the electroencephalogram signals of the visual evoked potential both in normal and abnormal conditions are calculated. The measures calculated from normal and abnormal signals are being analyzed for the prediction of the level of infection of the patients.

Keywords: Generalized fractal dimension, complexity, Irregularity, Electroencephalogram, Normal and Abnormal Visual evoked potential, Renyi dimension.

INTRODUCTION

The word "fractal" was first exercised by the mathematician Benoit Mandelbrot in the year 1975. The prescribed mathematical definition for fractal is given by Benoit Mandelbrot, and he says that a fractal is a set for which the Hausdorff Besicovich dimension strictly exceeds its topological dimension [1, 12]. However, this is a very abstract definition. In general, fractal is defined as a rough or fragmented geometric shape which can be subdivided into parts, each of which is (at least approximately) a reduced-size copy of the whole [2-4]. Fractals are generally self-similar and independent of scale. Lots of people are fascinated by the beautiful images termed fractals. Moving further than the typical perception of mathematics as a body of complicated, fractal geometry combines art with

mathematics to exhibit that equations are more than just a set of numbers. This makes fractals yet more interesting is that they are the greatest on hand mathematical images of many natural appearances, such as coastlines, mountains and parts of living organisms [5, 7]. In 1930s, during the early years of clinical EEG, Adrian and Matthew demonstrated changes of the occipital EEG that can be observed under stimulation of light. These evoked potentials needed to be took out from EEG and amplified, this was made by the 'signal averaging' device initially demonstrated by Dawson in 1951. At present, different computer programs are on hand that keep a described time interval of EEG activity after a visual stimulus, which is then repeated multiple times and the signals added together. The random EEG movement averages beyond, leaving the visual evoked potential [6]. Ciganek developed the first nomenclature for occipital EEG components. In 1965, Spehlmann make use of a checkerboard stimulation to explain human VEPs. Halliday et al brings out the principal clinical investigation using a VEP by recording delayed VEPs in a patient with retro bulbar neuritis in 1972 [8].

The Visual evoked potential is primarily a relatively large, positive polarity wave generated in the occipital cortex in response to visual stimulation. It measures the condition time of neuronal activity from the retina to the occipital cortex and is used clinically as a measure of the integrity and function of that pathway [10]. The optic nerve is the primary structure examined. The Evoked potentials are measures that record the brain's electrical response to visual, auditory, and sensory stimuli, which is obtained by placing recording sensors on the patient's scalp. The visual-evoked cortical potential (VEP) is an important electrophysiological test in the investigation of suspected optic nerve disease. The stimulus for diagnostic VEP is usually a reversing black and white checkerboard or grating, but an appearance stimulus can also be used [9]. VEP results are a representation of the functional integrity of all levels of the visual pathway including the anterior segment, retina, optic nerve, optic chiasm, lateral geniculation nucleus and visual cortex [8]. This refers to inflammation of the optic nerve, associated with swelling and progressive destruction of the sheath covering the nerve, and sometimes the nerve cable [11]. As the nerve sheath is dented, the time it takes for electrical signals to be carried out to the eyes is extended, resulting in an abnormal VEP. The VEP is a painless, safe, non-invasive vision test used to objectively measure neurological responses of the entire visual pathway. VEP calculates neurological responses by calculating the electrical movement in the vision system.

Generalized Fractal Dimension

A fractal dimension is an index for characterizing fractal patterns or sets by measuring their complexity as a ratio of the transform in detail to the transform in scale. In calculating the generalized fractal dimension, the following algorithm is being followed. The total database of the signal is divided into N parts according to the maximum and minimum values of the signal data, such that

If $X=(x_1, x_2, \ldots, x_N)$ is the given data set of the signal then $x_{max}=\max(x_i)$ and $x_{min}=\min(x_i)$ for $1 \le i \le N$.

$$r = \frac{(x_{max} - x_{min})}{n},$$ where the uncertainty factor n is assumed to be very small based on the device used to measure the signal.

N_i=the number of data samples of the signal belongs to the i-th bin. The probability of the data belonging in the i-th bin is defined as,

$$p_i = \frac{N_i}{N}.$$

The generalized fractal dimension or the Renyi dimension of order $q \in (-\infty, \infty)$ for the above probabilities p_i is defined by the formula,

$$D_q = \lim_{r \to 0} \frac{1}{q-1} \frac{\log_2 \sum_{i=1}^{N} p_i^q}{\log_2 r}.$$

In the equation Dq;

If q=0, the Hausdorff dimension or the fractal dimension D_0 is obtained, is defined by

$$D_0 = -\frac{\log_2 N}{\log_2 r}.$$

If q=1, the information dimension D_1 is obtained, is defined by

$$D_q = \lim_{r \to 0} \frac{\sum_{i=1}^{N} p_i \log_2 p_i}{\log_2 r}.$$

If q=2, the correlation dimension D_2 is obtained, is defined by

$$D_q = \lim_{r \to 0} \frac{\log_2 \sum_{i=1}^{N} p_i^2}{\log_2 r}.$$

Experiment of EEG signal of VEP

The visually evoked potential (VEP) computes the electrical reaction from the brain's primary visual cortex to a visual stimulus. For measuring the electrical response of the brain's primary visual cortex, three electrodes are placed on the scalp. An evoked potential test computes the time taken in the process which the brain responds to sensory stimulus through the processes like touch, sight or sound. Experts utilize the test for facilitate diagnose multiple sclerosis (MS) and other circumstances that can make a person's reactions to slow. The test can detect unusual responses to stimulation. Suppose if there is damage in the nerve sheath, the time period taken by the process for electrical signals to be carried out to the eyes is expanded, which will result in an abnormal VEP. It can be viewed in several scleroses and in the most common causes of optic neuritis which is discussed earlier. This damage to the optic nerve, leads to expanded VEPs. In this modality, a concise flash of space 5 ms subtending a image field for minimum of 20 degrees is the stimulus. Using flashing screen this result can be attained, otherwise either keeping a stroboscopic light in hand or by placing a Ganzfeld bowl before of the patient. The flash rate 1.0Hz ± 0.1 is recommended for the experiment, which means the flash rate 1 per second is recommended. The wave forms of flash VEP is given in figure 1. The first identifiable wave appears 30 ms after the stimulus and latter components are recordable up to 400 ms. Peaks are then assigned as positives & negatives in a numerical series progression. A nomenclature distinguishes the flash VEP and the pattern reversal VEP. The amplitude of positive peak is taken at around 220 ms and the negative peak at around 140 ms. The Generalized fractal dimension is calculated by using the graph and the absolute values taken in this experiment. In order to differentiate normal and abnormal VEP signals, let

$$Dim = \frac{1}{q-1} \log_2 \sum_{i=1}^{N} p_i^q.$$

Then for the given EEG of the VEP signal, the graph is plotted and the graphs are analysed for the difference between normal and abnormal VEP signals.

The above figures are the signal representation of the Normal and Abnormal Visual evoked potential of the right and the left eye observation. The VEP clinical data composed in this study consists of four signals of two kinds and which was obtained from the EEG database available with the website Physionet.org. For different q values the fractal dimensions were calculated and the values of D_q are observed. The fractal dimension D_q against the scaling factor q, for both Normal and Abnormal VEPs were plotted.

The fractal dimension values like Hausdorff dimension when q=0, information dimension when q=1, correlation dimension when q=2 and multifractal dimension values for other q values calculated and the same are tabulated as follows:

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From the above calculation, the deviation values on both left and right eyes are taken as data x and data y respectively, and correlation between both the left and right eye data is calculated. The detailed calculation is given in table 2.

In the above table 2 , The assumptions taken are *X:* Deviation Values from left eye and *Y:* Deviation Values from right eye, M_x: Mean of X Values, M_y: Mean of Y Values, *Also X - M_x* and *Y - M_y*: Deviation scores and $(X - M_x)^2$ and $(Y - M_y)^2$: Square of Deviation Scores, $(X - M_x)(Y - M_y)$: Product of Deviation Scores.

The Calculated values from the table are,
From X Values,

$\sum X$ = 2.364 and Mean, $\dfrac{\sum X}{n}$ = 0.296, and $\sum(X - M_x)^2$ = 0.269

From Y Values,

$\sum Y$ = 2.195 and Mean, $\dfrac{\sum Y}{n}$ = 0.274, and $\sum(Y - M_y)^2$ = 0.42

From X and Y Combined Values,
N = 8, $\sum(X - M_x)(Y - M_y)$ = 0.303

The correlation coefficient r is given by the Calculation,

$$r = \frac{\sum (X - M_x)(Y - M_y)}{\sqrt{\sum (X - M_x)^2}\ \sqrt{\sum (Y - M_y)^2}}$$

$$= \frac{0.303}{\sqrt{0.269}\sqrt{0.42}}$$

$$= 0.9023$$

This is a strong positive correlation, which means that high X variable scores go with high Y variable scores and vice versa. Comparing left and right normal eyes, the fractal dimension values are close to one another. But on the abnormal or affected eyes there is a huge variation between the values of dimension. It seems that when the eyes are affected, the infection level is not same for both left and right eyes. The calculations are done by using MATLAB Software. The probability values for each bin is calculated for the considered VEP signals and using the multifractal dimension formula the generalized fractal dimension is calculated for the entire signals.

CONCLUSION

The VEP is a promising expertise which helps the world in realizing the neural technology in the evoked biologic signals with reaction to visual stimulation. In this discussion, we have analysed the Electro encepelogram of the Normal and Abnormal Visual Evoked Potentials using the generalized fractal dimension. The GFD is useful to distinguish between the Normal and Abnormal VEP signals. Hence we conclude that the generalized fractal dimension method plays an important role in analysing the signals to detect and predict the illness of the patients. The Multifractal GFD analysis method is significant tool in all type of complex and irregular signals.

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Table 1: Fractal Dimension Values for the normal and abnormal VEP from Left and right eyes

Sl.No.	Dimension constant, q	Left Eye				Right Eye			
		Normal	Abnormal	Deviation	Affected level %	Normal	Abnormal	Deviation	Affected level %
1	0	1.623	1.127	0.496	30.56	1.602	1.006	0.596	37.20
2	1	1.345	0.753	0.592	44.01	1.375	0.758	0.617	44.87
3	2	0.8125	0.521	0.2915	35.88	0.9375	0.5015	0.436	46.51
4	3	0.6250	0.1875	0.4375	70.00	0.6345	0.3625	0.272	42.87
5	4	0.3752	0.1406	0.2346	62.53	0.3364	0.2436	0.0928	27.59
6	5	0.2503	0.07813	0.17217	68.79	0.2570	0.1563	0.1007	39.18
7	6	0.1250	0.0469	0.0781	62.48	0.08323	0.0375	0.04573	54.94
8	7	0.0938	0.0313	0.0625	66.63	0.05625	0.02125	0.035	62.22

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Table 2: The Correlation between the deviation of data from the Left and right eyes

SI.No.	Left Eye (X)	Right Eye (Y)	$X-M_x$	$Y-M_y$	$(X-M_x)^2$	$(Y-M_y)^2$	$(X-M_x)(Y-M_y)$
1	0.496	0.596	0.200	0.322	0.040	0.103	0.064
2	0.592	0.617	0.296	0.343	0.088	0.117	0.102
3	0.2915	0.436	-0.004	0.162	0.000	0.026	-0.001
4	0.4375	0.272	0.142	-0.002	0.020	0.000	0.000
5	0.2346	0.0928	-0.061	-0.182	0.004	0.033	0.011
6	0.17217	0.1007	-0.123	-0.174	0.015	0.030	0.021
7	0.0781	0.04573	-0.217	-0.229	0.047	0.052	0.050
8	0.0625	0.035	-0.233	-0.239	0.054	0.057	0.056
Total			0.296	0.274	0.269	0.420	0.303
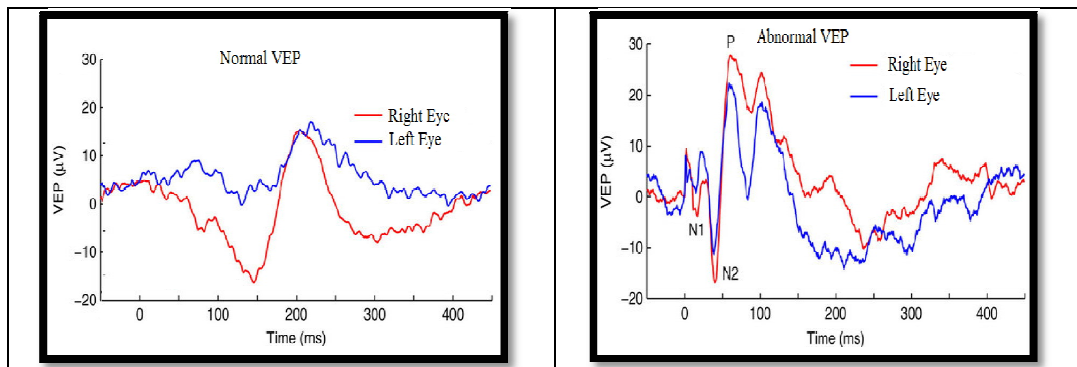
Fig 1 : VEP of a patient with normal and abnormal wave forms of left and right eyes
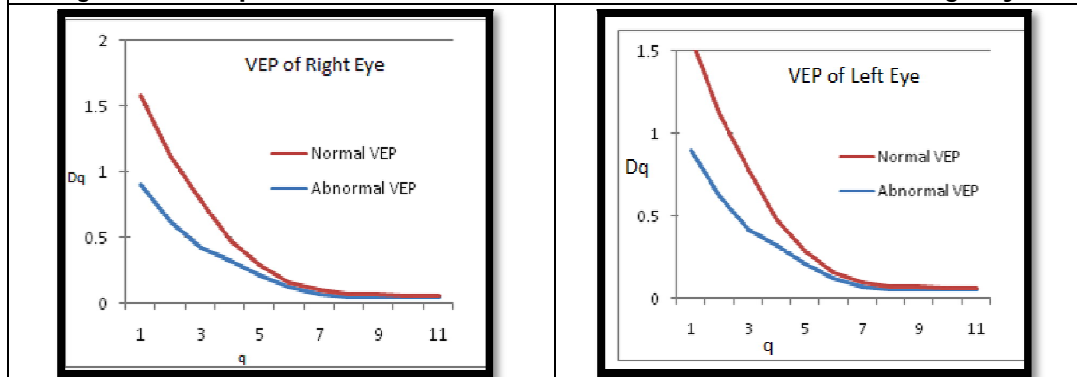
Fig 2 : Graphs between q versus Dq of the normal and abnormal VEP from Left and right eyes

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RESEARCH ARTICLE

Effectiveness of Warm up Exercises and Post Isometric Relaxation in Improving Hamstring Flexibility in Middle Aged Women

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ABSTRACT

The purpose of the study is to evaluate the effect of Warm Up Exercises and Post Isometric Relaxation on Hamstring Flexibility. 10 middle aged women between the age group of 35 and 45 years in and around Salem who had Hamstring Tightness were selected as samples using simple random sampling technique. Pre-test measurements of Hamstring Flexibility were collected using Passive Knee Extension Test. After the Pre-test assessment, all the subjects received Warm up exercises and Post Isometric Relaxation for a period of 15 days and on the 15th day, Post-test measurements were collected similarly to that of Pre-test measurement. The results showed that warm up exercises and post isometric relaxation were significantly effective in improving hamstring flexibility in middle aged women.

Keywords: Post Isometric Relaxation, Hamstring Flexibility, middle aged women

INTRODUCTION

Many of the middle-aged women have problem in some of the activities of daily living because of the hamstring muscle tightness. If the muscle flexibility is improved and maintained, their performance will be good without any pain or hassle in their day-to-day activities. So, our study mainly aims on improving and maintaining the hamstring muscle flexibility in middle aged women as most of their life style is sedentary. Hamstrings is an important muscle in the body which has an integral role in maintaining the body in the upright posture. Hamstring flexibility is very vital as tightness could lead to altered pelvic rotations. Muhammed Şeref Yıldırım *et al.* showed that the cut-off values for the diagnosis of hamstring tightness in case of passive knee extension angle >32.2° for males and >19.2° for females [1]. Warm up Exercises increases flexibility as well as lowers the risk of injury and improves the performance and increases the blood circulation. Muscle Energy Technique (MET) is a manual therapy technique which has two types post isometric relaxation and reciprocal inhibition which uses a muscle's own energy in the form of gentle

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isometric contractions to relax the muscles through autogenic inhibition or reciprocal inhibition, and lengthen the muscle. This is done by giving gentle isometric contractions which uses the muscles own energy. Post-Isometric Relaxation (PIR) is one of the types of muscle energy technique in which the therapist stretches and lengthens a muscle, and it relaxes after the contraction. This procedure lengthens and relaxes the muscle fibres.

Adaptability and flexibility are ideal characteristic of musculoskeletal system. It is additionally basic in the recovery and avoidance of skeletal wounds, and in the improvement of performance in our life even after our age increase. Hamstring muscle tightness is fairly common in a female population. Harreby *et al.* exhibited that tightness of hamstring as a danger factor for low back torment [2]. An absence of hamstring extensibility is thought to prompt changes in lumbopelvic cadence. Kendall *et al.* have revealed the relationship between unreasonable lumbar spinal flexion and diminished hamstring extensibility [3]. White *et al.* in their examination found that patients with patellofemoral pain had shorter hamstring muscles with asymptomatic controls [4]. Reduced hamstring extensibility is likewise thought to incline competitors to injury. Every strategy has certain preferences, unique highlights, and physiologic impacts. A few investigations have analysed the viability of these techniques. There is no consistent agreement on the best extending strategy with very much characterized boundaries. A few investigations have underscored the adequacy of latent stretches on the adaptability of tight muscles by changing the mechanical properties of the muscle and its flexibility. The aim of the study is to find out the effectiveness of Warm Up Exercises and Post Isometric Relaxation in improving hamstring flexibility in middle aged women. The need of the study is as many of the middle-aged women who have a sedentary life style have a tight hamstrings problem, which hinder their works in day-to-day activities and end with back pain, knee pain and all. So, improving the flexibility and maintaining that will reduce their problem to maximum level. Hence, we work on finding whether introducing newer physiotherapeutic technique like Post Isometric Relaxation when given in addition to Warm Up Exercises will improve and maintain the hamstring flexibility.

MATERIALS AND METHODS

The study design is a Pre and Post experimental trial. 10 samples were selected using simple random sampling method. The subjects who had hamstring tightness and are medically and psychologically fit and also who fulfil the selection criteria of between age group of 35 and 45 in and around Salem were only included for the study. The parameter for hamstring tightness was kept as passive knee extension angle above 19.2 degrees. Informed consent was obtained from the subjects and then only they were included for the study. Pre-test assessment was taken using passive knee extension test for all the 10 samples for both the legs using goniometer for both groups in the following fashion. The subjects were positioned in supine lying with hip and knee at 90-degree Flexion. Neutral hip rotation was maintained throughout the test. The fulcrum of the goniometry was placed over lateral intercondylar area of knee joint. The stable arm is placed along the lateral border of femur and the movable arm is placed along the lateral border of fibula and with this alignment of the goniometer passive ROM of knee extension was measured. Then all the subjects were given warm up exercise for 10 to 12 mins with rest periods in between. The warm up exercises included a) Marching in the standing position itself with upper limb reaching outwards b) moving back and front from the standing position one step ahead with upper limb reaching forwards c) moving one step side wards from the standing position and coming back to normal with both upper limbs reaching sidewards.

After the Warm up exercises Post Isometric Relaxation Technique for hamstring muscle was given. The treatment technique was given to both the legs. The subjects were made to lie in supine lying with contralateral hip and knee supported by a pillow below the knee. The therapist stood on the side of the leg to which post isometric relaxation need to be given. The hip is flexed to 90 degrees and knee is fully flexed. Then the knee was extended until the restriction barrier was identified. Then the leg was placed on the shoulder of the therapist and the patients was asked to contract the muscle sub maximally by pushing downwards on the shoulders of the therapists whereas the therapists give a submaximal resistance (a counter force). The contraction is kept held for 7-10 counts and then it is

relaxed for 10 counts. After that again the limb was passively moved by the therapists and new barrier was identified. Then the procedure was repeated from the new barrier. The technique was given 6 times. The same protocol was repeated for the other leg. Treatment technique was given once daily for 15 days for both the sides. At the end of the 15th day post-test was taken using Passive Knee Extension Test. The collected data was subjected to statistical analysis using paired "t" test for both the legs separately (Table No. 1).

RESULTS AND DISCUSSION

The data subjected to statistical analysis using paired "t" test for 9 degrees of freedom at 95% confident level had revealed that the Warm up exercises and Post Isometric Relaxation was found to be significantly effective in increasing Hamstring Flexibility in Middle Aged Women. The results of the study shows that Warm up exercises and Post Isometric Relaxation is significantly effective may be because, the principles of autogenic inhibition are employed in post isometric relaxation. In this Contraction of the facilitated muscle from a lengthened position generates sufficient tension to activate the Golgi endings in the tendon. This reflex inhibits both the gamma and the alpha motor neurons. This results in lengthening of the muscle upon relaxation. Accurately localized, low-intensity, isometric contractions of the agonist or antagonist segmental muscles are the most effective for restoring mobility and thereby reducing pain. Francis P *et al.* I have assessed changes in hamstring flexibility utilizing different extending strategies. Nonetheless, the viability and clinical utilization of these procedures stay disputable [5]. In the current study, the impact of warm up exercises and one of the muscle energy technique post isometric relaxation was analysed on hamstring flexibility. The outcomes showed that the two techniques essentially improved the hamstring flexibility. Agarwal Sonal *et al.* proved that there is a significant improvement in Hamstring Flexibility, when Post Isometric Relaxation was given[6]. Shadmehr *et al.* demonstrated that Hamstring Flexibility improves following Post Isometric Relaxation in women[7]

CONCLUSION

The result of the study makes us to conclude that Warm Up and Post Isometric Relaxation are significantly effective in improving hamstring flexibility in middle aged women.

ACKNOWLEDGEMENT

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Table 1. Passive Knee Extension test data with paired 't' test

Test	Variable	t calculated value	t table value
Paired "t" test for Warm up exercises and Post Isometric Relaxation for Right leg	Flexibility	14. 78	2.262
Paired "t" test for Warm up exercises and Post Isometric Relaxation for Left leg	Flexibility	13.62	2.262

RESEARCH ARTICLE

Panic Buying and Price Variation

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ABSTRACT

Crisis situation like COVID pandemic resulted in announcement of lockdown to contain the spread of virus. The restrictions on the operations of the shopping centers and markets created panic among the consumers. The current study focused on finding the impact of this panic among the consumers. Since the elasticity of demand has its effect on price, the impact of panic among consumers was studied comparing the prices of essential commodities during pre-lockdown, lockdown and post-lockdown. The study found that price of the most commonly consumed essential commodities increased lockdown.

Keywords: Panic buying, price, essential commodities, consumer behaviour

INTRODUCTION

Crisis situations leads the common public stranded without cues. Lack of exposure to such situations makes the situation complicated for the common public and the administering machinery. Lockdown was imposed among the public with restrictions for the operations of the shopping centers. Especially these crisis situations creates panic among the consumers and make them end up with panic buying.

Literature review

Panic buying was defined as "the unusual bulk purchase by the consumers in expectation of shortage or price hike."
Parveen Roja M (2020) found that "security was the most important aspect of consumer behaviour."S.M. Yasir Arafat et al. (2020) found that "a high proportion of panic buying were reported from developed countries."
Catherine Prentice et al. (2020) concluded that "a causal relationship existed between the timing of government measures and panic buying."

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Samuel Lins and **Sibele Aquino** (2020) found that "men buy more in panic than women; panic buying scale was positively correlated with impulse buying, past and future temporal focus and risk perception and it was negatively correlated with optimism and age."

Mary Loxton et al. (2020) concluded that "panic buying, herd mentality and discretionary spending as anticipated by Maslow's Hierarchy of Needs were witnessed among the consumers that appears to align with their behaviours exhibited during shocks and crises of the past."
Tahir Islam et al. (2021) found that "during COVID, information on scarcity of quantity and time caused perceived arousal leading to impulsive and obsessive buying."
Muhammad Naeem (2021) found that "proof of uncertainties and insecurities, persuasive buying, proof of product unavailability, authorities' communication, global logic, and expert opinion are some of the causes on social media platforms that developed the situation of consumer panic buying during Covid-19 crisis."

METHODOLOGY

The study focused on comparison of prices of essential commodities for the time span of 3 months in each of the three time periods COVID lockdown (March -June, 2020), pre-lockdown (December 19 - February 2020) and post-lockdown (July-September, 2020). The secondary data on prices of essential commodities was referred from the Indian Government consumer affairs official website. The Department of consumer affairs is monitoring the price of 23 essential commodities for some selective cities across different states of India. The current study referred the details of prices of 21 essential commodities given for 9 cities in Tamilnadu. These essential commodities include rice, wheat, wheat(atta), gram dal, tur dal, urad dal, moong dal, masoor dal, groundnut oil (packed), mustard oil (packed), vanaspati (packed), sunflower oil (packed), palm oil (packed), potato, tomato, onion, sugar, gur, milk, tea loose and salt pack (iodised).

