Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Antimicrobial Activities of *Terminalia catappa* Leaves' Extracts against Bacterial and Fungal Pathogens

G. Muthulakshmi* and P. Neelanarayanan

PG and Research, Department of Zoology, Nehru Memorial College (Autonomous and Affiliated to Bharathidasan University), Puthanampatti, Tiruchirappalli, Tamil Nadu, India.

Received: 12 Nov 2020 Revised: 24 Nov 2020 Accepted: 17 Dec 2020

***Address for Correspondence**
G. Muthulakshmi
PG and Research, Department of Zoology,
Nehru Memorial College (Autonomous and Affiliated to Bharathidasan University),
Puthanampatti, Tiruchirappalli, Tamil Nadu, India.
Email: lakshmikannanur@gmail.com

ABSTRACT

In the present study an attempt was made to identify the presence of phytochemicals in the Aqueous, Ethanol, Methanol and Petroleum ether extracts of leaves' extracts of *Terminalia catappa*. The leaves extracts were studied qualitatively to ascertain the presence of phytochemicals such as phenols, alkaloids, terpenoids, saponins, flavonoids, tannins, carbohydrates, glycosides, oils, proteins, resins and amino acids by adopting standard methods. In this study antibacterial and antifungal activity of leaves extracts of *T.catappa* were tested against five pathogenic bacterial strains such as *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumoniae* and *Salmonella paratyphi A.* by agar well diffusion method. *T.catappa* were tested against five pathogenic fungal strains such as *Candida albicans, Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. The methanol leaves extracts of *T.catappa* demonstrated higher antibacterial activities against *Klebsiella pneumonia* (29±0.57µl) in 500 µl concentration. A higher antibacterial effect was observed with the methanol leaves extract with an inhibitory halo of *Aspergillus flavus* (28.67±0.66µl) in 500 µl concentration.

Keywords: *Terminalia catappa,* Phytochemical screening, Antibacterial activity, Antifungal activity.

INTRODUCTION

Traditional medicine has been in practice worldwide for centuries, particularly the application of herbal plants for therapeutic purposes. Philippines is endowed with a rich source of medicinal plants, although not as extensive as India or China, enough to provide us with alternative remedies [1]. Traditional medicine has been used in several countries over the years, due to its low cost and high efficacy for certain bacterial diseases [2]. The huge number of medicinal plants has been investigated worldwide by scientists for their biological activities. Most scientists are

interested to find out the naturally occurring new drugs with significant medicinal values from nature without side effects for the treatment of diseases. The plant extracts contain several chemical compounds that are responsible for biological activities. The selected medicinal plant species showed several biological activities like antioxidant, antimicrobial, anticandidal, antidiabetic, antifungal, and antidiarrheal activities [3-4] and the plant is used traditionally for the treatment of different diseases. The use of medicinal plants to cure specific diseases has been in practice since ancient times. *Ayurveda, Siddha* and *Unani* systems of medicines have been in existence in India for several centuries. These systems of medicine fulfill the need of nearly seventy percent of the human population residing in the villages. New drugs from plant origin with scientific validation are boon to mankind to cure various microbial diseases. The investigations on the efficacy of plant-based drugs have been paid immense attention because of their very less side effects, cheap and easy availability [5]. According to the WHO, approximately 80% of the world population rely on plants or derived products for their treatment [6]. Medicinal plants are considered as important sources of new chemical substances with potential therapeutic effects [7].

Medicinal plants possess a rich source of antimicrobial agents among them. Plant origin chemicals with possible antimicrobial activities need to be tested scientifically against some pathogenic microbes to confirm their effectiveness. Infectious diseases represent a critical problem to the health and wellbeing of organisms and they are one of the main causes of morbidity and mortality worldwide. These microbial organisms can cause serious diseases in plants, animals, and humans. The conventional drug therapy currently available for the treatment of diseases caused by microorganisms has limitations such as the high rate of resistance besides numerous side effects [8]. The decoction of *T. catappa* leaves' extracts contains the number of medicinally important Phyto-constituents such as alkaloids, flavonoids, tannins, saponins and glycosides which showed antimicrobial properties [9]. *T. catappa* belongs to the family Combretaceae and it is a large deciduous tree naturally occurring and widespread in the subtropical and tropical zones of the Indian and Pacific Oceans. It is also planted extensively in many countries as an ornamental tree. Most of the research works carried out on *T. catappa* has mainly focused on biological and phytochemical studies of leaves, bark, and whole fruit extracts as a database for medicinal benefits, not in the realm of food application [10- 14]. *T. catappa* leaves contain several bioactive compounds responsible for antiviral, antibacterial, antifungal and anticancerous activities [15].Therefore, in the present investigation, an attempt was made to study the phytochemical constituents of the plant *T.catappa* by using antibacterial and antifungal activities of the extract.