RESULTS AND ANALYSIS

Table 1: Correlations
The variation of prices during the three time periods were subjected to Karl Pearson correlation analysis. The correlation analysis showed that there pre-lockdown price variation and lockdown prices variation were positively but moderately related but the pre-lockdown price variation (r=.299, p(.002)<0.05) and post-lockdown prices variation (r=-.580, p(.000)<0.05) were negatively but strongly related.

Table 2: ANOVA
 The ANOVA results showed that variation of prices during pre-lockdown, lockdown and post-lockdown were significantly different (F(pre-lockdown)=38.366, p(.000)<.05, F(lockdown)=4.923, p(.000)<.05, F(post-lockdown)=14.096, p(.000)<.05)

Table 3: Paired Samples Test
 The paired samples T test showed that price variation during lockdown and post lockdown for rice, gram dal, tur dal, moong dal, tomato (t(rice)=4.193, p(.009)<.05, t(gram dal)=4.298, p(.008)<.05, t(tur dal)=3.242, p(.023)<.05, t(moong dal)=9.768, p(.000)<.05, t(tomato)=-.201, p(.008)<.05) were significantly different. The paired samples T test showed that price variation during pre-lockdown and lockdown for tur dal, groundnut oil (packed) palm oil (packed), tomato, onion (t(tur dal)=-6.279, p(.003)<.05, t(groundnut oil (packed))=-3.171, p(.034)<.05, t(palm oil (packed))=6.244, p(.003)<.05, t(tomato)=-8.401, p(.001)<.05, t(onion)=-4.504, p(.011)<.05) were significantly different. The paired samples T test showed that price variation during pre-lockdown and post-lockdown for moong dal, sunflower oil (packed), tomato, onion (t(moong dal)=11.383, p(.000)<.05, t(sunflower oil (packed)=3.112, p(.036)<.05, t(tomato)=-4.965, p(.008)<.05, t(onion)=-4.843,p(.008)<.05) were significantly different.

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Table 4: Paired Samples Test
The paired samples T test showed that price variation of essential commodities during pre-lockdown and lockdown for Chennai, Tiruchirapalli, Coimbatore, Tirunelveli (t(Chennai)=-2.156, p(.021)<.05, t(Tiruchirapalli)=-2.221, p(.039)<.05, t(Coimbatore)=-2.393, p(.027)<.05, t(Tirunelveli)=-2.102, p(.048)<.05

DISCUSSION

The price variation during pre-lockdown and lockdown for tur dal, groundnut oil was significantly lower than lockdown except palm oil (packed) where the price variation during pre-lockdown was significantly higher than lockdown. The price variation during lockdown was significantly higher than post lockdown for rice, gram dal, tur dal, moong dal except tomato where the price variation during lockdown was significantly lower than post lockdown. The price variation during pre-lockdown was significantly higher than post-lockdown for moong dal, sunflower oil (packed) except tomato, onion where the price variation during pre-lockdown was significantly lower than post lockdown. The price variation of essential commodities during pre-lockdown was significantly lower than lockdown for Chennai, Tiruchirapalli, Coimbatore, Tirunelveli. The prices of rice, gram dal, tur dal, moong dal and groundnut oil (packed) increased during the lockdown period but the price of tomato decreased during the lockdown period. The most commonly used cereals, pulses and edible oil in Tamilnadu increased during the lockdown period. These commodities could be stored and used for a period of time span more than 6 months. The price of essential commodities increased during lockdown in Chennai, Tiruchirapalli, Coimbatore, Tirunelveli. The price increase was high in Chennai followed by Coimbatore, Tiruchirapalli and Tirunelveli. The price increase was high among the Tier 1 cities than the Tier 2 cities.

Managerial Implications
In handling crisis situations, the administration could consider the extension the timing of the operations of wholesale and retail stores to avoid the panic among the buyers regarding the restriction on buying. For tier 1 cities additional time of operation of these stores and modes of delivery could be worked out during the crisis. The celebrity endorsements could be used to instill confidence among the consumers regarding the availability of essential commodities. Awareness on how the panic buying would affect the price and create loss to the consumers could be brought through the frequent communications about the safety precautions to be adopted during the crisis.

CONCLUSION
The restricted buying situation creates panic buying among the consumers during the lockdown situation. The hype in demand situation caused a spurt of rice hike during the lockdown period. The consumers express their security needs and buy in bulk quantities these essential commodities in anticipation of unavailability of these commodities and increase of prices. However the price of these commodities increased due to sudden increase of demand during lockdown period and later subsided when the demand normalised during the post-lockdown period.

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Table 1: Correlations

		PRELOCKDOWN %VARIATION	LOCKDOWN %VARIATION	POSTLOCKDOWN %VARIATION
PRELOCKDOWN % VARIATION	Pearson Correlation	1	.299[**]	-.580[**]
	Sig. (2-tailed)		.002	.000
	N	102	102	102
LOCKDOWN % VARIATION	Pearson Correlation	.299[**]	1	-.003
	Sig. (2-tailed)	.002		.970
	N	102	124	124
POSTLOCKDOWN % VARIATION	Pearson Correlation	-.580[**]	-.003	1
	Sig. (2-tailed)	.000	.970	
	N	102	124	186
[**]. Correlation is significant at the 0.01 level (2-tailed).				

Table 2: ANOVA

		Sum of Squares	df	Mean Square	F	Sig.
PRELOCKDOWN%VARIATION	Between Groups	31300.089	20	1565.004	38.366	.000
	Within Groups	3304.114	81	40.792		
	Total	34604.203	101			
LOCKDOWN%VARIATION	Between Groups	18756.624	20	937.831	4.923	.000
	Within Groups	19620.151	103	190.487		
	Total	38376.775	123			
POSTLOCKDOWN%VARIATION	Between Groups	143956.122	20	7197.806	14.096	.000
	Within Groups	84253.565	165	510.628		
	Total	228209.687	185			

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COMMODITYCODE			Paired Differences							Sig. (2-tailed)
			Mean	Std. Deviation	Std. Error Mean	95% Confidence Interval of the Difference		t	df	
						Lower	Upper			
RICE	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	9.422	5.503	2.247	3.646	15.197	4.193	5	.009
GRAM DAL	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	16.720	9.528	3.890	6.721	26.719	4.298	5	.008
TUR DAL	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-13.418	4.778	2.137	-19.351	-7.485	-6.279	4	.003
	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	14.085	10.643	4.345	2.915	25.255	3.242	5	.023
MOONG DAL	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	25.093	6.292	2.569	18.490	31.697	9.768	5	.000
	Pair 3	PRELOCKDOWN% VARIATION - POSTLOCKDOWN% VARIATION	23.568	4.630	2.071	17.819	29.317	11.383	4	.000
GROUND NUT OIL (PACKED)	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-9.156	6.457	2.888	-17.174	-1.138	-3.171	4	.034
SUNFLOWER OIL	Pair 3	PRELOCKDOWN% VARIATION - POSTLOCKDOWN% VARIATION	3.262	2.344	1.048	.352	6.172	3.112	4	.036
PALM OIL (PACKED)	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	19.566	7.006	3.133	10.866	28.265	6.244	4	.003
	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	-14.330	7.143	2.916	-21.826	-6.834	-4.914	5	.004
POTATO	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-12.036	13.507	6.041	-28.808	4.736	-1.992	4	.117
TOMATO	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-26.420	7.033	3.145	-35.152	-17.688	-8.401	4	.001
	Pair 2	LOCKDOWN%VARIATION - POSTLOCKDOWN% VARIATION	-83.117	48.461	19.784	-133.974	-32.260	-4.201	5	.008

Table: 3 Paired Samples Test

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			Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig.
	Pair 3	PRELOCKDOWN% VARIATION - POSTLOCKDOWN% VARIATION	-118.660	53.444	23.901	-185.020	-52.300	-4.965	4	.008
ONION	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-72.832	36.161	16.172	-117.732	-27.932	-4.504	4	.011
	Pair 3	PRELOCKDOWN% VARIATION - POSTLOCKDOWN%VARIATION	-129.856	59.958	26.814	-204.304	-55.408	-4.843	4	.008

Table 4: Paired Samples Test										
			Paired Differences			95% Confidence Interval of the Difference				
CITYCODE			Mean	Std. Deviation	Std. Error Mean	Lower	Upper	t	df	Sig. (2-tailed)
CHENNAI	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-6.021	10.967	2.393	-11.013	-1.029	-2.516	20	.021
THIRUCHIRAPALLI	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-13.859	27.910	6.241	-26.922	-.797	-2.221	19	.039
COIMBATORE	Pair 1	PRELOCKDOWN% VARIATION - LOCKDOWN%VARIATION	-8.424	15.741	3.520	-15.791	-1.058	-2.393	19	.027
TIRUNELVELI	Pair 1	PRELOCKDOWN%VARIATION - LOCKDOWN%VARIATION	-10.636	23.186	5.059	-21.190	-.082	-2.102	20	.048

RESEARCH ARTICLE

A Study on Role of Work Place Autonomy and its Impact on Quality of Work Life

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ABSTRACT

Improving autonomy and independence in the workplace can cause a revolution of the work experience leading to better performance and, as such, feature in the literature on organisational development produced as guidance for local government bodies (Allington and Morgan, 2002a). The study aims to analyse the role of implementation of autonomy programmes and its impact on Quality of work life. It also compared public sector and private sector bank's professionals in analyzing the implementation of autonomy programmes and its impact on Quality of Work Life. It also found that autonomy is higher in public sector banks than in private sector banks.

INTRODUCTION

An employed person have much of his or her job autonomy from the characteristics of work, which provide feelings of control over work and independence of decision-making. A group of senior professional staff with management experience were approached to give their view on what degree of autonomy and independence they perceived they had, and therefore, what degree of QWL. Quality of work life (QWL) is a philosophy a set of principles, which holds that people are the most important resource in the organisation as they are trustworthy, responsible and capable of making valuable contribution and they should be treated with dignity and respect (Straw and Heckscher 1984). QWL encompasses mode of wages payment, working conditions, working time, health hazards issue, financial and non-financial benefits and management behaviour towards employees (Islam and Siengthai 2009). According to Gadon (1984), QWL programmes have two objectives: a) to enhance productivity and (b) to increase the satisfaction of employees. Behram et al., (2012) revealed that there is a significant relationship between the variables namely salary

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and benefits, job security, healthy and second work environment, autonomy at work, providing the basis for skills education, and determining the job development direction with the employees effectiveness.

Conceptual Framework

Creating autonomy and independence involves not just removing layers of management, but also involves fundamentally changing how essential layers of management operate (Boyne, 1999). Not only should lower-level employees be given decision-making power, but they also need the training that will provide them with the ability to make good decisions (Boyne, Poole and Jenkins, 1999). Many practices have been used in recent years in both the private and public (Boyne, Poole and Jenkins, 1999) sectors to transform the workplace and improve performance. These include: increasing worker autonomy through flattening hierarchies; decentralizing authority; instituting quality programs; upgrading workers' skills through training; increasing flexibility in deployment of labor and establishing self managing work teams (Geary, 2002).The concept of quality of work life is a multifaceted concept. It is an impact of work on people as well as on organizational effectiveness (Sen Gupta, 1985). It covers a person's perception or feeling about every dimension of work included economic reward and benefits, security, working conditions, organizational and interpersonal relationship and intrinsic meaning in a persons' life (Karrir and Amulya, 1997). Jain (1984) identified eight conceptual areas in QWL from the concepts developed by Walton (1974). Lawler et al., (1982) identified eleven issues related to QWL. The increasing interest in human resources is due to the assumption that employees and the way they are managed is critical to the success of organization and can be a source of sustainable competitive advantage (Sabarirajan and Geethanjali 2011). These are related to pay, work, management, occupational stress, health and safety, recognition, supervisor – subordinate relation, grievance handling, adequacy of resources, promotion and security.

Objective of the study

The study aims to exhibit the implementation of autonomy programmes and the impact on QWL at the banks in Dindigul.

Research Methodology

The study was designed as a descriptive study with the intention to look at the phenomenon of QWL as it is in the banking industry. A survey method was chosen to carry out the study. Since there is no in depth study on this aspect and also no reliable scale was available, the questionnaire was prepared after an in depth literature review on the topic in keeping with the local context. The sampled employees were distributed among the public and private sector banks at Dindigul. The collected data were processed with the help of SPSS. First, the descriptive statistics were found out. Secondly, the validity and reliability of the measurement scales were done with the help of confirmatory factor analysis (Carmines and Zeller, 1988).

Implementation of Programmes in Autonomy

In total, there are three programmes in autonomy since their factor loadings are higher in autonomy compared with other important QWL. The included three programmes in autonomy explain it to the extent of 77.87 per cent since their respective reliability coefficient is 0.7787. The present study has made an attempt to analyse the reliability and validity of programmes in autonomy with the help of CFA. The result of CFA is given in Table 1.

Rate of implementation of 'Autonomous' programmes in banks

The rate of implementation of various autonomous related programmes at banks have been computed by the mean score of the implementation of the various programmes in 'autonomous' among the employees in banks. It is denoted by RIAP. In the present study, the RIAP is confined to less than 2.00, 2.00 to 3.00, 3.01 to 4.00 and above 4.00. The distribution of banks on the basis of their RIAP is given in Table 2.

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Findings

The standardized factor loading of the programmes in autonomy are greater than 0.60 which reveals the content validity of the consultant. The 't' statistics of the standardized factor loading of all programmes in autonomy are significant at five per cent level which indicates the convergent validity. It is also supported by the composite reliability and average variance extracted since they are greater than its respective minimum threshold of 0.50 and 50.00 per cent. The important RIAP in the banks are 2.00 to 3.00 and 3.01 to 4.00 which constitutes 29.12 and 32.42 percent to the total respectively. The important RIAP in PUSBs are 3.01 to 4.00 and above 4.00 which constitutes 37.59 and 26.24 percent to its total. In the PRSBs, these are 2.00 to 3.00 and less than 2.00 which constitutes 41.46 and 34.15 per cent to its total respectively. The analysis reveals that the rate of implementation of autonomous related programmes is higher in PUSBs than that in PRSBs.

CONCLUSION

It is clear from the results of the survey set out above and the interviews that autonomy and independence are not just key for QWL characteristics for staff but also improve organizations cost-efficiency. Organisations should be leading on developing technical and management skills to support programmes which improve levels of autonomy and independence for staff. As such, councils should be investing in programmes of training and development, which increase autonomy and independence, ensuring staff are properly trained and developed to take on higher responsibility and raising staff levels of technical ability, discretion and autonomy (Gallie et al., 1998). While, to some extent, this recognition has been reflected in the rising investment in qualifications and training by professional staff in the last few years (Green and Felstead,1994) the investment has been poorly used because of poor planning and a failure to develop integrated and targeted programmes of development.

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Table 1: Reliability and Validity of programmes in 'Autonomy' Programmes

SI.No.	Programmes in Autonomy	Standardized factor loading	't' statistics	Composite reliability	Average variance extracted
1.	Autonomous work groups	0.8732	3.6994*	0.7502	53.17
2.	Benefit options to employees	0.7609	2.8103*		
3.	Project teams	0.6453	2.2561*		
Overall reliability coefficient efficient: 0.7787.					

* Significant at five per cent level.

Table 2: Rate of Implementation of Autonomous Programmes (RIAP) at banks

SI.No.	RIAP	Number of banks in		Total
		PUSBs	PRSBs	
1.	Less than 2.00	15	14	29
2.	2.00 – 3.00	36	17	53
3.	3.01 – 4.00	53	6	59
4.	Above 4.00	37	4	41
	Total	141	41	182
	Overall Mean Scores	3.4964	2.7606	

RESEARCH ARTICLE

Service Quality of Health Care Centers and its Consequences: An Empirical Study

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ABSTRACT

The service sector is increasing at a faster rate and becoming more competitive today (Akter, et al., 2008). The health care consumers in developing countries are becoming more aware on the facilities at health care industries at the international level (Nketiah – Amponsah and Hiemenz, 2009). As the standard of living of the consumer, urbanization and increase in information and technology, there is a demand for better medical care to improve the life style of consumers (Alhashem, et al., 2011). The role of service quality at health care centers is widely recognized as being a critical determinant for the success and survival of the health care centers in the competitive environment. (Zain, et al., 2010). Many stakeholders in health care sectors are now emphasising service quality delivery (Lepsley, 2000) as a mechanism to meet consumer demand and value of money (Smith et al., 2006). Patient satisfaction has emerged as an important measure of the quality services offered by the health care centers (Noor, 2010). The understanding of patients' satisfaction on the Health Care Centers service quality will improve the outcome of healthcare system and enhance better service quality. (Arasli et al., 2008). In addition, patients' satisfaction are more likely to generate patent loyalty towards their Health Care Centers (Kessler and Mylod, 2011). Hence it is essential to study the patients view on the service quality of health care centers and its consequences for future policy implications.

Literature Review

Health Care Centers Service Quality
The Health Care Centers service quality have examined with the help of seven dimensions namely personnel quality, infrastructure, administrative process, process of clinical care, safety, overall medical care and social responsibility

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(Duggirala et al., 2008). Lani and Kunz (2004) examined the admission process, physician care, nursing care and discharging process to measure Health Care Centers service quality. Butt and Cyril (2010) and Sohail (2003) tested the basic servqual measures to measure the service quality in Health Care Centers. Chahal and Kumari (2010) analysed physical environment, interaction quality and outcome quality regarding the Health Care Centers service quality. Suki et al., (2011) focused on the interaction between patient and doctors and the confidence of the patients in the quality of medical services at Health Care Centers regarding this aspect.

Patient Satisfaction

The patient are satisfied if their perceived performance matches their expectations of services from the health care centers (Witlz, and Lee, 2003). Wirtz and Mattila (2001) measured the patient satisfaction by disconfirmation model. Patient's satisfaction is mediated by a patients' personal belief and values about a Health Care Centers (Kim et al., 2008). Grogan et al., (2000) identified the important role of patients' satisfaction in the measurement of quality care in Health Care Centers industry. Alhashem et al., (2011) revealed that there is a direct impact of relationship between patients and doctors on the patients' satisfaction. Esch et al., (2008) correlated the technical and interpersonal care with the patient satisfaction.

Patient Loyalty

Zithaml et al., (1996) mentioned that the patient loyalty as a signal the customers will remain in with the service provider. Ladhari (2009) measured the patients loyalty by positive word of mouth and repurchase intention Finkalstein et al., (1999) measured the patient loyalty by the recommendation of the treatment to others. Kessler and Mylod (2011) measured by higher satisfaction and revisit of the Health Care Centers. Amin et al., (2011) revealed that he patient loyalty was developed by the previous experience in the Health Care Centers. Gaur et al., (2011) found a significant relationship between the patient satisfaction and patient loyalty. Even though, there are so many studies related with the service quality, patient satisfaction and patient loyalty in health care industry, there is no exclusive study in the Dindigul district. Hence the present study has made an attempt to fill up the research gap with the help of proposed research model. Based on the proposed research model, the present study confine its objectives to (i) to test the validity and reliability of variables in each measurement scale; (ii) to examine the direct and indirect impact of Health Care Centers' service quality on patient loyalty.

Hypothesis

The formulated hypotheses of the present study are:
1. There is no significant direct effect of Health Care Centers' service quality on the patient loyalty;
2. There is no significant mediator role of patient satisfaction in between Health Care Centers' service quality and patient loyalty.

Research Methodology
Sample

The sample size of the study was determined as 532 patients with the help of formula of $n = \left[\dfrac{Z\sigma}{D} \right]^2$. The 532 patients were equally distributed to public and private health care centers in Dindigul district, Tamilnadu. Out 532 patients, only 296 patients were responded the schedule at the reusable level. Hence, these 296 schedules were taken for further analysis.

The Instrument

The interview schedule was used as an instrument to collect the relevant data from patients. Three dimensions of Health Care Centers service quality, patient satisfaction and patient loyalty along with their profile were incorporated in the interview schedule as per the recommendation of Aagja and Garg (2010); Choi and Kim (2012). A

scale ranging from '1' very low to '5' very high was used to measure Health Care Centers service quality, patient satisfaction and patient loyalty. To avoid the issues of conceptual and psychometric property raised by Cronin and Jaylor (1992), the researchers used only perception of quality (Yavas et al., 2004).

Conceptual Frame Work of the Study

Service Quality in Health Care Centers
The service quality in health care centers represents the quality of service offered by the health care centers to the patients (Muslem 2008). It is based on the original service quality factors carried by Parasuraman et al., (1988). These are reliability, responsiveness, assurance, empathy and tangibles. Lynch and Schuler (1990), Sadig (2003) and Zim et al., (2004) also supported the core service quality factors to measure the Health Care Centers service quality. It was extended to value added service quality by Studer (2003), Massaro (2003) and Deman et al., (2002). Hariharan et al., (2004) supplemented the core service quality factors long with the previous two to measure the health care centers service quality. The service quality in health care centers are classified into outcome quality, (Kang and James, 2004), interaction quality (Jap, 2001) and Peer-to-peer quality (Payne et al., 2008). The outcome quality is the quality of the outcome of service act and what the patient is left with after service delivery is complete (Groonroos, 1984). It reflects the patient's perception of the superiority of service experience (Brady and Cronin, 2001). Interaction quality is related to patient's perception of the interactions with service providers (Bitner et al., 1994). It shows the manner in which the service is delivered doing service encounters (Lemke et al., 2011). Peer-to-peer quality has been defined as a perceived judgement about an entity's excellence and superiority (Verhoef et al., 2009). In the present study, the above said three service qualities are measured with the help of relevant variables. These are listed in Table 1. Patient's satisfaction shows the overall attitude of the patients on the Health Care Centers services (Boyer et al., 2006). It includes the performance of Physician, Nurses, para medical staffs, administrative staffs and also their process (Crowe, 2002). Patient's Loyalty is the positive attitudes based on cumulatively satisfying usage occasions (Oliver, 1999). It is defined as "an intention to perform a diverse set of behaviour that signal a motivation to maintain a relationship with the focal firm, including allocating a higher share of the category wallet to the specific service provider, engaging in positive words of mouth, and repeat purchasing (Sirdeshmukh et al., 2002). The variables included in these two are presented in Table 1.

Data Analysis
The collected data were processed with the help of confirmatory factory analysis (Sureshkumar et al., 2002) and Structural equation model (Alrubaiee, L. and Alkaa'ida, 2011) in order to justify the validity of and reliability of measurement scale and the role of patient satisfaction as a mediator role in between Health Care Centers service quality and patient loyalty.

RESULTS

Descriptive Statistics
Majority of patients are belonging to private Health Care Centers. The dominant gender among the patients is female. The important level of education among the patients are at the school level and under graduation. The important nativity among the patients is semi-urban whereas the dominant family size among them is four members. The dominant family income per month among the patients is Rs.10,000 to 20,000 whereas the important years of experience in this present Health Care Centers is 5 to 7 years.

Reliability and Validity of variables in the Construction
The constructs developed to test the hypotheses in the present study are outcome quality, interaction quality, peer-to-peer quality, patients' satisfaction and patients' loyalty. The variables in the constructs are varying from 3 to 5. The present study has made an attempt to examine the reliability and validity of variables in each construct with the

help of confirmatory factor analysis (Anderson and Gerbing, 1998) and the Cronbach alpha (Nunnally, 1978). The results are shown in Table 2. The standardised factor loading of the variables in each construct is greater than 0.60 which reveals the content validity (Byrne, 2001). The significance of 't' statistics of the standardised factor loading of the variables in each construct reveal the convergent validity (Arun, 2012). It is also supported by the composite reliability and average variance extracted since these are greater than its minimum threshold of 0.50 and 50.00 per cent respectively (Carmines and Zeller, 1988). The Cronbach alpha of each construct exceeded the minimum standard of 0.70 (Nunnally and Bernstein, 1994). All these results indicate the reliability and validity of the constructs developed in the study.

Discriminant Validity among the Constructs

The discriminant validity among the five constructs have been tested with the help of the mean of AVE and square of correlation coefficient between all possible pair of the constructs developed in the present study. If the mean of AVEs is greater than its square of correlation coefficient between the pair, its discriminant validity will be assured (Fornell and Larcker, 1981). The results are given in Table 3. The mean of AVEs of all pair of the constructs are greater than its respective square of correlation co-efficient. It shows the discriminant validity among the constructs. For example, the mean of AVEs between outcome quality and interaction quality (0.5357) is higher than its square of correlation coefficient (0.5041). The sample types of results are seen in all possible pair of the constructs.

Patient's Perception on HCCSQ and its Consequences

The patent's perception on health care centers' service quality and its consequences namely patient satisfaction and patient loyalty have been measured by the mean score of the variables in each construct. The mean of each construct among the patients in private and public health care centers have been computed separately. The 't' test has been administered to find out the significant difference among the two group of health care centers regarding their patient's view on HCCSQ and its consequences. The results are given in Table 4. The highly perceived HCCSQ by the patients in private health care centers is outcome quality since its mean score is 3.9097. It is followed by interaction quality since it's mean score is 3.6118. Among the patients in public health care centers, these are outcome quality and Peer-to-peer quality since it's mean scores are 2.3417 and 2.2784 respectively. The patients satisfaction and loyalty are higher among the patients in private health care centers compound to patients in public Health Care Centers. The patient loyalty is very poor on public Health Care Centers. Regarding the view on HCCSQ factors and it's consequences, there is a significant difference among the patients in private and public health care centers since then respective 't' statistics at five per cent level.

Direct and Indirect effect of HCCSQ factors on Patient's Loyalty

The Health Care Centers service quality may have some significant direct effect on Patient Loyalty. But, the insignificant direct effect of HCCSQ on patients loyalty may involve some indirect effect through the mediator variable namely patient satisfaction. The present study has made an attempt to examine the direct, indirect and total effect of HCCSQ factors on patients loyalty with the help of structural equation modeling (Bagozzi and Yi, 1988; Amderson and Gerbing, 1988). The results are given in Table 5. Initially, the fitness of data in the measurement model was justified since the chi-square is significant at Zero per cent level, the Root Mean Square Residual (0.0174), Goodness-of-fit-Index (GFI), Adjusted Goodness-of-fit-Index (AGFI) and comparative Fit index (CFI) are better than it respective standard minimum level (Gerbing et al., 1987). The significant direct effect of HCCSQ on patients loyalty is made by outcome quality alone since its path coefficients (0.1344) is significant at five per cent level. But all the three HCCSQ factors are having a significant indirect impact on patient loyalty since their path coefficients through patient satisfaction are significant at five per cent level. The higher indirect effect is made by interaction quality. The higher total effect of HCCSQ on patient loyalty is noticed in the case of interaction quality since its coefficient is 0.3866. The analysis reveals the importance of patient satisfaction as a mediator variable in between HCCSQ and patient loyalty.

Research Implications

The Health Care Centers' service quality measured by the three dimensions namely outcome, interaction and Peer-to-peer quality reveal the findings of Beom and Kim, (2012); Peyrot et al., (1993) and Amin and Siti (2013). The patient satisfaction and loyalty in public health care centers are lesser than that in private health care centers recall the findings of Arasli et al., (2008); Lim and Tang (2000) and Angelo Ponlon et al., (1998). The patient loyalty on health care centers' services among the patients is lesser than the patient satisfaction on Health Care Centers services is similar with the findings of Ambori et al., (2010) and Srideshmukh et al., (2002). The significant indirect effect of service quality on patient loyalty i.e. through the patient's satisfaction indicates the result of previous findings of Abuosi and Atinga, (2012) and; Hussein and Amal, (2013).

Managerial Implications

Own empirical findings should focus important managerial implications. First the health care centers authorities are advised to design their Health Care Centers' service quality in order to satisfy their patients. The important health care centers' service quality factors to be focused are outcome and interaction quality. The outcome quality reveals the recovery of the patient whereas the interaction quality create a faith among the patients. Even though the interaction quality and Peer-to-peer quality have no significant direct impact on patient's loyalty, these factor have a significant indirect impact on patient's loyalty. Hence, the Health Care Centers administrators are advised to provide an conductive environment to their employees which motivate them to offer a high quality services to patients. Even though the peer-to-peer quality has no direct effect on patient loyalty, it has a significant indirect effect an patient loyalty through patient satisfaction. It implies that the Health Care Centers authorities should facilitate the interaction among the patients to improve patients satisfaction and then patients loyalty. Since the patients satisfaction is a key mediator, the Health Care Centers administrators may need to choose a proper combination of Health Care Centers' service qualities to ensure patient satisfaction.

Limitations and Future Research

Since the present study limited its scope to health care centers only at Dindigul district, in future research, the scope may be extended. The study on service quality may be focused by core, value added, critical and innovative service quality in Health Care Centers industry at near future. The present study treats the patients satisfaction as only mediator variables in between service quality and patient loyalty. It may extended to patient value and patients faith on the health care centers in future research work. There may be scope of the study on comparative analysis on Health Care Centers service quality in corporate health care centers and poly clinics. The role of demographic profile in Health Care Centers service quality gaps may be analysed in future research work.

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Table 1

Variables in Service Quality Factors in Health Care Centers and its Consequences

SI.No	Variables	SI.No.	Variables
I	**Outcome quality** (Furguson et al., 1999)	2.	Superior interaction
1.	Effective delivery of the treatment	3.	Total contact
2.	Accurate diagnosis	IV	**Patients Satisfaction** (Fornell et al., 1996)
3.	Best treatment option	1.	Satisfaction on infrastructure
4.	Correct follow-up	2.	Satisfaction on HR at Health Care Centers
II	**Interaction quality** (Sharma and Patterson, 1999)	3.	Satisfaction upto my expectation
1.	Interaction with physician	4.	Better than others Health Care Centers
2.	Interaction with Medical Staffs	V	**Patients Loyalty** (Yim et al., 2008)
3.	Interaction with nurses	1.	Continue to reuse the service in future
4.	Courteous and friendly service	2.	Recommend to others
5.	Emphasized service	3.	Recommend to family members
III	**Peer-to-peer quality** (Lekme et al., 2011)	4.	Ignorance of high price
1.	Interaction with other patients		

Table 2

Reliability and Validity of variables in Constructs

SI. No.	Constructs	No. of variables in	Range of Standardised factor loading	Range of 't'statistics	Cronbach alpha	Composite reliability	Average variance Extracted
1.	Outcome quality	4	0.8443-0.6337	3.3969*-2.2676*	0.7441	0.7201	52.44
2.	Interaction quality	5	0.8901-0.6809	3.8901*-2.6917*	0.7886	0.7614	54.69
3.	Peer-to-peer quality	3	0.8573-0.6518	3.5089*-2.4089*	0.7709	0.7542	54.03
4.	Patients Satisfaction	4	0.8676-0.6473	3.6117*-2.3884*	0.7302	0.7163	52.04
5.	Patients Loyalty	4	0.8502-0.6917	3.4108*-2.7343*	0.7611	0.7421	53.09

* Significant at five per cent level.

Table 3

Discriminant Validity among the Constructs

SI. No.	Square of correlation coefficient	Mean of Average Variance Extracted				
		1	2	3	4	5
1.	Outcome quality		.5357	.5324	.5224	.5277
2.	Interaction quality	.5041		.5436	.5337	.5389
3.	Peer-to-peer quality	.4887	.5244		.5304	.5356
4.	Patient satisfaction	.5042	.4911	.5096		.5257
5.	Patient Loyalty	.4807	.4986	.5244	.5108	

Table 4

Patient's Perception on Health Care Centers' Service Quality (HCCSQ) and its Consequences

Sl. No.	Constructs	Mean score in		't' Statistics
		Private Health Care Centers	Public Health Care Centers	
1.	Outcome quality	3.9097	2.3417	3.5889*
2.	Interaction quality	3.6118	2.1189	3.4401*
3.	Peer-to-peer quality	3.5082	2.2784	3.1772*
4.	Patients Satisfaction	3.3917	2.2545	3.0886*
5.	Patients Loyalty	3.0079	1.8967	3.3997*

* Significant at five per cent level.

Table 5

Direct and indirect effect of HCCSQ on Patient's Loyalty

Sl. No.	HSQ factors	Direct effect	Indirect effect through patient satisfaction	Total effect
1.	Outcome quality	0.1344*	0.1901*	0.3245
2.	Interaction quality	0.0897	0.2969*	0.3866
3.	Peer-to-peer quality	0.0451	0.1471*	0.1922
Goodness of fit statistics: Chi-square = 108.11; p:0.0177 RMR = 0.0174 GFI = 0.9241 AGFI = 0.9027 CFI = 0.9409				

* Significant at five per cent level.

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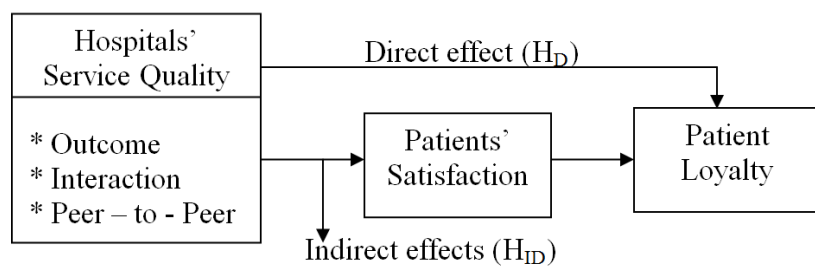
Graphical Study of Magneto Hydrodynamic Boundary Layer Flow of a Micropolar Fluid with Uniform Suction/Injection

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ABSTRACT

In the present study, a problem of heat transfer in a laminar stagnation point flow (steady) of an incompressible electrically conducting micropolar fluid is considered and which is impacting on a flat plate (permeable) with uniform suction or injection. A magnetic field of uniform strength is applied in normal direction to the plate and the effect of viscous dissipation is considered. Differential equations in the present study, which are in partial in nature are transformed into differential equations of ordinary nature. A numerical solution is obtained for the ordinary differential equations. Results are shown graphically. The results of effect of the magnetic and suction parameter on the flow are shown and discussed properly.

Keywords: micropolar fluid, laminar stagnation, suction

INTRODUCTION

The micropolar fluids can be categorized in the non-Newtonian fluids and that consists of a deferment of colloidal fluid elements and body fluids (small). For the study concerned with theory of micropolar fluids, the effects due to the intrinsic motion and microstructure of the fluid elements are important for consideration. The study of stagnation point flow of micropolar fluid have been analysed by researchers Nazar et al. [1] and Attia [2]. Arabay and Hassan [3] disscused the effect of suction/injection on the micropolar fluid flow in the presence of radiation. Salem and Odda [4] analysed the effects of thermal conductivity with variable viscosity on the micropolar fluid flow in presence of suction or injection. Attia [5] studied the stagnation point flow of a micropolar fluid with suction or blowing of uniform strength. Ishak and Nazar [6] and Kishan and Deepa [7] have analysed the micropolar fluid theory with suction or injection through diversed surfaces.

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Formulation of the Problem

In the present problem, we consider a steady flow (two-dimensional) of a viscous incompressible electrically conducting micropolar fluid in the perpendicular direction to a horizontal flat plate with permeable nature and placed at y=0. It separates in two streams on the plate and leave in both directions around a stagnation point. A transverse magnetic field of strength Bo (uniform) is applied normal to the plate. The x-axis is taken along the plate and y-axis is chosen normal to it. Let u and v be the x- and y components of velocity near to stagnation point respectively and N be the component of the micro-rotation vector normal to the plane of xy. A uniform strength suction/injection is applied at the plate with a transpiration velocity at the boundary of the plate given by $-v_0$ (v_0 >0 is for suction). We consider potential flow velocity outer to the boundary layer as U(x) = ax. Fluid properties are assumed to be constant for the whole motion.

The governing equations in presence of viscous dissipation are given by:

$$\frac{\partial u}{\partial x} + \frac{\partial v}{\partial y} = 0 \tag{i}$$

$$u\frac{\partial u}{\partial x} + v\frac{\partial u}{\partial y} = U\frac{dU}{dx} + \frac{(\mu_0 + r_0)}{\rho_0}\frac{\partial^2 u}{\partial y^2} + \frac{r_0}{\rho_0}\frac{\partial N_0}{\partial y} - \frac{1}{\rho_0}\sigma_0 B_0^2 u \tag{ii}$$

$$\rho_0\left(u\frac{\partial N_0}{\partial x} + v\frac{\partial N_0}{\partial y}\right) = \frac{e_0}{j_0}\frac{\partial^2 N_0}{\partial y^2} - \frac{r_0}{j_0}\left(2N_0 + \frac{\partial u}{\partial y}\right) \tag{iii}$$

$$\rho_0 c_p\left(u\frac{\partial T}{\partial x} + v\frac{\partial T}{\partial y}\right) = \kappa_0\frac{\partial^2 T}{\partial y^2} + (\mu_0 + r_0)\left(\frac{\partial u}{\partial y}\right)^2 + \sigma_0 B_0^2 u^2 \tag{iv}$$

The boundary conditions are

$$y = 0 : \ u = 0, \ v = -v_0, \ T = T_w \ , \ N_0 = -m\frac{\partial u}{\partial y}$$

$$y \to \infty : \ u = U(x) = ax, \ T = T_\infty \ , \ N_0 \to 0 \tag{v}$$

T = Temperatures of fluid

T_w = Temperatures of plate

T_∞ = Temperature of the fluid far away from the plate

μ_0 = Viscosity

ρ_0 = Fluid density

σ_0 = Electrical conductivity

κ_0 = Thermal conductivity

c_P = Specific heat at constant pressure

j_0 = Micro-inertia per unit mass

e_0 = Spin gradient viscosity

r_0 = Vortex viscosity

m = Boundary parameter (0≤ m≤1)

Here e_0, j_0 and r_0 are assumed to be constants and e_0 is assumed to be given by Nazar et al. [1]

$$e_0 = \left(\mu_0 + \frac{r_0}{2} \right) j_0 \qquad \text{(vi)}$$

We take $j_0 = \dfrac{\upsilon_0}{a}$ as a reference length, where υ_0 is called the kinematic viscosity.

Analysis

The equation (1) (continuity) is identically satisfied by stream function ψ_0 and defined as

$$u = \frac{\partial \psi_0}{\partial y}, \quad v = -\frac{\partial \psi_0}{\partial x} \qquad \text{(vii)}$$

For the solution of momentum, micro-rotation (spin) and the energy equation given by (ii) to (iv), the following similarity transformations are used to convert the partial differential equations into the ordinary differential equations

$$\psi_0(x, y) = x\sqrt{a\upsilon_0}\, f(\eta), \quad N_0(x, y) = ax\sqrt{\frac{a}{\upsilon_0}}\, g(\eta) \text{ and } \theta = \frac{T - T_\infty}{T_w - T_\infty},$$

where $\eta = y\sqrt{\dfrac{a}{\upsilon_0}}$ 　　　　　　　　(viii)

Assumptions

$$D = \frac{r_0}{\mu_0} \qquad \text{(Material parameter)}$$

$$B = \frac{\sigma_0 B_0^2}{\rho_0 a} \qquad \text{(Magnetic parameter)}$$

$$\Pr = \frac{\mu_0 c_p}{\kappa_0} \qquad \text{(Prandtl number)}$$

$$C = -\frac{v_0}{\sqrt{a\upsilon_0}} \qquad \text{(Suction/Injection parameter, +ive /-ive)}$$

Skin-friction coefficient (C_f) and the Local Nusselt number (Nu) which are known as physical quantities of interest are given by

$$C_f = \frac{\tau_w}{\dfrac{\rho_0 U^2}{2}} \quad \text{and} \quad Nu = \frac{x q_w}{\kappa_0 \left(T_w - T_\infty \right)}$$

Here $U\left(x \right) = ax$ is called a characteristic velocity while τ_w is known as wall shear stress.

Using basic definitions of τ_w and q_w , we get

$$C_f = \frac{2\left(1+\dfrac{D}{2}\right)}{\sqrt{\text{Re}}} f''(0) \quad , \quad Nu = -\sqrt{\text{Re}}\,\theta'(0)$$

where $\text{Re} = \dfrac{xU}{\upsilon_0}$ is known as the local Reynolds number.

RESULTS AND DISCUSSION

The reduced ordinary differential equations obtained from (ii) and (iii) subject to the reduced boundary conditions of (v) were solved by numerical procedure with the help of computer language Matlab. Velocity profiles are explained in figures (1) and (2). It is evident from these graphs that the velocity decreases with the increasing values of the magnetic parameter B and it increases with the increasing values of the suction/injection parameter C. Profiles of temperature distribution are shown in figure (3) and (4). It can be analysed from these graphs that the temperature decreases with the increasing values of the suction/injection parameter C but it increases with the increasing values of magnetic parameter B. Figure (5) shows the profile of the skin friction coefficient (which is proportional to $f''(0)$). It is clear from this graphs that the coefficient of skin friction increases with the increasing values of the parameter C. Figures (6) shows the profile of the Nusselt number (which is proportional to $-\theta'(0)$) and this graph shows that the Nusselt number decreases with the increasing values of the parameter B.

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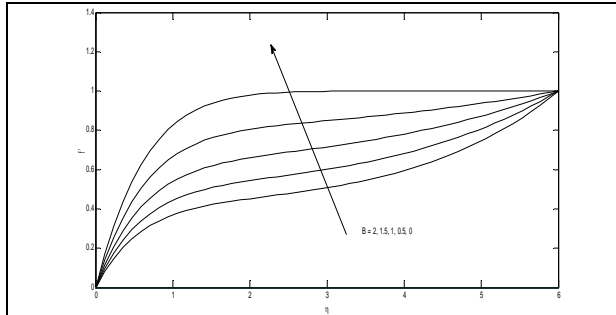**Figure1.Profile of Velocity against η for various values of magnetic parameter B when C=2 and D=2**

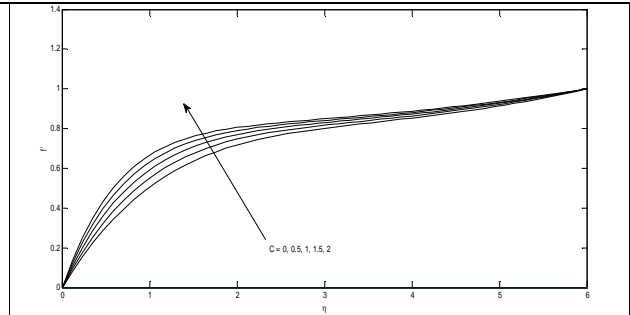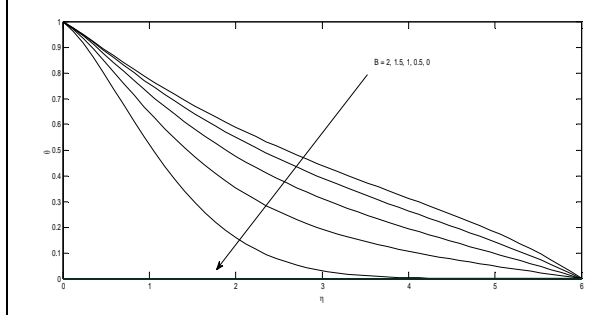**Figure 2. Profile of Velocity against η for various values of parameter C when B=0.5 and D=2**

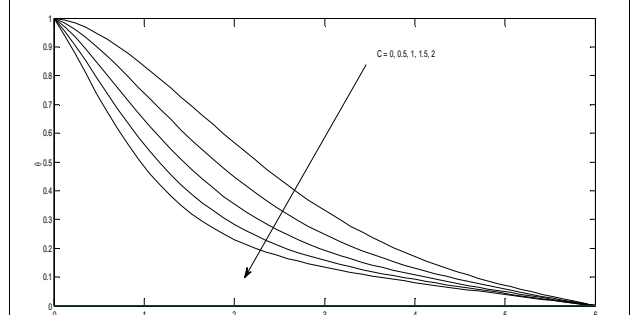**Figure 3. Profile of Temperature against η for various values of parameters B when D=2, Pr=0.5, Ec=0.5 and C=1**

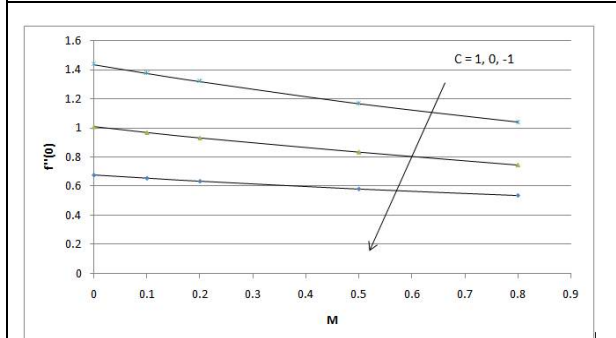**Figure 4. Profile of Temperature against η for various values of parameter C when B=0.5, D=2, Pr=0.5 and Ec=0.5**

Figure 5. Profile of Skin friction coefficient (f ''(0)) against M for various values of parameter C when D=1
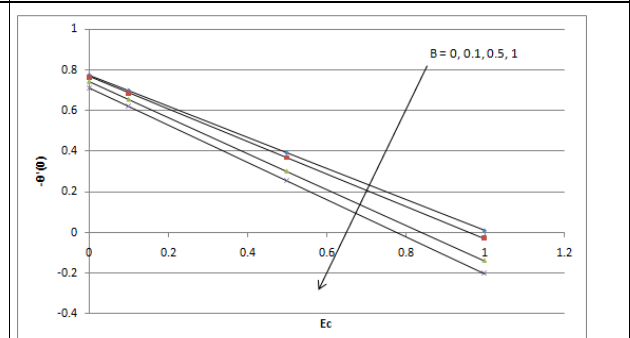
Figure 6. Profile of Nusselt Number (which is proportional to $-\theta$ '(0)) against Ec for various values of parameter B when Pr =0.5, C=1, D=1.

RESEARCH ARTICLE

Investication of Phytocomponents and GC- MS analysis of Ethyl Acetate and Ethanolic Extract of Aerial Part of *Leonotis nepetifolia* Linn.

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ABSTRACT

Objective: To qualitative and analysed the chemical composition in ethyl acetate and ethanolic extract from the aerial part of *Leonotis nepetifolia* Linn. by gas chromatography-mass spectrometry (GC-MS). **Methods:** The shade dried aerial part powder was extracted with ethanol by using Soxhlet extractor and crude ethyl acetate and ethanolic extract was obtained. **Results:** The extractive values were recorded in pet.ether, ethyl acetate, chloroform, methanol, acetone, ethanol and aqueous. Physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, foreign organic matter, loss on drying, swelling index and foaming index. The extractive values such as alcohol soluble extractive and water soluble extractive are also determined. GC-MS analysis of ethyl acetate and ethanolic extract of leaf showed several active components. Among the identified compounds of ethyl acetate was six phytochemical compounds were Phenol, 2,6-dinitro-4-(trifluoromethy)- was found to be the major compound attained the largest peak (26.62 %) with the retention time (13.603) minutes. Another major compound 2-Heptanone, 6-methyl- having peak area of (26.19 %) with retention time (7.798) minutes and ethanol was one phytochemical compounds were Tetrasiloxane, decamethyl- was found to be the major compound attained the largest peak (100.00 %) with the retention time (16.856) minutes. **Conclusion:** The studies performed on various parts of this plant are food additives, flavoring agents, colouring agents, cosmetics, chemical data reporting rules, adhesive & fragrance, functional fluid, lubricant, personal care additives and commercial activity.

Jeyaraman Amutha Iswarya Devi *et al.*,

INTRODUCTION

Leonotis nepetifolia, (also known as klip dagga, Christmas candlestick, or lion's ear), is a species of plant in the genus *Leonotis* and the family Lamiaceae (mint). It is native to tropical Africa and southern India. It can also be found growing abundantly in much of Latin America and the West Indies. It has been found growing on road sides, rubbish heaps or waste land. It grows to a height of 3 metres (9 ft 10 in) and has whorls of striking lipped flowers, that are most commonly orange, but can vary to red, white, and purple. It has drooping dark green, very soft serrated leaves that can grow up to 10 centimetres (4 in) wide. Sunbirds and ants are attracted to the flowers. The stems are strongly angled (square in cross section) and the leaves are in pairs opposite each other. The leaves are smooth with coarsely toothed margins triangular in shape. The flowers are borne in rounded spiny clusters that encircle the stems so that it looks like the stems are growing right through the middle of the cluster. As the stems elongate, new flower clusters continue to develop above the older ones. The tubular flowers that peck out of the spiny heads are orange and furry like a lion's ear. An infusion of the roots is used to treat stomach disorders while a decoction of the leaves is drunk for stomach. The stems are relatively robust, sparsely branched, and sparsely covered in small whitish hairs (i.e. they are puberulent). These stems are four-angled (i.e. quadrangular) and usually have a distinct groove running lengthwise (i.e. longitudinally) down the centre of each side. The leaves (4.5-20 cm long and 2-15 cm wide) are oppositely arranged along the stems and borne on stalks (i.e. petioles) 2-10 mm long. The lower leaves, which are somewhat egg-shaped in outline (i.e. oblong-ovate or ovate), are larger and broader than those towards the top of the plant. All leaves have distinctly toothed (i.e. crenate or serrate) margins, pointed tips (i.e. acute apices), and are sparsely hairy. The flower clusters form a hard and somewhat spiky ball when mature, and consist mainly of the persistent calyx tubes (which enlarge to 15-25 mm long in fruit). Each individual fruit is a four-lobed 'capsule' (i.e.schizocarp) that separates into four 'seeds' (i.e. nutlets) when mature. These 'seeds' are dark brown or dull black in colour, and either somewhat egg-shaped (i.e. oblong-ovoid) or triangular in shape (2.5-4.3 mm long and 1-1.9 mm wide).