MATERIALS AND METHODS

T. catappa leaves were collected from Kannanur, Tiruchirappalli District and Tamil Nadu and subsequently, they were authenticated at St. Joseph's College (Autonomous), Tiruchirappalli, Tamil Nadu. The air-dried leaves of *T. catappa* (150g) were utilized for aqueous, ethanol, methanol and petroleum ether respectively for extraction by using Soxhlet apparatus for 800 ml of solvent 24 to 48 hrs. Later, each extract was transferred to airtight bottles, labelled and stored at 4^{o}C until further analysis was performed.

Preliminary phytochemical analysis

Preliminary phytochemical screening was performed in all the four extracts, individually, to identify the phyto-chemical constituent's *viz.*, alkaloids, phenols, flavonoids, steroids, terpenoids, saponins, tannins, proteins, carbohydrates, glycosides, gums, oils, resins, and amino acids by adopting standard protocols [16].

Microorganisms used

The microorganisms used in this study were obtained from K.A.P. Viswanatham Medical College, Tiruchirappalli and Tamil Nadu. The bacterial strains used for the study were *Staphylococcus aureus, Proteus mirabilis, Pseudomonas aeruginosa, Klebsiella pneumonia* and *Salmonella paratyphi A.* The fungal strains used for the study were *Candida albicans*, *Aspergillus niger, Aspergillus flavus* and *Aspergillus fumigatus*. All these microbial isolates were subculture and utilized for antimicrobial assessment using leave's extracts individually.

Minimum Inhibitory Concentration (MIC)

***In vitro* antibacterial activity**

The leaves' extracts of aqueous, ethanol, methanol and petroleum ether were evaluated for their antibacterial activity against the chosen pathogenic microbial strains. The agar dilution method was performed using Muller-Hinton agar (Hi-Media). Suspension of each microorganism was made and applied to plates with serially diluted compounds to be tested and incubated for 24 h at 37°C. The compounds were tested at concentrations of 100, 250 and 500 µl. Chloramphenicol was used as a reference standard. The zone of inhibition developed surrounding the discs was measured and recorded in millimeter diameter [17].

***In vitro* antifungal activity**

The leaves' extracts of aqueous, ethanol, methanol and petroleum ether were evaluated for their *in vitro* antifungal activities against the selected pathogenic fungi using agar diffusion method with Saburoud's dextrose agar (Hi-Media) medium. Suspension of each fungal pathogen was prepared and applied to agar plates with serially diluted compounds to be tested and incubated for 26 °C for 72 h. The compounds were tested at three concentrations such as 100, 250 and 500 µl. Nystatin was used as a reference standard. The zone of inhibition developed surrounding the discs was measured and recorded in millimeter diameter [18]. All these experiments were performed in triplicates. The inhibition zone data were expressed as mean ± standard error.

RESULTS AND DISCUSSION

The preliminary phytochemical screening was done for aqueous, ethanol, methanol and petroleum ether leaves' extracts of *T. catappa* and the results thereof are given in Table 1. Among the four types of methanol leaves' extracts of *T. catappa* had a maximum of twelve phytoconstituents like alkaloids, phenols, flavonoids, steroids, terpenoids, tannins, proteins, carbohydrates, glycosides, oils, resins, and amino acids. These phytochemicals are known to be biologically active. Babayi *et al* [19] reported the presence of these components and in addition saponins, glycosides and phenols in *T.catappa*. The *T. catappa* leaves have been reported to be maturate and emollient, used in the treatment of wound and ulcerations, the bark rich in tannins, the fruits rich in ascorbic acid and seeds contain oil [20].In the present study antibacterial activities of aqueous, ethanol, methanol and petroleum ether extracts of *T. catappa* was evaluated against five bacterial pathogens such as *S.aureus, P. mirabilis, P. aeruginosa, K. pneumoniae,* and *S. paratyphi A.* Four different concentration (100µl, 250µl, 500 µl and control) of the test drugs were chosen. Of all the antimicrobial tests the highest antibacterial activity was observed with aqueous, ethanol, methanol and petroleum ether extracts of *T. catappa* against *K. pneumonia* (27±1.00, 28.67±0.66, 29±0.57,25.67±0.33) in 500 µl concentrations, respectively(Table 2-5 and Fig.1).