MATERIALS AND METHODS

Collection and preparation of plant material

The aerial part of *Leonotis nepetifolia* was collected from Rajapalayam, Virudhunagar District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu. The plant of *Leonotis nepetifolia* and were dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The plant were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Extractive values of *Leonotis nepetifolia* Linn

Extractive values were recorded in pet. ether, ethyl acetate, chloroform, methanol, acetone, ethanol and aqueous with a view to study the distribution of various constituents of *Leonotis nepetifolia*. Accurately weigh 0.5gm of coarsely powdered air dried material was placed in a glass stoppered conical flask and macerated with 25ml of different solvents for 6 hours. Shacking frequently and then allowed to stand for 18 hours. The mixture was filtered rapidly. Then it was transferred to a tarred flat bottomed dish and evaporate to dryness on water bath. The residue was dried at 105°C for 6 hours. Cooled in a desiccator for 30 minutes and weighed without delay.

Physicochemical Investigation

Physicochemical screening of *Leonotis nepetifolia* revealed that, it possess good physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, foreign organic matter, swelling index and foaming index.

Gas-chromatography-mass spectrometry analysis

GC-MS analysis of various extract and aqueous extract of *Leonotis nepetifolia* Linn was performed using a Perkin-Elmer GC clauses 500 system and Gas Chromatograph interfaced to a mass spectrometer (GC-MS) employed a fused silica capillary column packed with Elite-1 (100% dimethyl poly siloxane, 30 nm × 0.25 nm ID × 1µm df) For GC/MS detection, an electron ionization system with ionizing energy of 70 eV was used. Helium gas (99.999%) was used as the carrier gas at constant flow rate 1 ml/min and an injection volume of 2 µl was employed(Split ratio of 10:1) injector temperature was set at 250°C; Ion-source temperature was set at 280°C. The oven temperature was programmed from 110°C (isothermal for 2 min) with an increase of 10°C/min to 200°C, then 5°C/min to 280°C, ending with a 9 min isothermal at 280°C. Mass spectra were taken at 70 eV; a scan interval of 0.5 seconds and fragments from 45 to 450 Da. Total GC detection time was completed in 36 minutes. The relative percentage amount of each component was calculated by comparing its average peak area to the total areas, software adopted to handle mass spectra and chromatogram was a Turbo mass.

RESULTS

Extractive values of *Leonotis nepetifolia* Linn

The extractive values are valuable to estimate the chemical constituents present in the crude drug and further more assist in evaluation of definite constituents soluble in particular solvent. Extractive values were recorded in pet.ether, ethyl acetate, chloroform, methanol, acetone, ethanol and aqueous with a view to study the distribution of various constituents of *Leonotis nepetifolia*. Highest range of extractive values obtained in aqueous extract is 5.0**%** w/w

Physicochemical parameters of *Leonotis nepetifolia* Linn

Physicochemical screening of *Leonotis nepetifolia* revealed that, it possess good physicochemical parameters such as total ash, acid insoluble ash, water soluble ash, sulphated ash, loss on drying, foreign organic matter, swelling index and foaming index. The extractive values such as alcohol soluble extractive and water soluble extractive are also determined.

Gas-chromatography-mass spectrometry analysis

From GC-MS analysis for 6 active components were detected from ethyl acetate extract and 1 active components were detected from ethanolic extract. The identification of phytochemical compounds was based on retention time, molecular formula, peak area, molecular weight are presented and medicinal activity and nature of compound were presented.

DISCUSSION

The study of aerial part of *Leonotis nepetifolia* by extractive values were recorded in pet.ether (3.1 % w/w), ethyl acetate (1.7 %w/w), chloroform (1.6 % w/w), methanol (4.5 % w/w), acetone (4.1 % w/w), ethanol (2.5 % w/w) and aqueous (5.0 % w/w). Physicochemical parameters such as total ash (6.0% w/w), acid insoluble ash (7.1 % w/w), water soluble ash (5.5 % w/w), sulphated ash (8.0 % w/w), loss on drying (8.0 % w/w), foreign organic matter (12.1 % w/w), swelling index (10.4 % w/w) and foaming index (less than 100). The extractive values such as alcohol soluble extractive (4.16 % w/w) and water soluble extractive (19.68 % w/w) are also determined.GC-MS analysis showed the existence of ethyl acetate extract with different phytochemical compounds are 2-Heptanone, 6-methyl- having peak

area (26.19%); 1,2,3,4-Butaneterol, [S-(R*, R*)]- having peak area (4.13%); 1,3-Propanediamine, N,N-dimethyl- having peak area (24.19%); Phenol, 2,6-dinitro-4-(trifluoromethy)- having peak area (26.62%); 2-Pentadecanone, 6,10,14-trimethyl- having peak area (5.27%); Cyclotrisiloxane, hexamethyl- having peak area (13.59%). GC-MS analysis showed the existence of ethanolic extract with different phytochemical compounds are Tetrasiloxane, decamethyl-having peak area (100.00%). The results from this use of *Leonotis nepetifolia* as a multi-purpose medicinal agent, while *Leonotis nepetifolia* has been used successfully in Siddha medicine for centuries, more clinical trials should be conducted to support its therapeutic use. Moreover, the therapeutic potential of the plant should also be checked when used in combination with other herbal drugs.

CONCLUSION

Traditionally, plants are used in the treatment of many infections and systemic disorders. More than hundreds of chemical compounds are derived from plants which have medicinal values due to their health-enhancing and therapeutic properties are referred as herbs. Through screening of literature available on *Leonotis nepetifolia* depicted the fact that it is a popular remedy among the various ethnic groups Siddha and Ayurvedic properties.

ACKNOWLEDGEMENTS

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Table 1: Extractive values of *Leonotis nepetifolia* Linn.

Experiment	% Values			Average yield (%) W/W± SEM
	1 (%) W/W	2 (%) W/W	3 (%) W/W	
Pet. Ether extract	2.7	3.1	3.5	3.1±0.02
Ethyl acetate extract	1.2	1.7	2.2	1.7±0.01
Chloroform extract	1.2	1.5	2.2	1.6±0.06
Methanol extract	4.2	4.5	4.9	4.5±0.01
Acetone extract	3.6	4.0	4.7	4.1±0.02
Ethanol extract	2.0	2.4	3.0	2.5±0.03
Aqueous extract	4.6	4.9	5.5	5.0±0.04

Table 2: Extractive values of *Leonotis nepetifolia* Linn.

S. No	Parameters	Observation
1.	Alcohol soluble extractive	5.16 % w/w
2.	Water soluble extractive	17.68 % w/w

Table 3: Physicochemical parameters of *Leonotis nepetifolia* Linn.

Experiment	% Values			Average yield (%) W/W± SEM
	1(%) W/W	2(%) W/W	3(%) W/W	
Total ash	5.7	6.0	6.3	6.0±0.04
Acid insoluble ash	6.4	7.0	7.9	7.1±0.06
Water soluble ash	5.1	5.5	6.0	5.5±0.04
Sulphated ash	7.3	8.0	8.7	8.0±0.05
Loss on drying	7.4	8.0	8.8	8.0±0.02
Foreign organic matter	11.0	12.5	12.9	12.1±0.001
Swelling index	10.1	10.5	10.8	10.4±0.01
Foaming index	Less than 100			

Table 4: Chemical composition of Ethyl acetate extract of *Leonotis nepetifolia* Linn.

S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	7.798	2-Heptanone, 6-methyl-	$C_8H_{16}O$	128	26.19
2.	11.760	1,2,3,4-Butaneterol, [S-(R*, R*)]-	$C_4H_{10}O_4$	122	4.13
3.	13.443	1,3-Propanediamine, N,N-dimethyl-	$C_8H_{19}OClN_2$	194	24.19
4.	13.603	Phenol, 2,6-dinitro-4-(trifluoromethy)-	$C_7H_3O_5N_2F_3$	252	26.62
5.	14.133	2-Pentadecanone, 6,10,14-trimethyl-	$C_{18}H_{36}O$	268	5.27
6.	17.953	Cyclotrisiloxane, hexamethyl-	$C_6H_{18}O_3Si_3$	222	13.59

Table 5: Activity of phytocomponents identified in Ethyl acetate extract of *Leonotis nepetifolia* Linn. by GC-MS.

RT	Name of the compound	Nature of compound	Activity
7.798	2-Heptanone, 6-methyl-	Ketone	Food additives and flavoring agent
11.760	1,2,3,4-Butaneterol, [S-(R*, R*)]-	Alcohol	Food additives and cosmetics
13.443	1,3-Propanediamine, N,N-dimethyl-	Amine	Commercial activity and Chemical data reporting rules
13.603	Phenol, 2,6-dinitro-4-(trifluoromethy)-	Aromatic alcohol	Pesticide transformation products
14.133	2-Pentadecanone, 6,10,14-trimethyl-	Ketone	Food additive, coloring agent and flavoring agent
17.953	Cyclotrisiloxane, hexamethyl-	Alkane	Adhesive and fragrance

Table 6: Chemical composition of ethanolic extract of *Leonotis nepetifolia* Linn.

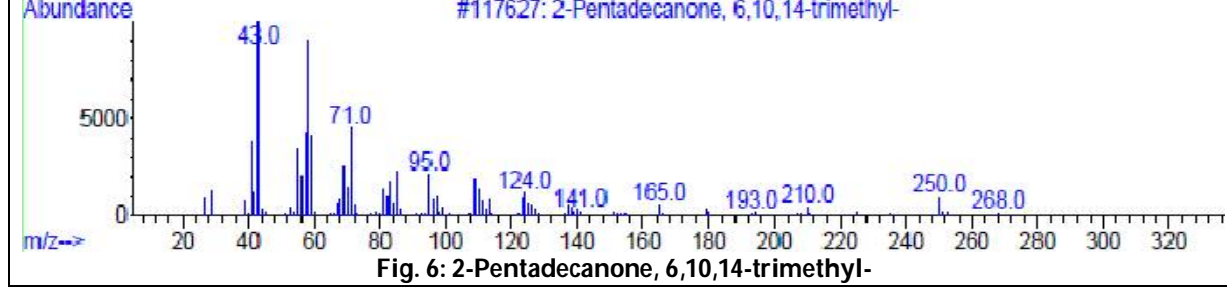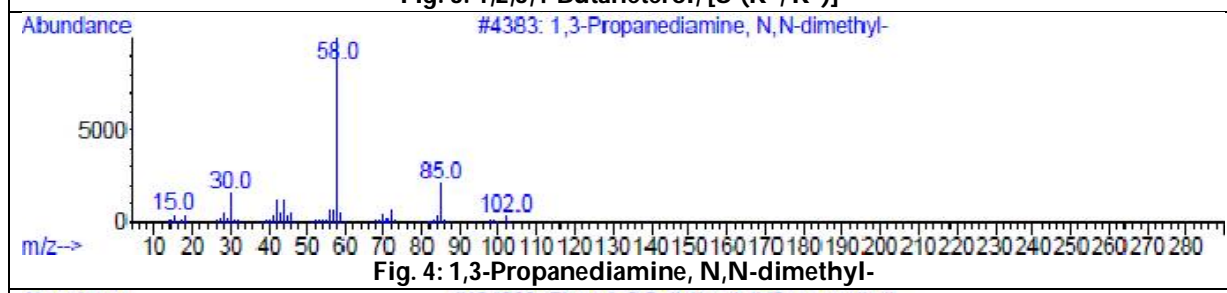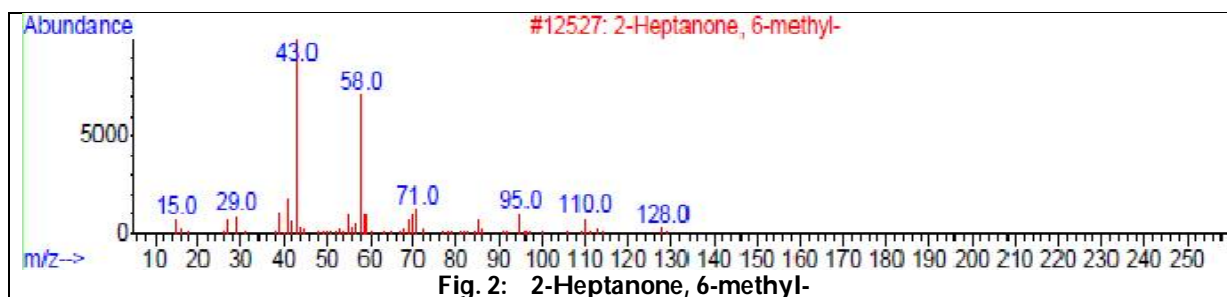| S. No.	RT	Name of the compound	Molecular Formula	MW	Peak Area %
1.	16.856	Tetrasiloxane, decamethyl-	$C_{10}H_{30}O_3Si_4$	310	100.00

Table 7: Activity of phytocomponents identified in ethanolic extract o f*Leonotis nepetifolia* Linn. by GC-MS.

RT	Name of the compound	Nature of compound	Activity
16.856	Tetrasiloxane, decamethyl-	Alkane	Functional fluid, intermediates, lubricant, personal care additive and solvent for cleaning & degreasing.

Fig. 1: GC-MS Chromatogram of Ethyl acetate extract of *Leonotis nepetifolia* Linn.

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Fig. 2:   2-Heptanone, 6-methyl-

Fig. 3: 1,2,3,4-Butaneterol, [S-(R*, R*)]-

Fig. 4: 1,3-Propanediamine, N,N-dimethyl-

Fig. 5: Phenol, 2,6-dinitro-4-(trifluoromethy)-

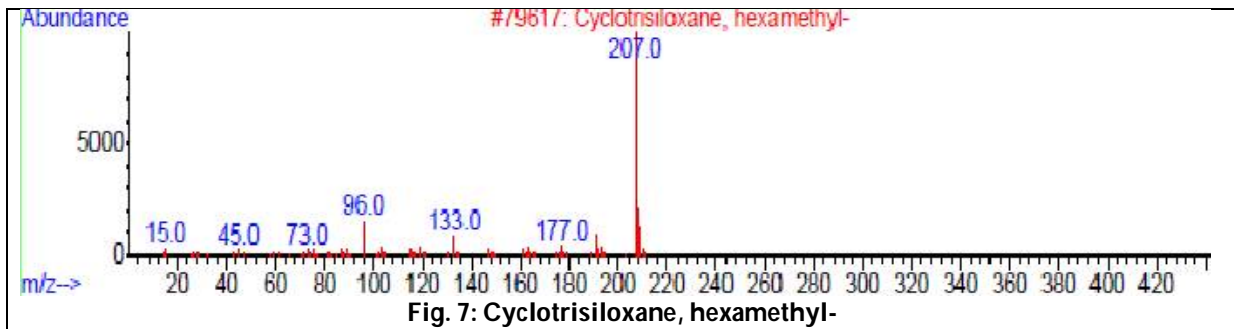**Fig. 6: 2-Pentadecanone, 6,10,14-trimethyl-**

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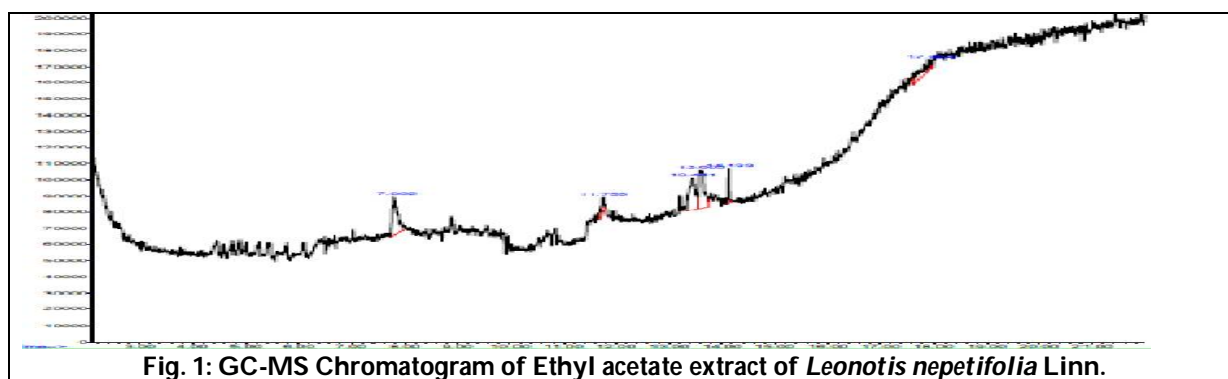
Fig. 7: Cyclotrisiloxane, hexamethyl-
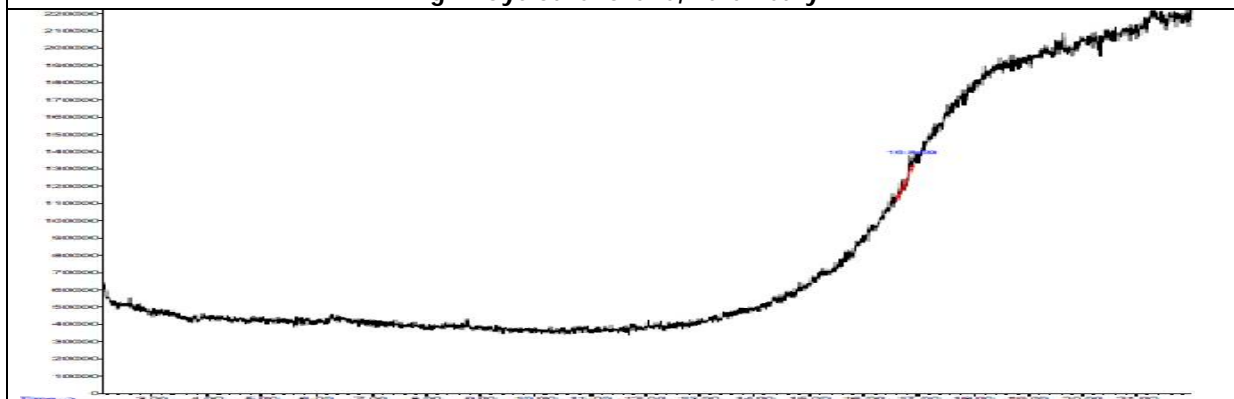
Fig. 8: GC-MS Chromatogram of ethanolic extract of *Leonotis nepetifolia*Linn.
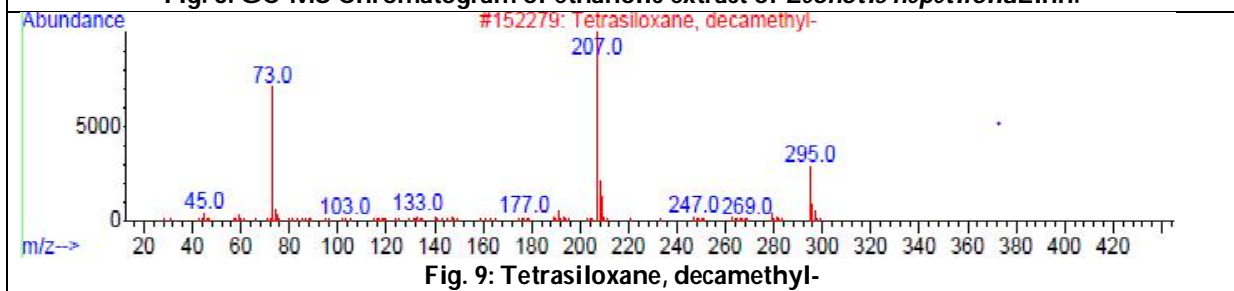

Fig. 9: Tetrasiloxane, decamethyl-

REVIEW ARTICLE

Natural Immunity Booster Foods used During Pandemic: A Review

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ABSTRACT

The Corona virus pandemic has the worst impact on the health in decades. Newer drugs have been trial to tackle the corona pandemic. It has been discussed about the immunity and corona survival rate. Various traditional foods like Citrus fruits, sitaphal, apple papaya have been found immunity booster with antiviral properties. Some vegetables like broccoli, onion, garlic and green leaves and dietary supplements like Nuts, ginger, turmeric, pepper, egg yolk, shell fish, mushrooms are also found to be immunity booster. One can say that diet and nutrition are the important supplement to counter this pandemic. We also tried our best to provide complete menu diet. The main hurdle is the non-availability of the food items. We also suggested some alternative to these food items. A chart is given for the normal adult. It should be compensated based on sex, age, body mass index and physical activities.

Keywords: pandemic, corona virus, nutrition, diet.

INTRODUCTION

The easily transmissible coronavirus disease (COVID-19) is caused by the novel coronavirus SARS-CoV-2 which was identified first in Wuhan state of china at the far end of 2019. On 11 March 2020, WHO declared it as a global pandemic(WHO, 2020). A lot of drugs have been tried in the setting of life-threatening respiratory disease and still the ideal combination remains elusive. Due to lack of scientific evidence to control the pandemic, diet and nutrition should be supplemented to these patients (Adhikari *et al.,* 2020). The beneficial live bacteria which are present in the gut builds the immune system and protects the human body from various diseases. Basically, the people with low or weak immune systems are more susceptible to COVID-19 or any other diseases and people especially people of under and over ages.

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Almost 85% of the body's immune system is due to the gut microbiome and the vegan foods increase and helps to grow the intestinal beneficial bacteria, and improves the overall gut microbiome health. Therefore, byeating a low-fat, plant-based vegetarian diet may boost the immune system. On the other hand, excess of animal foods consumption depletes the body's good bacteria, promote inflammation, and are the underlying cause of diabetes, chronic obstructive pulmonary disease cardiovascular diseases, hepatitis B, cancer, and chronic kidney diseases. Vegetarians have more effective white blood cells compared to non-vegetarians, due to higher intake of vitamins and lower intake of fat (Davison *et al.,* 2016). In this short communication review, we have mentioned about the food items with its properties. The main problem is the non-availability of many nutritious supplements due to the government measure taken to control the spread of the pandemic. We also prepared a menu with easily preparable recipes.

Foods That Increase Immunity And With Possible Anti-Viral Properties:

Citrus Fruits
The most popular and globally available world's major fruit crop is citrus fruits. It is also known as argumes (sour fruits). The important citrus fruit crops are oranges, grapefruit, limes, lemons, pomelos, and tangerines (Liu *et al.,* 2012). Citrus fruits have nature's best and easily available source of Vitamin C, a key nutrient in supporting our immune system. Citrus fruits have other benefits like antioxidants, anti-tumour, neuroprotective and cardioprotective effects. Citrus fruits are also a rich source of fibre content. But what makes them significant is their immune boosting potential. The most important chemical present in the citrus is the flavonoids. The whole citrus fruit is more nutritious than its juice especially with sugar (Lv *et al.,* 2015).

Other Fruits
Every fruit is good for health and improves human immunity. Apple, papaya and sitaphal have antiviral effects against specific viruses (Suchitra and Parthasarathy, 2015; Konowalchuk and Speirs, 1978).

Nuts and Seeds
Seeds and nuts rich in proteins, fat, minerals, vitamins and fibres. Seeds are botanically mature ovules and rich in proteins, healthy fats, fibres and minerals such as magnesium potassium, calcium, iron and zinc and contain vitamins such as B1, B2, B3 and vitamin E. Oily seeds are enriched with antioxidants which prevent fats from rancidity. Commonly edible seeds include pumpkin seeds, opium seeds, flax seeds, sesame seeds, sunflower seeds, mustard seeds, amaranth seeds, oat seeds, barley seeds, black rice seeds, brown rice seeds, quinoa seeds, nigella seeds and millet seeds.

Sunflower Seeds
Sunflower seeds are loaded with the lots of the nutrients. These seeds are rich in mono and polyunsaturated fats which improves the heart health and reduces the risk of cardiovascular disease. These seeds are also a rich source of vitamins and minerals, including folate for DNA synthesis, Vitamin E for anti-inflammatory properties and minimize the risk of heart disease, B6 for the cognitive development and its function. Zinc for the metabolism and immune system. Phosphorus for the development of bone health, Selenium, an antioxidant that protects against cell damage, manganese having bone production capacity, copper improves the heart health and the immunity.

Pumpkin Seeds
Pumpkin seeds are the dark green seeds, flat in shape, with some encased in a yellow white husk. They are chewy in texture, sweet in taste and have pleasant nutty flavour. The consumption of pumpkin seeds improves prostate health because they are highly rich in zinc content. Consumption of quarter cup of pumpkin seeds containing half of the RDA of magnesium is essential for maintenance and formation of heart. It also helps to prevent hypertension, sudden cardiac arrest, stroke and heart attack. These seeds also boost immunity system and fertility. Sterols present

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in seeds are linked with the protection against hormone-based cancers. Pumpkin seeds decrease oxidative stress by the regulation of insulin which helps in preventing diabetic complications. Tryptophan is present in seeds and it is important for the production of serotonin, which is responsible for happy moods. Consumption of pumpkin seeds avoids depression, keeps up spirits high and restful night sleep. In addition, Pumpkin seeds also rich in vitamin E, folate, omega 3 fatty acids and magnesium which is helpful in maintaining heart health.

Hemp Seeds
It is an excellent source of vegetarian protein. These seeds are idea to be added into the diets of vegans and vegetarians. Hemp seeds have more than 30% of the protein and other important nutrients. These seeds also contain high amount of every essential amino acid that cannot be synthesized by our body. Hemp seeds also contain gamma linolenic acid which have anti-inflammatory properties.

Flax Seeds
These seeds are also known as linseeds. They are considered as the most nutrient dense seeds. Flax seeds reduce the cholesterol levels in the body effectively as they contain antioxidants like lignans and omega 3 fatty acids. As a result, consumption of flaxseeds reduces the risk of getting heart disease to the minimum. It also controls blood pressure and also prevents the growth of cancerous tumours in the body.

Poppy Seeds
It provide 523 calories and rich source of thiamine, folic acid and several essential minerals such as manganese, calcium, zinc, phosphorus, iron and magnesium. The seeds contain 6% water, 28% carbohydrates, 42% fat, 28% carbohydrates and 6%fat. It has analgesic properties as it contains papaver alkaloid.

Sesame Seeds
It is used in various dishes worldwide such as bread meals, crackers, soups and meat cuisines. Sesame seeds are the good source of protein, dietary fibre, copper, vitamin B, manganese, magnesium and calcium. It is helpful in reducing the risk of cancer, preventing diabetes, reducing signs of aging, protecting DNA from radiation damage, improves oral health and digestion and also minimizes the risk of cardiovascular disease.

Amaranth Seeds
These are the good sources of fiber, protein, vitamin B, manganese, folate, phosphorus, magnesium and iron. They are helpful in lowering cholesterol, aiding weight loss, reducing inflammation, improves the immunity, eliminating constipation and bloating, reduces risk of colon cancer and strengthens the heart health. It can be consumed by the gluten intolerant people.

Oat Seeds
It is a type of cereal grain; oats are very popular amongst us. They are commonly used to make oatmeal, cookies, cakes, cereal and flour. Oats contain highest protein and the lowest carbohydrates amongst all the cereals. It is helpful in lowering cholesterol levels, preventing heart disease, increasing appetite control hormones, reducing the risk of type 2 diabetes and improves the immune system.

A **nut** is a simple dry fruit. It is consisting of one or two edible kernels inside a hard shell. Nuts are rich in high calorific value, unsaturated fatty acids, dietary fibres, proteins, antioxidants, vitamins E, B6, folic acid, niacin and minerals such as magnesium, zinc, iron, copper, selenium, phosphorus and potassium and low in saturated fats and cholesterols. Popular edible nuts include almonds, cashew nut, Brazil nuts, pistachio nuts, walnuts and pea nuts. Vitamin E is a lipid-soluble antioxidant which is commonly present in the membrane of all cells including immune cells. This is supposed to prevent stress induced damage to cells. Almonds and peanut skin have significant antiviral effects against specific viruses. Common flu symptoms had been treated by eating almonds (Makau *et al.,* 2018).

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Almonds

Almonds are the type of dry fruits and consumed to prevent and fight off colds. They have small size but packed with a lot of nutrients which are beneficial to the body, and its whole-body pulp is made up of healthy fats and vitamins and minerals. Almonds have several health benefits including controlling blood sugar, lowering blood pressure, alleviating constipation, regulating cholesterol level, anaemia and respiratory disorders; help in nail strengthening, hair repair and growth, and dental strength and care. Almonds contain Vitamin E and C which are amazing immunity boosters. Almond oil is used for massaging and improves skin appearance and reverses the signs of aging. It also prevents the colon cancer.

Brazil Nuts

They are the rich source of selenium, iron and zinc which are the vital for the immunity. Brazil nuts are highly famous due to its high selenium content. Selenium is an essential microelement that is important for a daily immune system and helps to prevent damage to our cells and nerves.

Peanuts

they are the good source of protein, monosaturated fats, manganese and niacin. They are helpful to fight against cancers, nerve diseases, heart diseases and viral and fungal infections. Consumption of peanuts reduces the low density lipoprotein cholesterol levels and therefore reduces the risk of coronary heart diseases. Peanuts improves the immune system in the body.

Green Tea

Camellia sinensis is the botanical name of green tea. It is composed of flavonoids that is called as catechins. These catechins found to be inhibit in case of viral infection because it blocks the enzymes. It plays an important role in replication and found to be effective in various diseases like herpes viruses, HIV and hepatitis B etc. (Chacko *et al.,* 2010).

Vegetables

Cruciferous vegetables and Broccoli found to be effective in boosting the immunity. Scientist claims that chemical i.e. sulforaphane found in the vegetablesis switched on the antioxidant genes and enzymes in specific immune cells. It makes our body free from radicals and also helps in preventing the various disease. Broccoli has antiviral properties that helps to fight against influenza viruses (Antonenko *et al.,* 2013).

Garlic

Due to various properties like anti-tumour effects, antioxidant and cardioprotective, garlic is the most popular. Bioactive chemical is present in the extraction of the garlic. It is also present in the raw material of the garlic homogenate. The enzyme alliinase is activated at the time of chopping and its produce allicin. Scientist also find that garlic has anti-viral against cytomegalo – virus, HIV, herpes, and the flu viruses (Bayan *et al.,* 2014). The important mechanism is unknown for the garlic.

Turmeric

Turmeric the family member of the ginger. It is a herbaceous perennial plant. Its medical properties, presence of bioactive compound like curcumin is known for thousands of years. But there is huge gap in understanding and various unknown applications make it a good choice for the researchers. Turmeric has the various bioactive components that are still need to know. It has various known compounds like antiviral, antibacterial, antioxidant, immunity and cardioprotective properties. Due to the addition of black paper, bioavailability of curcumin is increased. Researchers have found that inflammatory cytokines like serum IL1 β and various growth factors are found significantly and it reduces the curcumin therapy (Hewlings and Kalman, 2017). It is assumed that due to the wake of corona pandemic, cytokine becomes worsening in the patients rather than replication of the virus.

Ginger

Ginger and the products made of ginger are used to boost the immune system. Ginger have antitumor effects, digestive and anti-inflammatory. Fresh ginger has anti-viral activity A against the Human Respiratory Syncytial Virus in the respiratory cell. It is found useful in exploration flu and its hit and trial becomes more nutritive diet. Ginger can extract the production of TNF alpha by immune system. Scientist also found that it has natural compound in curing the H1N1 influenza and the inhibition of viral infection (Mashhadi *et al.,* 2013; Chang *et al.,* 2013).

Miscellaneous

Beta-carotene boost the immunity and reduce the inflammation by increasing the leucocytes in the body. It is the most powerful antioxidant and have the excellent source of the beta carotene that also found in sweet potatoes, green leafy vegetables and carrots (Grune *et al.,* 2010). Coconut water contains niacin, riboflavin, folates and thiamine. It contains antibacterial and viral properties that increases the body's immune system and also increase the capacity to fight against viral infection like flu (Chauhan *et al.,* 2014). It also contains organosulfur compounds like allicin and quercetin that are helpful to fight against viral infection (Sharma, 2019). These bioactive compounds also helpful to hinder attachment of the virus with the cell. It also helps in changing the transcription and translation of the genome inside the cell. Tamarind leaves, seeds and fruits have also been found to be antiviral properties (Caluwé *et al.,* 2010). If someone intake the probiotics regularly, it allows intimate interaction with mucosal immune system. It further modulates the inflator and immune response with the gut epithelial cells. It signifies the presence of the gut brain axis. probiotics (Meydani and Ha, 2000) is also found in the curd. Egg yolk, although β-carotene, α-carotene, and β-cryptoxanthin contains the Lutein and zeaxanthin at the lower levels. The avidin becomes destroyed at the time of cooking inside the eggs and it is made available in the body (Andersen, 2015). To increase the immunity, cardioprotective plays an important role in addition to the vitamins (Venugopal and Gopakumar, 2017; Hosomi *et al.,* 2012). Sesame also contains nutrient food with ample zinc content. It also has antibacterial and antiviral properties (Suchitra and S, 2020). It also has positive impact on body immune system. Oats also contains fibre with vitamin D. It has antioxidant effects and not much antiviral effects (Rasane *et al.,* 2015). Bread jam is found to be toxic as it contains maida during this pandemic. Whey protein has antiviral properties and it is not advisable to be used in the night diet (West *et al.,* 2017). Mint family tree's plant leaves have shown anti-viral effects (Herrmann and Kucera, 1967). Ragi is not known and popular for anti-viral effects. It can provide 1800-2200 kilocalories. Based on the cooking techniques, its size varies. Based on the patient age, sex, weight and daily activities, size of its ingredient can be change. Due to the pandemic, its taste and size may decrease the availability of the nutrients.

CONCLUSIONS

Our immune system needs to fighting off foreign cell to protect us against illness. Antibodies are the proteins developed in our body that eliminate the abnormal cells. Vitamins A, D,E, C, B6 and minerals like zinc, selenium are the nutrient profiles which are necessary for developing immunity in human body. Keeping in view the grave situation of COVID 19 edible seeds and nuts can be included in our diet to boost up immunity of individuals until the suitable vaccine is available. Proper planning is necessary to tackle any disease. To counter any viral disease, nutrient supplements are necessary. One can conclude that a good diet with immunity booster and antiviral effects are more important.

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Table 1: Showing Menu With Nutritious Diet Plan

Time	Recipe	Remarks	Alternative
Early morning	Green tea75 ml , almonds with skin 2 pieces	Immune boosting Antiviral	Warm water with honey
Breakfast	Idly- 4/dosa 3 Onion-tomato chutney – 1-2 table spoon	carbohydrate and protein Immune booster with antiviral	Vegetable ragiuppma – 1 katori Mint chutney
Midmorning	Sprouted green gram dhal with lemon – 20 grams	Protein Immune booster	Egg white /mushroom pepper fry / Coconut water 100 ml Ground nuts – 1 small cup
Lunch	Rice – garlic sambar – 2katori Broccoli saute – 20 grams /other cruciferous vegetables /carrot curry + Curd – 150 ml	Immune boosting /antiviral Calorie intake	Shell fish soup Rice Sweet potato rasam,-2katori Carrot and green leafy vegetables – cooked – 2 cups Ginger raita
Evening	Papaya -1 and apple -1 (small)	Immune boosting /antiviral	Sitaphal 10 cusps /orange juice/grapes
Dinner	Chapathi 3 -4 + Vegetable subjee /peas masala – 1 cup	Immune boosting /antiviral	Sesame rice 2katori cups/lime sevai2katori
Bedtime	Turmeric milk with black pepper 150 ml	Immune boosting /antiviral	Combining garlic, ginger, lemon and honey as a single juice 150 ml

Source: immunity%20review%20paper/immunity%20boosted%20diet%20for%20a%20pandemic.pdf

RESEARCH ARTICLE

Growth Rate of Area, Production and Productivity of Maize in Tamil Nadu

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ABSTRACT

The growth of agricultural construction is generally judged by the performance of food grains and non-food grains production. In India there had been increasing trend in the production of food grains. Based on the significance of crop present study was accompanied to know the growth rate of area, production and yield of the maize in Tamilnadu. Secondary data was used for this study. Simple statistical tools like compound annual growth rate, percentage change were used in this study. Time series data has been used in this study (1968-2018). This study exposed that compound annual growth rate for maize in case of area, production and yield showed positive sign. The positive sign shows that there was an increasing growth rate of maize production pattern.

Keywords: Compound growth rate, Productivity, Compound annual growth rate.

INTRODUCTION

Maize is grown in a wider range of environmental and socio economic conditions than other major crops .The area under maize in India is nearly 93 lakh hectares with a production of 198.9lakh tonnes according to the First Advance Estimates of Ministry of Agriculture and Farmers Welfare (2019-20).The leading states in the country for maize production are Maharashtra, Madhya Pradesh, Karnataka, Bihar, Telangana and Andhra Pradesh. The major maize growing districts of Tamil Nadu are Salem, Dindigul, Namakkal, Pudukottai, Tirupur, Villupuram, Perambalur and Ariyalur. Favourable climatic condition prevailed during current season and extended monsoon which reduced the attack of fall Army Worm along with adequate precautionary measures taken by one maize farmer facilitated better crop growth and yield. Since the arrival from Tamil Nadu has already started, the maize prices started declining and the prices will not increase further, if the supply is adequate.

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MATERIALS AND METHODS

The study was based on secondary data collected from various institutes. The compound annual growth rate, percentage change or annual growth rate was calculated. Compound annual growth rates (CAGR) was worked out to study about the changes in area, production and yield of maize over a period of time. The compound annual growth rate was calculated by fitting the following equation in the time series data, production and yield.

$Y_t = Y_0 (1 + r)^t$ …………(1)

Taking log on both sides, we will get

$L_nY_t = L_nY_0 + t L_n (1 + r)$

$L_nY_t = a + bt$ …………(2)

Where

$a = L_nY_0$

$b = L_n (1 + r)$

Y_t = area/production/ yield

Y_0 = Constant

t = time period in years

b = regression coefficient

% compound growth rate = (Anti log b – 1) x 100 …………(3)

Percentage change in yield is given by

% Change in yield = (current year yield – pervious year yield)+ previous year.

RESULTS AND DISCUSSION

The major cultivation of crops in Tamilnadu is food crops, cash crops and plantation crops. So, it is important to study the production yield and area of maize in the state. During 1990 – 91 area under maize was 27466 thousand ha .This area increased to 355064 ha in 2015 – 16.As shown in the (Table 1). Over the years area, production and yield was increased from 1990 – 2015.The production increased from 43820 thousand tonnes to 2532330 thousand tonnes in 1990-91 and 2015 – 16 respectively. The productivity of maize was minimum 1189 t/ha in 2005 – 06 and maximum was 8224 t/ha in 2015- 2016. Annual growth rate of area of maize was found to be 65.93 percent (highest). There was negative growth rate of production in 2012-2013 which was highest over a period of time, whereas maximum positive growth rate (65.00) was found in 2011-12. Productivity growth rate was decreased and increased with the change in the area. Trends in area, production and yields of maize in Tamilnadu. Compound annual growth rate was calculated on area, production and yield of maize for about fifty years in Tamilnadu to know about the growth rate over a period of time. Compound annual growth rate (%) of area increases upto 1965. There was negative growth rate of area in 1975 -1985, but there is a sharp decrease in the compound annual growth rate (CAGR) to 3.13. Overall CAGR in area, production and yield were found to be 5.28%, 8.13% and 2.92%. CAGR of area showed increasing trend till 1975. Compound annual growth rate for production was highest (15.90) in between 2005-2015. There was positive growth rate of yield in 2005-2015, which was highest over Period. From the result of the investigation it was found that area in the maize was found to be increased by 5.28% over 65 years, whereas production and yield increased at 8.13% and 2.92%. Area was found to be stable in a trend, whereas production and yield was increased because of various incentives, high yielding varieties etc.

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Table - 1: Descriptive statistic of Maize crop in Tamilnadu (1990-91 to 2015-16)

Year	Area	Production	Yield
1990-91	27466	43820	1595
1991-92	33747	54170	1605
1992-93	43338	70420	1625
1993-94	37619	60850	1618
1994-95	46527	73640	1585
1995-96	46842	74880	1598
1996-97	49009	80390	1640
1997-98	58045	94940	1636
1998-99	55666	88340	1587
1999-20	88066	137380	1609
2000-01	81467	139916	1717
2001-02	72956	118463	1624
2002-03	121057	191646	1583
2003-04	160159	250992	1567
2004-05	189893	294717	1552
2005-06	202830	241217	1189
2006-07	197782	759112	3838
2007-08	223428	810057	3626
2008-09	286639	1257882	4388
2009-10	244159	1138126	4661
2010-11	230489	1027536	4458
2011-12	280629	1695467	6042
2012-13	291052	946363	3252
2013-14	380429	2245216	5902
2014-15	321952	2647751	8224
2015-16	355064	2532330	7132
2010-11	230489	1027536	4458
2011-12	280629	1695467	6042
2012-13	291052	946363	3252
2014-15	321952	2647751	8224
2015-16	355064	2532330	7132

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Table – 2: Percentage change in area, production and yield of maize in Tamilnadu (1990-91 to 2015-16)

| Year	Area ('000 ha)	Production ('000 t)	Yield (t/ha)
1990-91	-20.84	-18.22	3.30
1991-92	22.87	23.62	0.63
1992-93	28.42	30.00	1.25
1993-94	-13.20	-13.59	-0.43
1994-95	23.68	21.02	-2.04
1995-96	0.68	1.68	0.82
1996-97	4.63	7.36	2.63
1997-98	18.44	18.10	-0.24
1998-99	-4.10	-6.95	-3.00
1999-20	58.20	55.51	1.39
2000-01	-7.49	1.85	6.71
2001-02	-10.45	-15.33	-5.42
2002-03	65.93	61.78	-2.52
2003-04	32.30	30.97	-1.01
2004-05	18.57	17.42	-0.96
2005-06	6.81	-18.15	-23.39
2006-07	-2.49	214.70	222.79
2007-08	12.97	6.71	-5.52
2008-09	28.29	55.28	21.01
2009-10	-14.82	-9.52	6.22
2010-11	-5.60	-9.72	-4.36
2011-12	21.75	65.00	35.53
2012-13	3.71	-44.18	-46.18
2013-14	30.71	137.25	81.49
2014-15	-15.37	17.93	39.34
2015-16	10.28	-4.36	-13.28

Table - 3: Compound annual growth rate of area, production and yield of Maize in Tamilnadu.

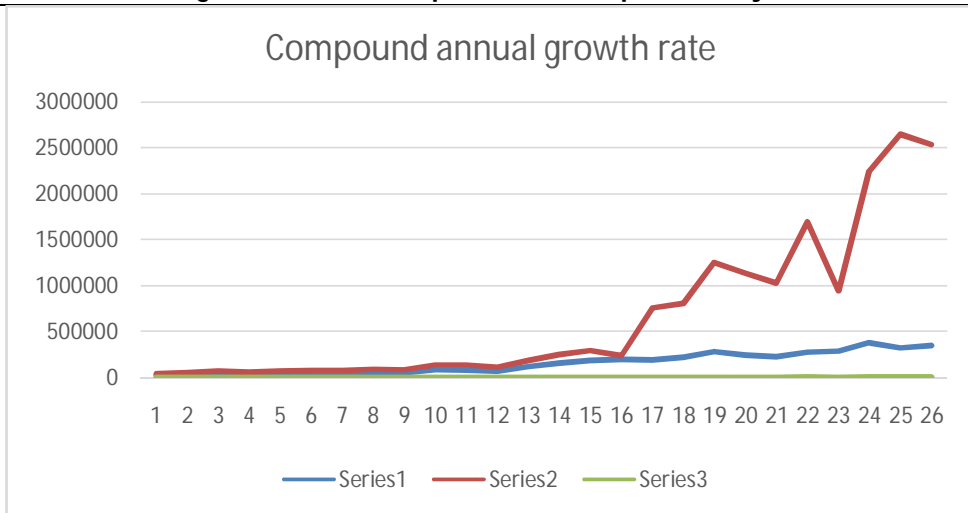| Year	Area	Production	Yield
1965-1975	10.32	11.45	0.58
1975-1985	-1.98	1.12	3.69
1985-1995	6.78	5.48	-0.61
1995-2005	8.17	6.68	-1.38
2005-2015	3.13	15.90	12.34
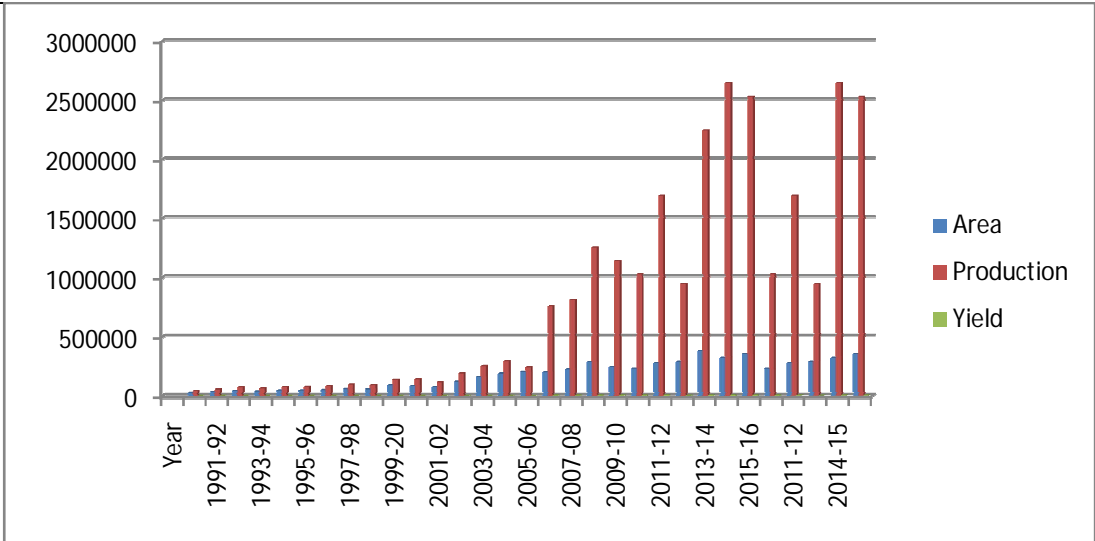
Annual growth rate of area, production and productivity of maize.

From the result of the investigation it was found that area in the maize was found to be increased by 5.28% over 65 years, whereas production and yield increased at 8.13% and 2.92%.

RESEARCH ARTICLE

In silico Molecular Docking Study on Phytochemical Components of *Leonotis nepetifolia* Linn against Enzyme Transport Protein.

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ABSTRACT

Aim: To analyze the cholesterol absorption inhibitory activity of phytoconstituents present in *Leonotis nepetifolia* Linn. using *In silico* docking study.
Methodology: The software Molegro Virtual Docker and tools were used for molecular docking v 6.0 along with Graphical User Interface, calculate dock score and evaluate conformers. Molegro virtual docker is a discriminatory programme to predict ligand interactions.
Results: The presence of six phytochemical constituents from ethyl acetate extract and one phytochemical constituents from ethanolic extract were identified by GC-MS. These components undergone *In silico* molecular docking studies using enzyme transport protein. The lead compounds were selected through the docking score. The compounds of ethyl acetate extract such as 2-Pentadecanone, 6,10,14-trimethyl- (-111.336); Phenol, 2,6-dinitro-4-(trifluoromethy)- (-80.7467); 2-Heptanone, 6-methyl- (-74.6784) and the compounds of ethanolic extract like Tetrasiloxane, decamethyl- (-91.463). Each extract have been shown three lead compounds from *In silico* molecular docking using standard atorvastatin (-104.402).
Conclusion: Further investigations on the above phytochemical constituents and *In silico* molecular docking study are necessary to develop potential chemical entities for the prevention and treatment of atherogenic activity.

Keywords: Anti-hyper cholesterolemia, Atorvastatin, *Leonotis nepetifolia*, Molecular docking, 3QNT.

INTRODUCTION

Cancer is a group of diseases involving abnormal cell growth with the potential to invade or spread to other parts of the body. These contrast with benign tumors, which do not spread. Possible signs and symptoms include a lump, abnormal bleeding, prolonged cough, unexplained weight loss, and a change in bowel movements. While these symptoms may indicate cancer, they can also have other causes. Over 100 types of cancers affect humans. Cancers are a large family of diseases that involve abnormal cell growth with the potential to invade or spread to other parts of the body. They form a subset of neoplasms. A neoplasm or tumor is a group of cells that have undergone unregulated growth and will often form a mass or lump, but may be distributed diffusely. Chemotherapy is the treatment of cancer with one or more cytotoxic anti-neoplastic drugs (chemotherapeutic agents) as part of a standardized regimen. The term encompasses a variety of drugs, which are divided into broad categories such as alkylating agents and antimetabolites. Traditional chemotherapeutic agents act by killing cells that divide rapidly, a critical property of most cancer cells. Radiation therapy involves the use of ionizing radiation in an attempt to either cure or improve symptoms. It works by damaging the DNA of cancerous tissue, killing it. Radiation therapy is used in about half of cases. The radiation is most commonly low energy X-rays for treating skin cancers, while higher energy X-rays are used for cancers within the body. Radiation is typically used in addition to surgery and or chemotherapy. *Leonotis nepetifolia*, (also known as klip dagga, Christmas candlestick, or lion's ear), is a species of plant in the genus *Leonotis* and the family Lamiaceae (mint). It is native to tropical Africa and southern India. It can also be found growing abundantly in much of Latin America and the West Indies. It has been found growing on road sides, rubbish heaps or waste land. It grows to a height of 3 metres (9 ft 10 in) and has whorls of striking lipped flowers, that are most commonly orange, but can vary to red, white, and purple. It has drooping dark green, very soft serrated leaves that can grow up to 10 centimetres (4 in) wide. Sunbirds and ants are attracted to the flowers.

MATERIALS AND METHODS

Molecular modeling studies
Molecular docking was performed using the software Molegro Virtual Docker (MVD) v 6.0 (www.molegr.com) along with Graphical User Interface (GUI), Molegro Virtual Docker tools was utilized to generate grid, calculate dock score and evaluate conformers. Molegro virtual docker is a discriminatory programme for predicting-ligand interactions.

Ligand preparation
The ligands used in this research were prepared using the chemical structure of phytochemical compounds was obtained from PubChem compound database. It was prepared by Chemsketch and MOL SDF format of this ligand was converted to 3D structure of ligand.

Protein preparation
The three-dimensional (3D) structure of transport protein (PDB-ID: 3QNT) was transformed from the RCSB protein Data Bank. Inhibition or depletion of NPC_1L_1 reduces intestinal cholesterol absorption, resulting in reduction of plasma cholesterol levels. If the reduction of plasma cholesterol level Niemann Pick C_1 Like$_1$ protein (NPC_1L_1). This protein is an established molecular target for the cholesterol lowering drug atorvastatin.

Structure based
Structure based drug design relies on knowledge of the 3D structure of the biological target obtained through methods such as X-ray crystallography or NMR spectroscopy. If an experimental structure of a target is not available, it may be possible to create homology model of the target based on the experimental structure of a related protein. Using the structure of the biological target, candidate drugs that are predicted to bind with high affinity and

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selectivity to the target may designed using interactive graphics and the intuition of a medicinal chemist. Alternatively, various automated computational procedure may be used to suggest new drug candidates.

RESULTS AND DISCUSSION

The molecular docking analysis were done with the targeted enzyme transport protein (PDB-ID: 3QND) was selected. The standard drug 5-fluorouracil (cancer drug) was used. This analysis of ethyl acetate extract contains 6 phytochemical compounds are 2-Heptanone, 6-methyl-; 1,2,3,4-Butaneterol, [S-(R*, R*)]-; 1,3-Propanediamine, N,N-dimethyl-; Phenol, 2,6-dinitro-4-(trifluoromethy)-; 2-Pentadecanone, 6,10,14-trimethyl-; Cyclotrisiloxane, hexamethyl. Then ethanolic extract contains 1 phytochemical compounds are Tetrasiloxane, decamethyl-.This docking score for all phytochemical compounds were compared with standard drug atorvastatin. From the results, we may observe that for successful docking, intermolecular hydrogen bonding and lipophilic interactions between the ligand and the receptor are very important.

CONCLUSION

The results of the present research study clearly demonstrated that, molecular docking score are best three phytochemical compounds of *Leonotis nepetifolia* aerial part of ethyl acetate extract and ethanolic extact such as 2-Pentadecanone, 6,10,14-trimethyl-; Phenol, 2,6-dinitro-4-(trifluoromethy)-; 2-Heptanone, 6-methyl- and then such as Tetrasiloxane, decamethyl-. This docking score for all phytochemical compounds were compared with standard drug atorvastatin. In the excellent binding sites and interactions with transport protein compared to the standard drug. Further investigations on the above phytochemical compounds in *In silico* molecular docking studies are necessary to develop potential chemical entities for the prevention and treatment of atherogenic activity.

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Table 1: Phytochemical compounds of ethyl acetate extract of *Leonotis nepetifolia*

Phytochemical compound names	Mol. Dock Score	Rerank Score	H Bond
2-Heptanone, 6-methyl-	-74.6784	-65.8695	-7.38436
1,2,3,4-Butaneterol, [S-(R*, R*)]-	-44.5505	-41.1454	-6.67057
1,3-Propanediamine, N,N-dimethyl-	-67.8518	-53.915	-4.97378
Phenol, 2,6-dinitro-4-(trifluoromethy)-	-80.7467	-76.0178	-4.31322
2-Pentadecanone, 6,10,14-trimethyl-	-111.336	-87.7138	-1.94869
Cyclotrisiloxane, hexamethyl-	-67.4469	-19.2996	0

Table 2: Phytochemical compounds of ethanolic extract of *Leonotis nepetifolia*

Phytochemical compound names	Mol. Dock Score	Rerank Score	H Bond
Tetrasiloxane, decamethyl-	-91.463	-29.227	-1.28835

Table 3: Molecular docking score of standard drug atorvastatin.

Standard drug	Mol. Dock Score	Rerank Score	H Bond
Atorvastatin	-104.402	-555.041	-8.45232

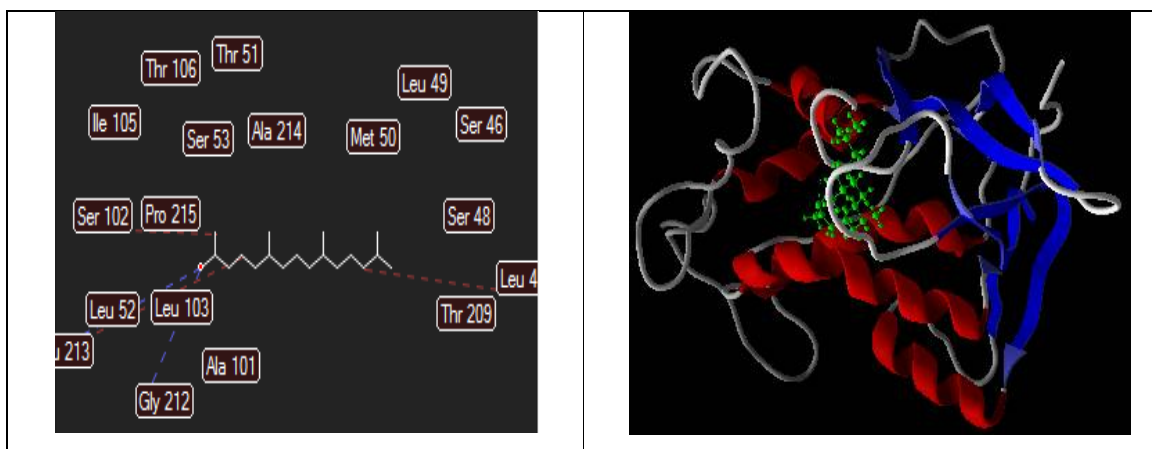**Jeyaraman Amutha Iswarya Devi** *et al.,*

Figure 1: Stearic interaction and docking pose of 2-Pentadecanone, 6,10,14-trimethyl-.
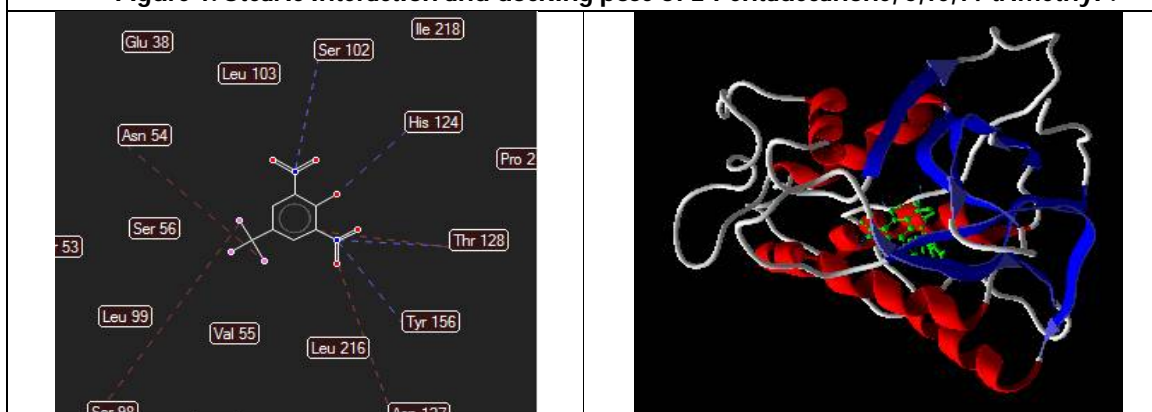

Figure 2: Stearic interaction and docking pose of Phenol, 2,6-dinitro-4-(trifluoromethy)-.
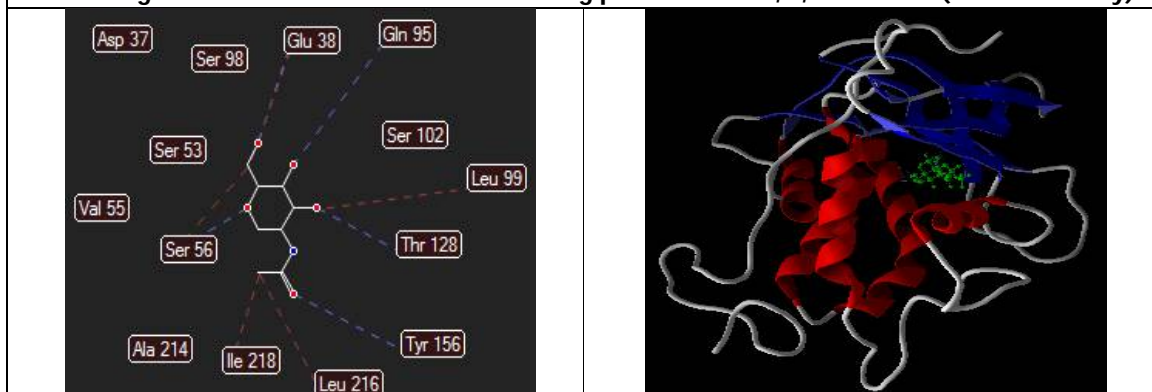

Figure 3: Stearic interaction and docking pose of 2-Heptanone, 6-methyl-.

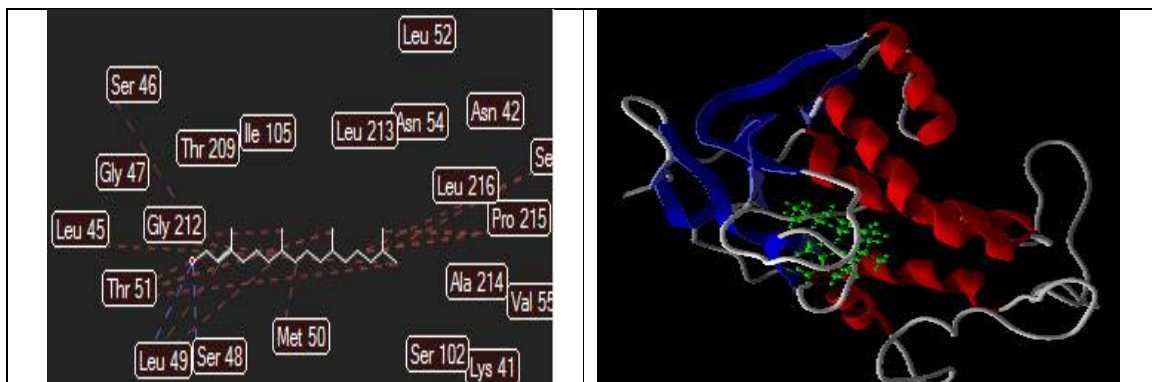**Jeyaraman Amutha Iswarya Devi** *et al.,*

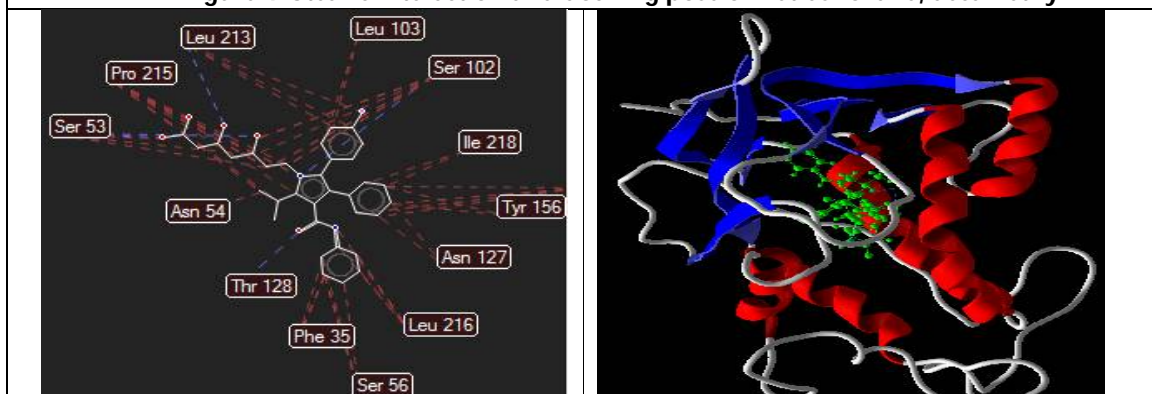**Figure 4: Stearic interaction and docking pose of Tetrasiloxane, decamethyl-.**

Fig. 5: Stearic interaction and docking pose of standard drug atorvastatin.

RESEARCH ARTICLE

Biomarkers for the Evaluation and Monitoring of Rheumatoid Arthritis - To make Things Real

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ABSTRACT

Introduction: One of the critical steps in RA patients' evolution is the identification and differentiation of the more severe symptoms of the disease by prognostic biomarkers for more systematic management of these cases. In order to guide clinical and therapeutic management of all phases of rheumatoid arthritis, biomarkers are critical because they can help predict disease progression in at-risk subjects, increase diagnosis by closing the serological gap, provide prognostic knowledge that is useful for making therapeutic decisions and assessing responses and effects of care, and allow for disease behaviour.
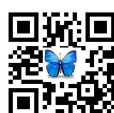**Objective:** The study aims to assess the role and critically evaluates the sensitivity, precision, a positive and negative predictive value of various biomarkers in rheumatoid arthritis patients.
Methods: Blood samples from RA patients were analyzed for RF, Anti-CCP, and Anti-Ds DNA AND CRP by respective immunologic and photometric assays.
Results: Anti-Ds DNA had a high uniqueness for RA with a reasonably low level of sensitivity. The mix of anti-Ds DNA and anti-CCP may raise a level of sensitivity and might be useful as diagnostic markers for rheumatoid arthritis.
Conclusion: The combination of conventional and novel biomarkers can be beneficial in predicting and very early medical diagnosis, primarily due to the variety of scientific look and inconsistency in the distribution of biomarkers in various individuals.

Keywords: Rheumatoid Arthritis, Rheumatoid factor, Systemic Lupus Erythematosus, Anti cyclic citrullinated peptide, Biomarkers.

INTRODUCTION

The major autoimmune disorders include Rheumatoid Arthritis (RA) and Systemic Lupus Erythematosus (SLE) [1]. These diseases are associated with various immunological, metabolic, haematological and genetic abnormalities. Just a few Indian-based studies have investigated the effectiveness of these biomarkers and their associations in treating these conditions [2]. RA is an inflammatory disease that requires some biomarkers to manage patients properly. Some of these markers help diagnose, whilst others assess the activity of the condition and the outcome of the treatment. Periodic antioxidant status monitoring and oxidative DNA damage assessment can help avoid long-term threats, such as malignancies [3]. In early diagnosis and treatment, combinations of novel and conventional biomarkers can be helpful, mainly due to the diversity of clinical presentation and inconsistency in the distribution of biomarkers in different patients. Increased oxidative stress and reduced antioxidant status may contribute to a deficit in the ability to restore DNA that may predispose patients to malignancy. The use of antioxidants can be recommended as a preventive measure. Rheumatoid factor (RF) and Anti cyclic citrullinated peptide (anti-CCP) are typically used to evaluate RA under the EULAR 2010 guidelines. Since 2010, new biomarkers have been reported and shown to be useful for early-stage RA diagnosis. Until the onset of symptoms such as rheumatoid factor and anti-citrullinated protein antibodies, various biomarkers can be used to identify subjects vulnerable to rheumatoid arthritis and those prone to pre-clinical rheumatoid arthritis [4]. They can be related to the risk of having rheumatoid arthritis and can predict more bone erosion and severe disease. Biomarkers such as the rate of erythrocyte sedimentation and the degree of C-reactive protein provide information on the disease's progression [5]. In contrast, predictive biomarkers allow clinicians to identify the potential for treatment response, particularly in the age of biological drugs, before starting a specific therapy [6]. This change from traditional stratification of patients to personalized healthcare approaches will significantly improve patient protection and reduce medical costs [7].

MATERIALS AND METHODS

Clinically reported RA situations with RA element and anti-CCP positive types of the study population. Normal individuals unfavourable for ANA, Anti- ds -DNA, RA, and Anti-CCP without any contagious or persistent conditions or autoimmune illness and not for any treatment, preferably the individuals' siblings, have been consisted of as the control. A total of 50 individuals with thought RA under the American Council of Rheumatology (ACR guidelines) were chosen for the research.

RESULTS AND DISCUSSION

Blood examples from RA individuals were assessed for RF and also Anti-CC serological markers. The physician offered the clients the disease occurrence rankings. The anti-ds DNA had a high uniqueness for RA with a reasonably reduced level of sensitivity. The mix of anti-Ds DNA and anti-CCP might raise the level of sensitivity and might serve as analysis pens for rheumatoid arthritis. This research study was conducted to assess the level of sensitivity, accuracy, the positive and adverse predictive worth of anti-cyclic citrullinated peptide in RA people. Researches done by several investigators have reported similar searching for as the most up to date disease-related research study primarily concerns the midlife group, and the majority gender is female. Reciprocal joint discomfort or irritability is generally approved as one of the most constant signs and symptoms in rheumatoid joint inflammation individuals, and the bulk may experience multiple joint pain. More individuals at the rheumatic facility dealt with both bilateral discomfort and minor joint swelling. Medical synovitis is a primary sign of rheumatoid arthritis and needs to be considered by physicians for dealing with rheumatoid arthritis people. Anti-CCP antibodies have a general sensitivity of 40%, a 100% favourable anticipating value, as well as a negative anticipating value of 60%. We located that anti-CCP antibody is associated with elevated danger for joint pain and joint pain, in proportion to joint involvement, enhanced CRP, and a rheumatoid factor.

CONCLUSION

RA is an inflammatory condition entailing a range of biomarkers for proper individual monitoring. Some of these pens aid with the medical diagnosis, while others assess medical events and patient results. The mix of unique and standard biomarkers can help anticipate and very early diagnosis, specifically as a result of the variety of medical appearance and variance in the distribution of biomarkers in various people.

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Author's contribution

S Santheep   : Manuscript preparation, Clinical Study, Literature Review
Preeti Mahawar   : Guide, Manuscript editing and review
P Rosh   : Study Design

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Table I: Group Statistics

Parameters	Serum RA factor	N	Mean	Std. Deviation	Std. Error Mean	P-Value
Serum Anti-CCP (u/ml)	Negative	39	13.41	1.996	.320	<0.01**
	Positive	50	366.68	187.366	26.498	
Serum Anti DS DNA (IU/ml)	Negative	39	63.67	29.762	4.766	<0.01**
	Positive	50	369.94	256.583	36.286	
CRP (mg/L)	Negative	39	7.108	4.6250	.7406	<0.01**
	Positive	50	21.220	22.4702	3.1778	
ESR	Negative	39	12.08	2.044	.327	<0.01**
	Positive	50	36.26	5.934	.839	

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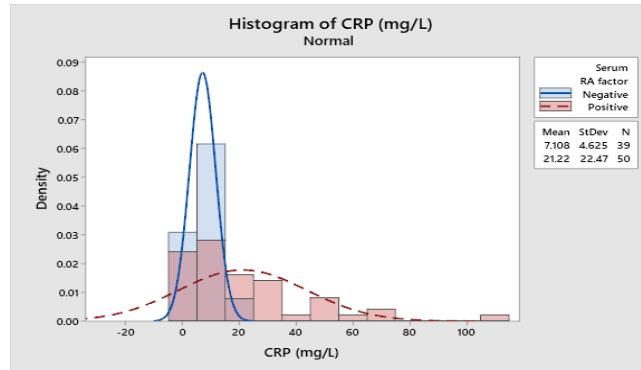
Figure I: Histogram of CRP

RESEARCH ARTICLE

Heterologous Expression and Immunological Characterization of *Mycobacterium tuberculosis* antigens HspX and Mpt51

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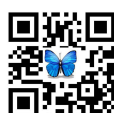Email: mohanshakila@yahoo.com

ABSTRACT

Tuberculosis (TB) is one of the most prevalent diseases in developing countries. In general, many individuals get infected with *Mycobacterium tuberculosis* (Mtb) but fail to develop active tuberculosis. In the case of latent TB, there will be an onset of active disease progression, in infected individuals after several years of infection due to various risk factors that include immunosuppression and immunodeficiency. The available vaccine *Mycobacterium bovis* Bacilli Calmette Guerin (BCG) confers poor protection against adulthood TB as it lacks many functional genes of Mtb. Thus the development of an effective and improved anti-TB vaccine has become an urgent need for adequate control and elimination of this disease. In this study, we attempted heterologous expression of two immunodominant antigens of Mtb namely HspX and Mpt51 that are associated with TB latency and re-activation of tuberculosis during HIV infection respectively. The prokaryotic expression vector containing Hsp X and Mpt 51 was transformed into *E.coli* BL21 DE3 expression vector and expression was optimized with 0.8mM IPTG at 20°C for Hsp X and at 37°C for Mpt 51. Expressed proteins were purified by Ni NTA column chromatography and quantified. Immunological characterization of the heterologously expressed HspX and Mpt51 was done using PBMCs and Rabbit animal models. The heterlogously expressed antigens HspX and Mpt51 stimulate significant immune response required to be a good quality vaccine candidate.

Keywords: Tuberculosis; HspX; Mpt51; Heterologous expression, Immune response

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INTRODUCTION

Mycobacterium tuberculosis (Mtb) is one of the major threats to the world population due to its mortality and morbidity. WHO's 2020 global TB report states an estimated 11.0 million TB incidences in the year 2019 among which 90% were adults, 56% were male, 32 % were female, 10 % were children, and 8.2% were HIV positive people. Besides Mtb's aerosolic spreading in the environment, its nature of host immune evasion, and emerging drug resistance to major anti-TB drugs make TB a most challenging disease to control (1). Available BCG vaccine shows its protective effect towards childhood tuberculosis while not giving desirable protection to the adults (2). The ineptitude of the BCG towards adult TB may be due to various reasons that include the missing region of some important genes that are present in Mtb or due to its genetic variance among individuals undergoing vaccination. Additionally, in predominant cases subsequently to the infection, Mtb reaches a dormancy state inside the host and resuscitate in the event of any favorable conditions like immune suppression, malnutrition, or co-morbid conditions like diabetes. This dormancy is called the latency period that happened since the TB bacterium has undergone various stress conditions like hypoxic conditions and nutrient-deprived stage (3). To survive such conditions, Mtb deploys various proteins that make survival possible during the period of dormancy. One among such proteins is HspX65 a 16 kDa heat-shock protein, an Alpha crystalline domain that helps Mtb with its chaperoning activity. The physiochemical requirements of HspX folding and its properties as a chaperone are still unexplored (4). HspX is an immunodominant antigen and it elicits B-cell and T-cell response in latently infected patients. Another important antigen of Mtb is Mpt51; a 27 kDa protein classified as a new family of non-catalytic alpha/beta hydrolases (5). It has been found in the Human Immuno Deficiency Virus (HIV) infected patient co-infected with TB (6). This study is an attempt to empower the current BCG vaccine with these immunodominant antigens to improve its vaccine efficacy to curtail TB at all stages of infection.

METHODOLOGY

Materials
All chemicals and reagents used in this study were biotechnology or molecular biology grade. IPTG-Isopropyl ß-D-1-thiogalactopyranoside from Medox biotech., Plasmids pMRLB.36 HspX and pMRLB.15 Mpt51 from BEI Resources, ATCC., USA.,

Heterologous expression of HspX and Mpt51

Heterologous expression of the HspX and Mpt51 genes in *Escherichia coli* BL21 was carried out using the methods as described elsewhere (7). In brief, *E.coli* BL21 competent cells were prepared using the calcium chloride method and transformed with 5μl of 10ng Plasmid DNA containing the functional genes of HspX and Mpt51. Screening of transformants was carried out using colony PCR and restriction digestion analysis using NdeI and HindIII restriction enzymes. The release of the gene product was checked on 1% agarose gel. To induce and optimize the culture conditions for the overexpression of these proteins, the transformed cultures were grown in LB media containing 0.1mg/ml Ampicillin and incubated at 37ºC, 180rpm till the optical density (OD) at 600nm reached 0.6. Then the culture was induced using IPTG at a final concentration ranging from 0.2mM to 1.0mM and incubated at different temperatures. The cell pellet was collected by centrifugation at 8000 rpm for 15 minutes at 4 ºC. To extract the proteins of interest, cell pellets were thawed and resuspended in cell lysis buffer (Tris-0.1M, NaCl 0.05M), vortexed vigorously, and sonicated at 72% power/9sec on – 5 sec off/ 2 mts. The supernatant was collected after centrifugation for 10 mts at 12,000rpm at 4 ºC and subjected to Ni-NTA column chromatography (HiMedia) by following the manufacturer protocol. Purified HSPX (16 kDa protein) and MPT51 (27 kDa protein) were run on a 12 % SDS PAGE to ascertain the efficiency of the purification process. The proteins were further purified by dialysis to remove sodium azide present in the commercial kit.

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Immunological Characterization of Heterologously over expressed HspX and Mpt51 Development of anti-HspX and anti-Mpt51 antibodies in Rabbit

New Zealand White rabbits of 3-4 months each weighing approximately 1.50 kg were chosen for developing anti-HspX and anti-mpt51 antibodies. The rabbits were acclimatized to the laboratory condition for seven days before subjecting it to the study. During the acclimatization period, the animals were observed for any sign of infection and infestation. Rabbits were fed with fresh leafy vegetables twice a day and provided with proper room conditions during the entire course of the study. This experiment was carried out with approval from the institutional ethical committee. This part of the study was approved by the Institutional Ethics Committee of Madurai Kamaraj University.

Immunization of Rabbits with HspX and Mpt51

Immunization of rabbits for developing Anti-HspX and Anti-Mpt51 antibodies was carried out by the prime-boosting method. Proteins at primary immunization were carried out using the antigens that were mixed with an equal volume of Freund's complete adjuvant (Sigma-Aldrich Co.). A total volume of one ml containing 0.47 mg of antigen was injected via the subcutaneous route at multiple sites not exceeding 100 µl per site. Three booster doses containing 0.4 mg/ml of the antigen mixed with an equal volume of Freund's incomplete adjuvant (Sigma-Aldrich Co.) were injected by a subcutaneous route at two weeks interval. Blood was collected from a marginal ear vein during the period of immunization. Utmost care was taken to prevent excess bleeding and the bleeding site was dressed with wound healing topical applications. Collection of less than 10% of total blood volume was ensured every time. After blood collection, Rabbits were transferred to a clean cage and observed for any sign of distress. Post 7 days of the third booster, blood was collected by cardiac puncture method from the anesthetized animal and it was sacrificed as per CPCSEA guidelines. The collected blood was immediately transferred to a sterile Falcon tube and stored at room temperature for one hour to facilitate blood clotting and serum separation. The clots were broken using a clean glass rod through swirling along the sides of the tube (Carried out to facilitate more serum collection) and then centrifuged at 3000 rpm for 10 minutes. Serum (supernatant) was collected into a fresh falcon without disturbing the RBC pellet and stored at -20ºC till further use.

Estimation of Anti-HspX and Anti-Mpt51 antibody titer using ELISA

To quantify the respective antibodies of the antigens 96 well ELISA plate was coated with 20 µg/ml protein (diluted in 1x PBS) and incubated at 4ºC overnight. the plate was then washed thrice with 200 µl washing buffer (1xPBS with 0.05% Tween). Blocking of un-occupied space was done using 100 µl blocking solution containing 1% Bovine Serum Albumin in wash buffer and incubated at 37ºC for 1 hour then washed thrice as mentioned previously. Serum collected at various stages of antibody production was diluted in the ratio of 1:200, 1:400, 1:600, 1:800, 1:1000 with 1x PBS. 100 µl of antibody dilutions were added to the specified wells and incubated at 37ºC for 1 hour and thoroughly washed as mentioned above. Incubated with 100 µl anti-rabbit IgG peroxidase conjugate (1:10,000, diluted) at 37ºC for 1 hour and washed thrice using the wash buffer. 100 µl of 0.4mg/ml O-Phenyl Diamine (OPD) substrate solution was added and incubated at 37ºC in dark till the development of color. The reaction was stopped by adding 50 µl 8N Sulphuric acid and the absorbance was measured using an ELISA plate reader (Thermo Scientific) at 450 nm and 490 nm.

Relative Quantification of Differential Cytokine Expression by Real-time PCR:

To detect the cell-mediated immune response inducing ability of the HspX and Mpt51 20µg/ml of these antigens that were heterologously expressed in *E.coli* was used to induce PBMCs collected from three healthy individuals. The cells were grown in presence of a 5% CO_2 atmosphere in the RPMI1640 medium supplemented with 10% FBS. Uninduced PBMCs were kept as controls. Post three days of stimulation the cells were harvested and subjected to RNA isolation by using HiPurA total RNA mini preparation kit (Hi-Media). The isolated total RNA was tested for its purity and integrity by using nano-drop spectrophotometer analysis. Complementary DNA was prepared from the total RNA using Protoscript II reverse transcriptase cDNA synthesis kit (Make: NEB) according to the manufacturer

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protocol. The real-time PCR reaction was carried out in the ABI-7000 real-time PCR machine (DST PURSE). The reaction mixture was prepared using a 2x Syber Green master mix (Make: Roche). The reaction following as denaturation for 60 seconds; annealing (60°C) for 45 sec and extension for 30sec. The dissociation constant was monitored to ensure the specificity of the reaction.

RESULTS

Heterologous Overexpression of Hsp X and Mpt 51 proteins of *Mycobacterium tuberculosis* in *E.coli* BI21 DL 3

E.coli BL21 DE 3 was transformed with pMRLB-Hsp X and pMRLB-Mpt 51 carrying hspX and mpt 51 respectively, screened by colony PCR and confirmed by restriction digestion analysis to release 440bp hsp X gene release and 880 bp Mpt 51 gene release.

Optimization of Culture Conditions for over expression of Mycobacterial Hsp X and Mpt 51 in *E.coli* BL 21

Standardization of culture concentration, inducer concentration and the temperature was done to overexpress Hsp X and Mpt 51 proteins. Both of these antigens were found to over-express when the LB media was supplemented with IPTG at a final concentration ranging from 0.8mM. Post-induction, expression was best at 20°C for HspX and 37°C for Mpt51 respectively. Intense overexpressed protein band 16kDa for Hsp X and 27kDa for Mpt 51 was found in 12% SDS PAGE. After purification of these antigens from the lysed cells through Ni-NTA column chromatography, the eluted samples were analyzed in SDS PAGE, and purification was confirmed by the appearance of a single intense band at 16kDa (Hsp X) and 27kDa (Mpt51). The purified protein concentration was analyzed in the non-chromogenic spectrophotometer method using a nanodrop analyzer. HspX was obtained at the concentration of 2.057mg/ml and Mpt 51 was at 0.922mg/ml concentration (Fig. 1&2).

Production of Anti-Hspx and Anti-Mpt51 antibodies in Rabbits

Antibodies against the HspX and Mpt51 were raised in rabbits immunized with respective proteins. Antibody titration was carried out by the ELISA method. The antibody titer for anti-HspX was found to be 1:800 after the third booster, similarly, the anti-Mpt51 antibody titer was found to be 1:1000 after the third booster. This shows the immunogenic nature of the heterologously expressed and purified antigens (Fig. 3&4).

Differential Cytokine Expression in the PBMCs

Post-six days of induction, the cells were harvested and RNA was isolated. cDNA was synthesized by reverse transcriptase PCR using random primers. An equal concentration of cDNA template was used to assess the differential cytokine expression HspX, Mpt 51, and PMA. The Hsp X induced monocytes showed an increased level of IL-2, IL-6, IL-8, and IFN-γ cytokine expression compared to Mpt-51. whereas Mpt 51 induced monocytes showed a higher fold of expression of TNF alpha and IL10 than the Hsp X induced monocytes besides IL-2 (Fig.5).

DISCUSSION

TB persists on the face of the earth for eons and human-kind still finds it as a great threat. Hence entire globe has now taken a stand to end TB by 2030. The major threat against this target is the fact that the host is being used as a TB reservoir in the form of latency. As mentioned previously currently available BCG vaccine does not address this problem (2) and hence an alternate vaccine is need of the hour. A good number of vaccine candidates are still in clinical trials and have not reached the end-user so far. Among different types of vaccines sub-unit vaccines that deliver vital antigens of Mtb are quite promising with significant efficiency (8).

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The first stepping stone in the development of subunit vaccines is the heterologous expression of the chosen antigens to achieve manageable quantities of antigens. In this study, we heterologously expressed two immunodominant antigens (HspX and Mpt51) of Mtb in *E.coli* that was transformed with an expression vector carrying the *hspx* and *mpt51*. HspX is associated with the growth of Mtb and is found to be differentially expressed during various stages of growth. Repression of HspX has lead to increased infection load in the host indicating its significance during the period of dormancy wherein the TB bacteria do not replicate (9). Besides, HspX was previously found to be involved in the polar localization of various proteins inside the Mtb cell (10). Hspx is considered as an immunodominant antigen as it leads to T-cell recognition and the anti-HspX antibodies could be demonstrated in the sera of latent TB patients who were recently infected (11,12). IL-10 an anti-inflammatory cytokine was previously reported to down-regulate the expression of HspX (13). Incoherence, we observed lower induction of IL-10 in the presence of heterologously expressed HspX (Fig. 5) and the development of specific antibodies in the rabbits (Fig.3). IL-8 was up-regulated in the presence of HspX displaying its potential to stimulate incursion of the site of infection by the leukocytes (14). These qualities of HspX suggest it as a promising vaccine candidate (15). However, recombinant HspX may require treatment with Mycobacterial cell lysate for better vaccine efficacy (16).

Three of four HIV-positive patients displayed the presence of anti-Mpt51 antibodies in their serum emphasizing its immuno dominance and significance during HIV infection (6) and was recommended to be an early target to detect TB-coinfection through serodiagnosis (17). Heterologously expressed Mpt51 antigen has increased the expression of a pro-inflammatory cytokine TNF-α, IL-6, and IL-8 in the PBMCs when compared to the uninduced PBMcs (Fig.5) and anti-Mpt51 antibodies in the rabbits. Increased expression of TNF-α is interlinked with activation of NF-$\varkappa$B and MAPK pathways. IL-6 has high anti-inflammatory properties, however, there are reports which states that IL-6 has context-dependent pro-and anti-inflammatory properties (18). IL-10 is another anti-inflammatory cytokine that was upregulated in Mpt-51 induced monocytes (19). Both HspX and Mpt51 elicited IL-2 cytokine that regulates T-cells by promoting its into effector T-cells and into memory T-cells during tuberculosis infection (20). There are only a few attempts were made by the researchers to heterologously express the HspX and Mpt51 antigens in *E.coli* (21) and BCG (16,22) among which one reported the significance of the chaperon function to confer protection in the host (16). Our current study highlights the immunological potency of these antigens expressed in *E.coli* that could be easily purifiable using the Ni-NTA column since the vectors we used were Histidine tagged. Hence it could be too easily scaled-up for mass production to meet industrial needs.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

AUTHORS CONTRIBUTION

VS and SH conceived the concept. VS, SER, and SH designed the experimental studies, wrote and edited the manuscript. VS and RA performed over-expression studies. VS, SER, and RA performed antibody production in the rabbits. VS and SER performed ex-vivo immunological characterization in the PBMCs. VS, SER, and SH analyzed the data. All authors read and agreed to publish.

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Table 1: List of PCR primers used for differential cytokine profiling in PBMCs induced with heterlogously expressed HspX and Mpt51 antigens

| Cytokines	Forward Primer (5′ – 3′)	Reverse Primer (5′ – 3′)
IL-6	AATTCGGTACATCCTCGACGG	GGTTGTTTTCTGCCAGTGCC
IL-8	GACCACACTGCGCCAACAC	CTTCTCCACAACCCTCTGCAC
IL-10	GGTTGCCAAGCCTTGTCTGA	AGGGAGTTCACATGCGCCT
TNF α	GGAGAAGGGTGACCGACTCA	CTGCCCAGACTCGGCAA
IFN γ	AGCTCTGCATCGTTTTGGGT	CGCTTCCCTGTTTTAGCTGC
β-ACTIN	TCACCCACACTGTGCCCATCTACG	CAGCGGAACCGCTCATTGCCAATG
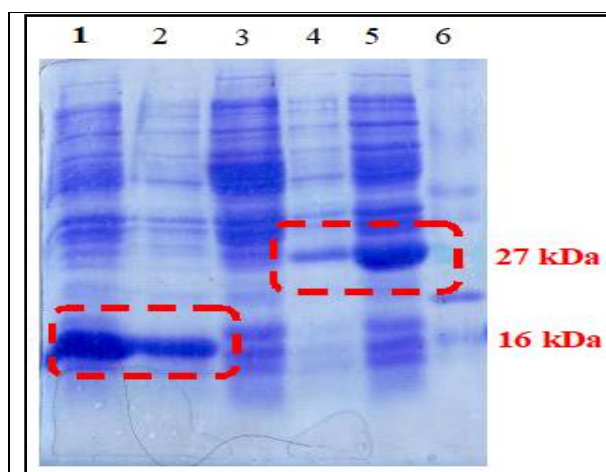
Figure 1: 12% SDS-Gel for over expression induced with 0.8mM IPTG at 20ºC for HspX and 37ºC for HspX
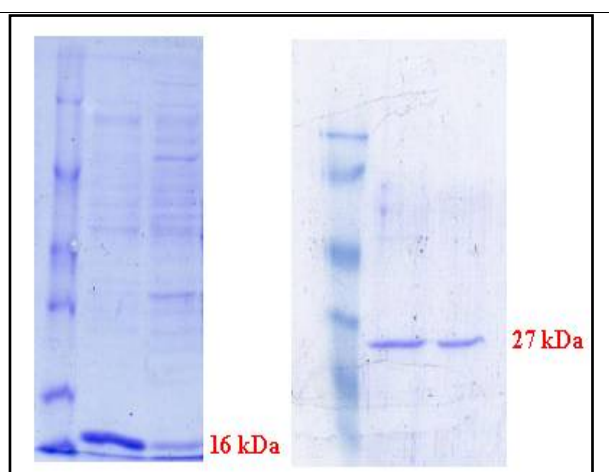
Figure 2: 10 % SDS gel of purified Mpt51 protein obtained from Ni-NTA gravity column

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Figure 3: Estimation of Anti-HspX antibody titer in Rabbit serum immunized with HspX antigen.

Figure 4: Estimation of anti-Mpt51 antibody titer in Rabbit serum immunized with the Mpt51 antigen.

Figure 5: Differential cytokine expression in the PBMCs induced with heterologously expressed Hsp X and Mpt 51 proteins.

RESEARCH ARTICLE

Evaluation of *In vitro* Antioxidant Activity of Aerial Part of Various Extract of *Leonotis nepetifolia* Linn.

Jeyaraman Amutha Iswarya Devi, Murugan Vannithurai* and Velayutham Mani Mala

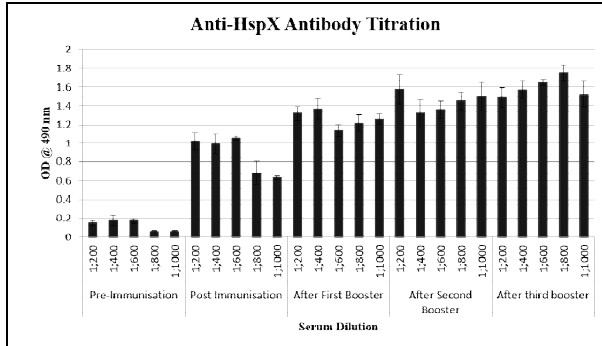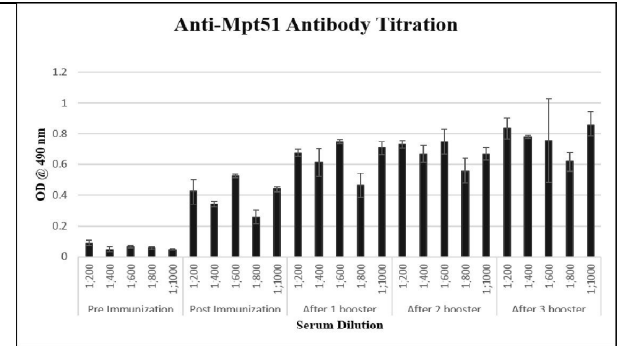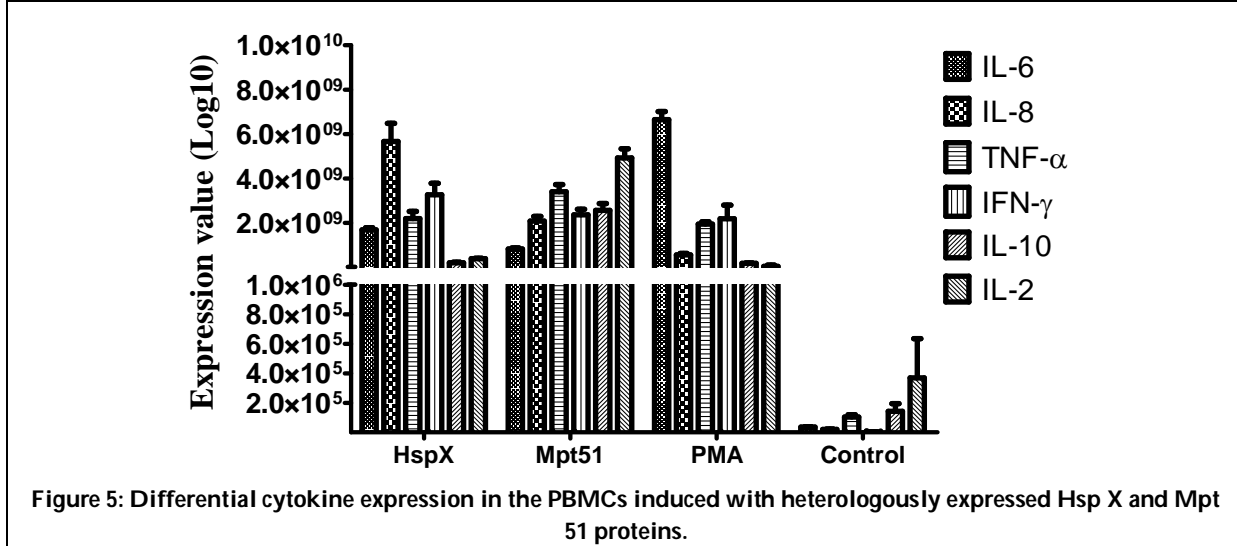Department of Pharmaceutical Chemistry, Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Krishnankoil-626126, Srivilliputtur (via), Virudhunagar, Tamil Nadu, India.

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ABSTRACT

Aim: To examine the *in vitro* antioxidant activities of pet.ether, ethyl acetate and ethanolic extract of aerial part of *Leonotis nepetifolia* Linn.
Method: The shade dried aerial part of powder was extracted with pet.ether, ethyl acetate and ethanol by continuous hot percolation method using Soxhlet apparatus.
Results: The antioxidant activity was determined by DPPH assay, Superoxide anion scavenging activity, iron chelating activity, hydroxyl radical scavenging activity, nitric oxide scavenging activity, FRAP assay, total antioxidant activity (phosphomolybdic acid method) at four different doses 125, 250, 500 and 1000 µg/ml with reference natural standard rutin, quercetin, EDTA and ascorbic acid respectively and total phenolic and total flavonoid content was analysed.
Conclusion: An IC_{50} values was found that ethanolic extract of *Leonotis nepetifolia* is more effective all antioxidant activity. These *in-vitro* assays indicate that this plant extract are better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Antioxidant, DPPH assay, *Leonotis nepetifolia, In-vitro* antioxidant, Total flavonoid content, Total phenolic content.

INTRODUCTION

Leonotis nepetifolia, (also known as klip dagga, Christmas candlestick, or lion's ear), is a species of plant in the genus *Leonotis* and the family Lamiaceae (mint). It is native to tropical Africa and southern India. It can also be found growing abundantly in much of Latin America and the West Indies. It has been found growing on road sides,

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rubbish heaps or waste land. It grows to a height of 3 metres (9 ft 10 in) and has whorls of striking lipped flowers, that are most commonly orange, but can vary to red, white, and purple. It has drooping dark green, very soft serrated leaves that can grow up to 10 centimetres (4 in) wide. Sunbirds and ants are attracted to the flowers. Antioxidants are man-made or natural substances that may prevent or delay some types of cell damage. Diets high in vegetables and fruits, which are good sources of antioxidants, have been found to be healthy; however, research has not shown antioxidant supplements to be beneficial in preventing diseases. Examples of antioxidants include vitamins C and E, selenium, and carotenoids, such as beta-carotene, lycopene, lutein, and zeaxanthin. Free radicals are highly unstable molecules that are naturally formed when you exercise and when your body converts food into energy. Your body can also be exposed to free radicals from a variety of environmental sources, such as cigarette smoke, air pollution, and sunlight. Free radicals can cause "oxidative stress," a process that can trigger cell damage. Oxidative stress is thought to play a role in a variety of diseases including cancer, cardiovascular diseases, diabetes, Alzheimer's disease, Parkinson's disease, and eye diseases such as cataracts and age related macular degeneration. Antioxidant molecules have been shown to counteract oxidative stress in laboratory experiments (for example, in cells or animal studies). However, there is debate as to whether consuming large amounts of antioxidants in supplement form actually benefits health. There is also some concern that consuming antioxidant supplements in excessive doses may be harmful. Vegetables and fruits are healthy foods and rich sources of antioxidants. Natural antioxidants are constituents of many fruits and vegetables and they have attracted a great deal of public and scientific attention. There are several common natural antioxidants which are found in everyday foods, the most common of which being Vitamin C (ascorbic acid), Vitamin E (tocopherols), Vitamin A (carotenoids), various polyphenols including flavonoids, and Anthocyanins (a type of flavonoid), Lycopene (a type of carotenoid) and Coenzyme Q also known as Ubiquitin, which is a type of protein. Synthetic antioxidants are chemically synthesized since they do not occur in nature and are added to food as preservatives to help prevent lipid oxidation. These antioxidants fall into two major categories depending on their mode of action Primary antioxidants and Secondary antioxidants.

MATERIALS AND METHODS

Collection and Identification of *Leonotis nepetifolia* (Linn)

The aerial part of *Leonotis nepetifolia* was collected from Rajapalayam, Virudhunagar District of Tamil Nadu, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu. The plant of *Leonotis nepetifolia* and were dried under shadow, segregated, crushed by a mechanical processor and went through a 40 lattice sifter. The plant powdered materials were put away in a hermetically sealed holder.

Preparation of Plant Extract

The aerial part powder samples of *Leonotis nepetifolia* were extracted with pet.ether, ethyl acetate and ethanol at temperature between 60-70ºC by using soxhlet extractor. The solvent was evaporated by rotavapor to obtained viscous semi solid masses.

In vitro Antioxidant Studies

DPPH Radical Scavenging Effect

The DPPH assay of aerial part of the plant was measured using the method described by (Mensor*et al.*, 2001).The ethanolic extract was taken in different concentrations varying between 125 to 1000µgmL and results showed that the antioxidant activity, the percentage of inhibition. The absorbance was measured at 518 nm and converted into percentage radical scavenging activity as follows.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\ Control - A_{518}\ Sample}{A_{518}\ Control} \times 100$$

Nitric Oxide Radical Scavenging Activity

The ability of the plant extracts to scavenge the nitric oxide radical activity were determined by the method described by (Green *et al.*, 1982). Nitric oxide radical generated from sodium nitroprusside in aqueous solution at optimum pH conditions interacts with oxygen to produce nitrile ions which can be estimated by the use of Griess Ilosvay reaction at 540 nm.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

Iron Chelating Activity

The iron chelating assay of aerial part of plant extracts of *Leonotis nepetifolia* were described by (Benzie and Strain, 1996).The principle is based on the formation of O-Phenanthroline-Fe2+ complex and its disruption in the presence of chelating agents. EDTA was used as a classical metal chelator. The experiment was performed in triplicates.

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

Hydroxyl Radical Scavenging Activity

The hydroxyl radical scavenging properties of ethanolic extract were determined by (Elizabeth and Rao, 1990).The assay is based on quantification of degradation product of 2-deoxy ribose by condensation with TBA. Hydroxyl radical was generated by the Fe^{3+}-Ascorbate-EDTA-H_2O_2 system (Fenton reaction). The scavenging activity on hydroxyl radical was calculated as follows:

Scavenging activity (%) = (1 - absorbance of sample/absorbance of control) × 100

Superoxide Radical Scavenging Activity

The assay for superoxide anion radical scavenging activity was supported by riboflavin-light-NBT system (Winterbourne *et al.*, 1975). Ascorbic acid was used as standard. The scavenging ability of the plant extract was determined by the following equation:

Scavenging effect (%) = (1 - absorbance of sample/absorbance of control) × 100

Total Antioxidant Activity (Phosphomolybdic acid Method)

The antioxidant activity of the ethanolic extract of *Leonotis nepetifolia* was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex (Prieto *et al.*, 1999). Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

$$Total\ antioxidant\ activity\ (\%) = \frac{A_{518}\ Control\ -\ A_{518}\ Sample}{A_{518}\ Control} \times 100$$

FRAP Assay

The FRAP assay of aerial part of plant extracts of *Leonotis nepetifolia* were described by modified method of (Benzie and Strain, 1996). Readings of the coloured product (Ferrous tripyridyltriazine complex) were taken at 593 nm. The standard curve was linear between 200 and 1000 μM $FeSO_4$. Results were expressed in μM (Fe (II) /g) dry mass and compared with that of ascorbic acid.

Estimation of Total Phenol Content

Total phenolic content of aerial part of the plant was measured using the method described by (Mallick and Singh *et al.*, 1980).

Estimation of Total Flavonoids Content

Total flavonoid content of aerial part of the plant was measured using the method described by (Cameron *et al.*, 1943).

RESULTS AND DISCUSSION

In vitro Antioxidant
DPPH Photometric Assay
The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge DPPH photometric assay was calculated as % inhibition and was compared with rutin used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 57.36% in pet.ether, 60.33% in ethyl acetate and 64.20% in ethanol when compared to rutin69.83% which is statistically significant at same concentration. The IC_{50} value was found to be 505 μg/mlfor pet.ether, 480 μg/ml for ethyl acetate and 425 μg/ml for ethanolic extract of *Leonotis nepetifolia* and for rutin it was 480 μg/ml.

Superoxide Anion Scavenging Activity
The ability of pet.ether, ethyl acetate and ethanolic extracts to superoxide anion scavenging activity was calculated as % inhibition and was compared with quercetin used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 51.56% in pet.ether, 56.75% in ethyl acetate and 66.56% in ethanol when compared to quercetin98.01% which is statistically significant at same concentration. The IC_{50} value was found to be 950 μg/mlfor pet.ether, 440 μg/ml for ethyl acetate and 285 μg/ml for ethanolic extract of *Leonotis nepetifolia* and for quercetin it was 60 μg/ml.

Iron Chelating Activity
The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge iron chelating activity was calculated as % inhibition and was compared with EDTA used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 49.88% in pet.ether, 56.30% in ethyl acetate and 68.54% in ethanol when compared to EDTA 97.90% which is statistically significant at same concentration. The IC_{50} value was found to be 1010 μg/mlfor pet.ether, 610 μg/ml for ethyl acetate and 390 μg/ml for ethanolic extract of *Leonotis nepetifolia* and for EDTA it was 65 μg/ml.

Hydroxyl Radical Scavenging Activity
The ability of pet.ether, ethyl acetate and ethanolic extracts to hydroxyl radical scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 48.35% in pet.ether, 55.22% in ethyl acetate and 65.24% in ethanol when compared to ascorbate75.23% which is statistically significant at same concentration. The IC_{50} value was found to be 1120 μg/mlfor pet.ether, 870 μg/ml for ethyl acetate and 405 μg/ml for ethanolic extract of *Leonotis nepetifolia* and for ascorbate it was 410 μg/ml.

Nitric Oxide Scavenging Activity
The ability of pet.ether, ethyl acetate and ethanolic extracts to nitric oxide scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 50.35% in pet.ether, 55.90% in ethyl acetate and 65.55% in ethanol when compared to ascorbate75.23% which is statistically significant at same concentration. The IC_{50} value was found to be 970 μg/mlfor pet.ether, 780 μg/ml for ethyl acetate and 420 μg/ml for ethanolic extract of *Leonotis nepetifolia* and for ascorbate it was 410 μg/ml

Total Antioxidant Activity (Phosphomolybdic Acid Method
The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 μg/ml of concentration, the percentage inhibition of plant extracts was found to be 51.61% in pet.ether, 52.20% in ethyl acetate

and 59.66% in ethanol when compared to ascorbate65.23% which is statistically significant at same concentration. The IC_{50} value was found to be 960 µg/ml for pet.ether, 780 µg/ml for ethyl acetate and 490 µg/ml for ethanolic extract of *Leonotis nepetifolia* and for ascorbate it was 410 µg/ml.

FRAP Assay

The ability of pet.ether, ethyl acetate and ethanolic extracts to scavenge Superoxide anion scavenging activity was calculated as % inhibition and was compared with ascorbate used as standard. It was observed that at 1000 µg/ml of concentration, the percentage inhibition of plant extracts was found to be 49.20% in pet.ether, 54.47% in ethyl acetate and 64.59% in ethanol when compared to ascorbate98.07% which is statistically significant at same concentration. The IC_{50} value was found to be 1015 µg/mlfor pet.ether, 780 µg/ml for ethyl acetate and 560 µg/ml for ethanolic extract of *Leonotis nepetifolia* and for ascorbate it was 50 µg/ml.

Estimation of Total Phenol Content

TPC showed a sharp pet.ether extract range of 1.234mg/g, ethyl acetate extract range of 2.412mg/g and ethanolic extract range of 3.840mg/g as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

Estimation of Total Flavonoids Content

The TFC showed a sharp pet.ether extract range of 0.967 mg/g, ethyl acetate extract range of 2.010 mg/g and ethanolic extract range of 1.450 mg/g as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

CONCLUSION

The present study was clearly indicated ethanolic extract of *Leonotis nepetifolia* showed strong antioxidant activity when compared with pet.ether and ethyl acetate extracts. Therefore, further investigations need to be carried out to isolate and identify the antioxidant compounds present in the ethanolic plant extract.

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Table 1: DPPH Assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Rutin)
1	125	16.34±0.01	16.70±0.04	16.96±0.05	18.85±0.076
2	250	32.54±0.06	33.67±0.03	38.98±0.02	22.08±0.054
3	500	49.56±0.76	51.77±0.79	54.54±0.90	52.21±0.022
4	1000	57.36±0.07	60.33±0.07	64.20±0.08	69.83±0.014
IC$_{50}$		**505µg/ml**	**480µg/ml**	**425µg/ml**	**480 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

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Table 2: Superoxide Anion Scavenging Activity

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Quercetin)
1	125	18.42±0.20	18.20±0.23	30.37±0.14	73.81±0.006
2	250	25.39±0.18	38.28±0.20	47.92±0.22	91.31±0.011
3	500	48.56±0.76	52.56±0.52	59.93±0.75	92.99±0.024
4	1000	51.56±0.09	56.75±0.07	66.56±0.05	98.01±0.012
IC_{50}		950µg/ml	440µg/ml	285µg/ml	60 µg/ml

*All the values are expressed as mean ± SEM for three determinations

Table 3: Iron Chelating Activity

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (EDTA)
1	125	14.60±0.02	16.85±0.02	20.18±0.07	58.68±0.007
2	250	28.85±0.20	30.79±0.30	38.57±0.22	65.87±0.018
3	500	40.54±0.05	44.16±0.03	56.90±0.03	83.83±0.012
4	1000	49.88±0.09	56.30±0.07	68.54±0.09	97.90±0.019
IC_{50}		1010µg/ml	610µg/ml	390µg/ml	65 µg/ml

*All the values are expressed as mean ± SEM for three determinations

Table 4: Hydroxyl Radical Scavenging Activity

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	13.40±0.01	19.30±0.02	22.64±0.02	26.87±0.076
2	250	22.5±0.30	30.53±0.25	38.90±0.02	30.30±0.054
3	500	38.09±0.22	36.76±0.33	60.87±0.08	60.64±0.022
4	1000	48.35±0.07	55.22±0.12	65.24±0.02	75.23±0.014
IC_{50}		1120µg/ml	870 µg/ml	405 µg/ml	410 µg/ml

*All the values are expressed as mean ± SEM for three determinations

Table 5: Nitric Oxide Scavenging Activity

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample Ethanolic extract)	Standard (Ascorbate)
1	125	13.55±0.02	18.10±0.09	20.09±0.03	26.87±0.076
2	250	21.90±0.20	24.64±0.33	32.60±0.10	30.30±0.054
3	500	30.85±0.02	45.35±0.03	60.35±0.04	60.64±0.022
4	1000	50.35±0.30	55.90±0.12	65.55±0.08	75.23±0.014
IC_{50}		970 µg/ml	780 µg/ml	420 µg/ml	410 µg/ml

*All the values are expressed as mean ± SEM for three determinations

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Table 6: Total Antioxidant Activity (Phosphomolybdic Acid Method)

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Petroleum ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	20.65±0.05	23.35±0.07	25.62±0.09	26.87±0.076
2	250	38.36±0.02	30.30±0.07	38.63±0.01	30.30±0.054
3	500	42.63±0.05	48.50±0.07	50.36±0.07	60.64±0.022
4	1000	51.61±0.30	52.20±0.03	59.66±0.08	65.23±0.014
IC$_{50}$		**960 µg/ml**	**780 µg/ml**	**490 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table 7: FRAP Assay

S.No	Concentration (µg/ml)	% of activity(±SEM)*			
		Sample (Pet. ether extract)	Sample (Ethyl acetate extract)	Sample (Ethanolic extract)	Standard (Ascorbate)
1	125	25.26±0.06	30.62±0.02	31.28±0.08	72.04±0.014
2	250	30.63±0.05	42.46±0.06	44.64±0.05	82.05±0.034
3	500	40.47±0.08	48.36±0.08	53.23±0.08	86.04±0.026
4	1000	49.20±0.04	54.47±0.05	64.59±0.04	98.07±0.041
IC$_{50}$		**1015 µg/ml**	**780 µg/ml**	**560 µg/ml**	**50 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table 8: Total Phenol

S.No	Extracts	Total phenolic content (mg/g of Catechol)(±SEM)*
1	Pet. ether extract of *Leonotis nepetifolia*	1.234±0.003
2	Ethyl acetate extract of *Leonotis nepetifolia*	2.412±0.004
3	Ethanolic extract of *Leonotis nepetifolia*	3.840±0.007

*All the values are expressed as mean ± SEM for three determinations.

Table 9: Total Flavonoids

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Pet. ether extract of *Leonotis nepetifolia*	0.967±0.08
2	Ethyl acetate extract of *Leonotis nepetifolia*	1.450±0.04
3	Ethanolic extract of *Leonotis nepetifolia*	2.010±0.03

*All the values are expressed as mean ± SEM for three determinations

RESEARCH ARTICLE

Transmission Power Line Fault Detection based on Deep Learning Methods- Survey on Recent Advancements

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ABSTRACT

This paper examines about the recent developments made for the automated identification of fault in Transmission power line using the recent advancements in the field of Deep Learning (DL). Over the years, the number of techniques has been proposed, and each method has its own merits and drawbacks. Uninterrupted electric power delivery to end-users is a challenging task for Power System Operators (PSO). Although intrusion of fault is beyond human control, it is fundamentally essential to detect, classify, and locate the fault location accurately. Initiatives have been taken to create an intelligent protection system capable of reliably identifying, classifying, and locating faults. Advances in signal processing techniques, Artificial Intelligence (AI), Machine Learning (ML) & Deep Learning (DL) have helped researchers to adopt a more detailed and dedicated approach in investigations associated with conventional strategies for fault detection.

Keywords: Transmission Lines, Artificial Intelligence, Machine Learning, Deep Learning, Fault Detection, Fault Classification, Fault Locating.

INTRODUCTION

The fault is nothing other than an abnormal state. The system quantities (voltage, current, phase angle, etc.) in power transmission systems surpass their threshold values if the system encounters an unusual situation called a fault. The maximum portion of the overhead transmission line was exposed to atmospheric conditions; therefore, the chances of faults occurring in overhead transmission lines were more significant than those of underground cables. Faults in the overhead transmission system can be classified into two types, as shown in Figure1 Series (open conductor) faults and Shunt (short circuit) faults. Series faults can be efficiently recognized by looking at the voltage of each phase. If the voltage values increase, it means that there is a fault with the open conductor. Such faults are classified into two types, one open conductor fault, and two open conductor faults. Such faults have happened very rarely. Short-circuit faults can be efficiently detected by examining the current of each phase. When the current values increase, this means the short circuit fault occurs. Short-circuit faults are split into two classes Symmetric faults and Asymmetric faults. Asymmetrical faults are Double Line to Ground (LLG), Line to Line (LL) and Line to Ground (LG), and symmetrical faults are Triple Line to Ground (LLLG) and Triple Line (LLL). In Figure1, the variables A, B, C, and G indicate phases A, B, C, and Ground, respectively. The most generally happened is LG fault and severity, and occurrence-based is LL fault. The complete system will collapse due to LLLG fault and 3- phase faults (LLL and LLLG). To prevent significant losses, we must locate the fault and identify the existence of the fault and location of the fault within less time.

Transmission Line Fault Analysis Techniques

A traditional fault analysis program detects, classifies, and locates faults. Some pre-set thresholds are needed in the detection and classification of faults, and the location of faults is based on a less accurate lumped parameter model. Within the literature, several methods of fault analysis are discussed. Figure 2 displays a tree diagram of the latest techniques for the study of fault line transmission. Detection of faults is usually performed before classification and estimate of the location. Fault detection is done based on the features extracted. Whenever a self-governing methodology is employed to detect faults, the classifier and the locator are activated after positively detecting a fault. Therefore, since classifiers and locators are skilled in distinguishing between safe and unhealthy situations, there is no need to formulate fault detection algorithms. However, some techniques for detecting faults are discussed here.

Fault Detection Techniques

The components of negative sequences for identifying faults are used by Martinez *et al.* [1]. Thus, decrease the chances of fault detection, a Joint Fault Indicator (JFI), is accomplished utilizing the convolution of partial differential over time of negative sequence components with a triangular wave. JFI based detection of faults is robust in cases of variation in amplitude and frequency deviation. In Transmission Lines, the wavelet-based method is proposed for real-time fault recognition [2]. Diverse research has been conducted to detect High Impedance Faults (HIF) [3-5] since conventional algorithms may not identify HIF. Wai *et al.* collected high-frequency data by utilizing DWT for HIF detection with a quadratic spline mother wavelet [3]. DWT wavelet coefficient and converted scale coefficients employed for HIF detection [4]. The mean of DWT coefficients is achieved via PCA in [5] to decrease the dimensionality of the features at various frequency bands. Raza, A *et al.* [6] summarize that the detection period for faults usually does not considerably impact the protection system's performance, which involves mechanisms for identification, classification, and location. Detection of faults is generally accomplished in 2–10 ms, compared to 30 ms for classification of fault type.

Fault Classification Techniques

Fault-type classification plays a crucial role in protecting transmission lines; it was shown increasing interest in developing stable, novel, and precise classification methods for faults. Some of the classification methods available are based on classifiers and theories of statistical learning [7], while others are based on logical approaches [8].

Developing fault detection techniques is highly dependent on advances in the methods of machine learning and pattern recognition. The following section provides a thorough analysis of the fault-type classification methods.

Wavelet-based methods fundamentally utilize the time difference between traveling wave reflections are considering higher sampling rates and synchronized terminal measurements. While this approach has a lower estimation error, it does have a computationally intensive burden [9]. Phasor unit measuring (PMU) based methods demand synchronized phasor quantities from every transmission line terminal. When implemented on transmission lines to identify and locate faults, they are often sluggish and complex to implement [10]. Support Vector Machines (SVM) are supervised learning models that map the input space of large dimensions to target space [11]. The key benefit is its regularization parameter, which tells the SVM not to misclassify each sample of the training [12]. SVM works well with continuing data and, therefore, can learn more from fewer samples. One positive sign is that researchers used SVM as a classifier in the transmission lines in the past to conduct fault classification [9]. Babu *et al.* [13] suggested classification of fault utilizing Empirical Mode Decomposition (EMD) and SVM's. A system for detecting and classifying faults based on Principal Component Analysis (PCA) and SVM is proposed by K. Li *et al.* [14].

A multi-class SVM approach to the classification of faults in transmission lines is proposed by Malathi *et al.* [15]. Wavelet decomposition utilizing Discrete Wavelet Transform (DWT) of post fault phase current signals are utilized as a feature set for the SVM, which predicts the fault condition. Dubey *et al.* [16] suggested a method for classifying fault using the Least Square SVM (LS-SVM) method. An approach was proposed to combine Discrete Wavelet Transform (DWT) with SVM for classifying faults by Hanif *et al.* [17]. DWT is employed to extract the post fault voltage and normalized energies, which are utilized as input to the four SVM classifiers. Youssef *et al.* [18] suggested detecting and classifying faults using SVMs in real-time. After the fault, the phase angle was registered for nine kinds of fault types and utilized to train the two SVMs. A Work has been carried out to implement SVM to detect and identify faults on a series compensated circuit [19-21] that is out of bounds for this study. A hybrid wavelet transform, and modular artificial neural network were suggested by Koley *et al.* [22]. It is based on fault detector, classifier, and locator for six-phase lines using a single end (single terminal) data. A hybrid wavelet transform, and modular artificial neural network were suggested by Koley *et al.* [22].

Fault Location Techniques

A comprehensive review of existing Fault Location (FL) finding methods is available in [8,23.24]. Fundamentals and new advances in fault location techniques are presented in this thesis, based on existing literature. Fault location methods can be classified based on data sources; dual-end, single-end, and wide-area. In this study, wide-area approaches are explored due to the demand and requirement for prospective smart grids. Likewise, compensated series and hybrid TLs are considered more than standard lines because of their unique properties. Traditional AI-based approaches are addressed due to their substantial success in finding fault Location and broad prospects for use.

Conventional fault location methods cannot trace faults if either of the monitoring devices installed at TL's end terminals fails to record changes in voltage and current profiles. For this kind of scenario, wide-area FL techniques may be a viable solution [25]. In wide-area FL techniques, each application/algorithm replica runs at different transmission substations [26], inhibiting the overloading of that specific station's available computing and communication resources. Thus, even with fewer devices mounted at different end-terminals of transmission links, fault can be located. Synchronized algorithms based on optimization are proposed for the location of fault [27, 28]. Travelling-Wave (TW) techniques are also implemented to determine the location of fault with single-end data and two-end data [29]. The Linear least square (LLS) approach is adopted to locate the position of the fault. Non-iterative substitution based synchronized voltage algorithm suggested in [30] for estimating the location of the fault. This approach is based on the impedance matrix of positive and negative sequences obtained through the network's topology. In a positive sequence network, the corresponding degree factor is equal to zero, reflecting the position of

the fault point [31]. Thus, the matching degree is utilized to point out the faulty bus throughout the system. In [32], an impedance-based hierarchical routine is employed to locate the defective zone, line, and point.

Neural Networks are the first choice when the intention is to model non-linear and complex relationships that follow the pattern of real life. They generalize well, which results in a model that allows good predictions of unseen data. Twafik *et al.* [33] suggested an Artificial Neural Network (ANN) for predicting the location of the fault on transmission lines. Prony technique is utilized to extract the modal voltage information or current signal information. Yadav *et al.* [34] have formulated a comprehensive and thorough review that will reduce new research complexity in evaluating several ANN-based methods with a collection of references for all the contributions concerned. They concluded from the survey that ANN is a robust, precise, and effective method for the detection of faults on the transmission line, classification, localization, direction discrimination, and faulty phase selection. Ray *et al.* [35] introduced Support Vector Machine (SVM) for classification of fault and Support Vector Regression (SVR) for locating the fault. Classification of fault consists of four SVMs. Each process predicts the involvement of each phase and location of fault consisting of SVR. In Table 1Raza, A *et al.* [6] compares the generalized strengths and weaknesses of several artificial intelligence and machine-based learning algorithms. It can help pick an effective system for identification, classification, and location of faults based on its strengths, functionality, and complexity levels.

PROPOSED METHODOLOGY

Based on the survey, it is observed that very few research papers use Partial Discharge (PD) data acquired in the field. Most data used were obtained from laboratory experiments or simulations that could lead to a lack of generality. The method would not be applicable to measurements made in real installations. In this work, a real-time dataset obtained by VSB - ENET Centre in the Czech Republic, is used, and a novel pattern recognition method is proposed to achieve automated identification of PD's.

Objective of the Proposed Work
1. To design a suitable Deep Learning framework for Transmission Power Line Fault Detection.
2. To investigate different pattern recognition methods to recognize and classify Partial Discharges activities on Insulated Overhead Conductors.
3. To propose suitable combinations of Deep Learning algorithms to enhance the performance of PD prediction and classification.

Scope of the Proposed Work
1. A standard real-time dataset is selected for the analysis of PD in this work.
2. A detailed review of the literature is accomplished to find further directions.
3. Various pattern recognition methods are analyzed to determine a suitable method that can recognize PD activities on IOC's caused by phase-to-phase, phase-to-ground fault.
4. The thesis investigates specific identified Deep Learning algorithms to enhance the performance of the PD recognition system.
5. With the analysis of various Deep Learning algorithms' performance, a suitable framework to enhance the performance of PD recognition is obtained.

CONCLUSION

This paper examines about the recent developments made for the automated identification of fault in Transmission power line using the recent advancements in the field of Deep Learning (DL). A literature survey on all three tasks, identification of faults, classification, and location is also analysed. Considering the characteristics, inputs, difficulty,

used system, performance, and need for the study is also discussed. The objectives and scope of the work is also proposed. Which provides an overview of the various contemporary methodologies and recently developed approaches and their similarities utilized to identify, classify, and locate the fault in the transmission power line.

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Table 1: Strengths and Weaknesses of various emerging Computational Intelligence Methods. [6]

| Technique	Strength	Weakness
ANN Technique	ANN is good in determining the exact fault-type and easy to implement. It is easy, with the adjustment of only a few parameters. It has a lot of applications in real-life problems. ANN learns and no need for reprogramming.	The training process is quite complex for high-dimension problems. A local optimum solution is provided by the gradient-based back-propagation technique for non-linear separable pattern classification problem. ANN offers slow convergence in the BP algorithm. Convergence is dependent on the selection of the initial value of weight constraints connected to the network.
PNN Technique	The learning process is not required. Determination of initial weights of the network is not needed. No correlation of the recalling process and learning process. Convergence in Bayesian classifier is certain. PNN show fast learning time.	It requires high processing time for large networks. Not easy to determine how many layers and neurons are required. Large memory space is required to save the model.
Fuzzy Methods	Simple 'if-then' relation is used to solve uncertainty problems.	No robustness is observed. Experts are mandatory in order to determine membership function and fuzzy rules, for large training data.
SVM Technique	SVM is a highly accurate approach. SVM works quite well even for non-linearly separable data in base feature space. The probability of misclassification is very low. To reduce error bound, it maximizes the margin. Upper bound error does not affect the space dimension.	Demands for more size and speed for the testing and training. Complexity is high in classification and thus large memory is required.
Decision Tree	Easy interpretation and understanding. Compatible with other available decision methods. Rules can be set easily.	When high uncertainty or several outcomes are involved, calculations become very complex. DT may suffer from over-fitting. Information gain in DTs is biased in favour of those features which have more levels.
Modal Transform	It is not dependent on electrical values and frequency. The single transformation matrix is for thethree-phase system (identical for current and voltages). Transposition and non-transposition of electrical values are done by simple multiplication of matrices. No convolution methods are required.	Modal parameters are required. Not reliable for complex structures.
Deep Learning	Best-in-class performance on problems that significantly outperforms other solutions in multiple domains. This is not by a little bit, but by a significant amount. DL reduces the need for feature engineering, one of the most time-consuming parts of machine learning practice. It is an architecture that can be adapted to new problems relatively with ease, e.g., time series, languages, etc., are using techniques like convolutional neural networks, recurrent neural networks, long short-term memory, etc	A large amount of data is required. DL is computationally expensive to train and takes weeks to train via hundreds of machines equipped with expensive graphical processing units (GPUs). Determining the topology/training method for DL is a black art with no theory.

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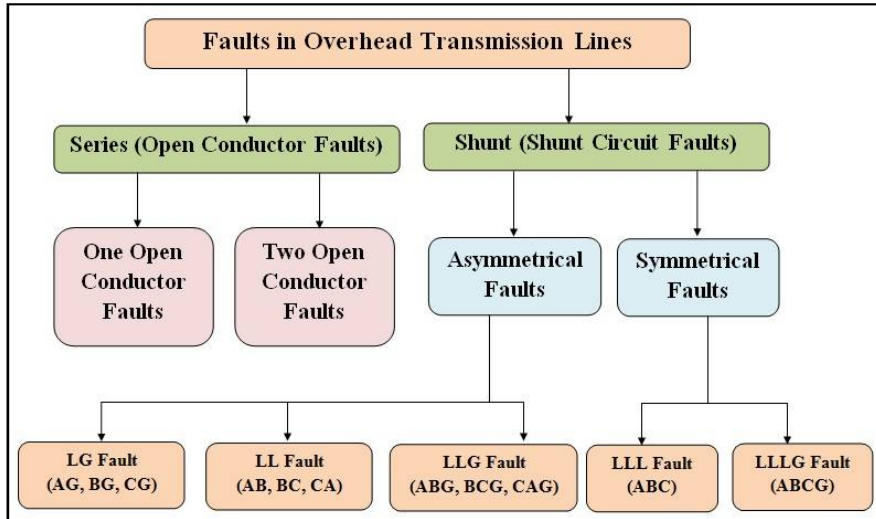
Figure 1 Types of faults occur in three-phase power transmission systems.
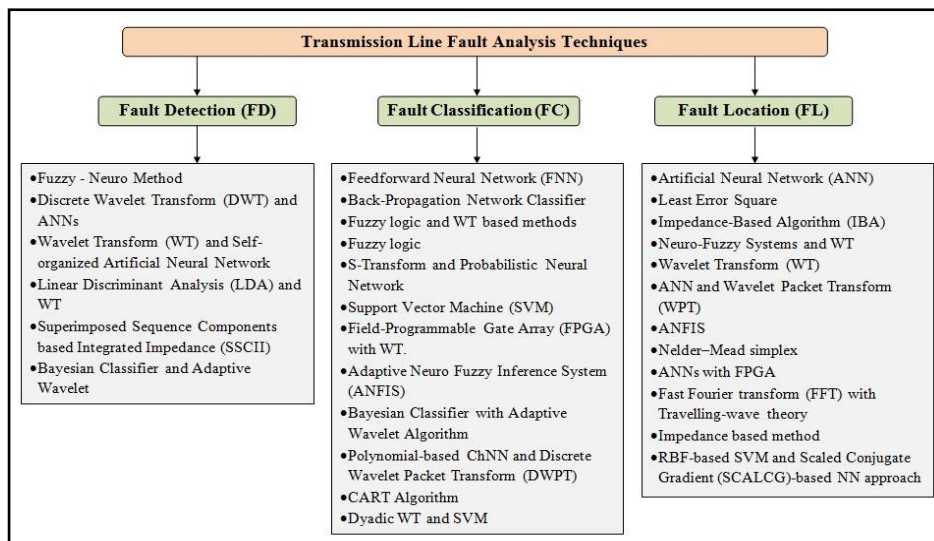

Figure 2 Tree diagram of the existing techniques in Transmission Line Fault Analysis