Among these, maximum zone of inhibition (29±0.57mm) was obtained against *K. pneumoniae*. Therefore, the methanol extract has been selected for investigating antimicrobial activity. The inhibitory effects of this medicinal plants on the microorganisms may, therefore be due to the presence of phytochemical components alkaloids, phenols, flavonoids and tannins. Though the leaf extracts showed limited activity against all the organisms, marked activity was exhibited against *K. pneumonia* and *S. aureus*. Similar low activity was also reported Babayi *et al,* [19]. They indicated that *S. paratyphi A, P. aeruginosa* and *P. mirabilis* were susceptible to methanolic extract of *T.catappa* . The antibacterial potential of the leaves extract of *T. catappa* has been elucidated by the result of this study, which could explain its traditional usage for treating bacterial diseases [21]. Therefore there is a need to develop alternative antimicrobial drugs for the treatment of infections using medicinal plants [22-23]. Similarly, Aqueous, ethanol, methanol and petroleum ether extracts of *T. catappa* was evaluated against four fungal pathogens such as *C. albicans, A. niger, A. flavus* and *A. fumigatus.* of all the highest antifungal activity was observed with aqueous, ethanol, methanol and petroleum ether extracts of *T. catappa* against *A. flavus* (18±0.58, 21±0.58, 28.67±0.66, 20.67±0.67) at500 µl concentration (Tables 6-9 and Fig. 2). A Maximum of 28.67±0.66mm zone of inhibition was obtained against *A. flavus*.

In general, the performance of antifungal activities, particularly at 500 µl, was found to be higher than the control. The results of these studies have shown that methanol extract of *T. catappa* has good antifungal activity. The earlier work on methanolic leaf extracts of *T. catappa* has also reported the presence of these components [19, 24]. Several studies have reported the presence of bioactive compounds that are responsible for the medicinal properties of the plant that is used for the treatment of different ailments. However, methanol extract of plants tested against all five fungi showed significant antifungal activity. Similarly, Xie, *et al* [25] reported that flavonoids are known antibacterial agents against a wide range of pathogenic bacteria. Also Fu *et al* [26] also revealed that phenolic extracts from some plants also have antibacterial effects against many kinds of bacteria. Some of the phytochemical compounds *e.g.* glycosides, saponins, tannins, flavonoids, terpenoids, alkaloids have variously been reported to have antimicrobial activity [27]. In this present study, methanol extracts of *T. catappa* leaves showed good inhibition against all the tested pathogens. Similar kind of report also given by Goun *et al* [28] and Babayi *et al* [19] However, Sumitra *et al* [29] showed that the extracts of *T. catappa* are more active against the gram negative organisms than the gram positive.

CONCLUSION

Leaves' extracts of *T. catappa* used in the present investigation contain numerous bioactive compounds that are responsible for their antimicrobial properties. The extracts have shown dose dependent as well as dose independent growth inhibition against different selected bacterial and fungal strains which can be justified the use of this plant in traditional medicine for the treatment of infections. However, further studies are required to identify the exact compounds in the extracts and to understand the exact mechanism of action against the chosen microorganisms.

ACKNOWLEDGEMENT

We are thankful to UGC-RGNF (F1-17.1/2016- 17/RGNF-2015-17-SC-TAM-27784 / (SA-III/Website) for extending financial assistance to do this work. The authors are thankful to the Management and Principal of our college for the facilities extended for doing this research work. We thank the Staff members of Zoology Department, Nehru Memorial College (Autonomous) for their help.

CONFLICT OF INTEREST

We declare that we have no conflict of interest

REFERENCES

1. Quisumbing E. Medicinal plants of the Philippines. Quezon: Katha Publishing Co., Inc:1978.
2. Pamo Reyna OG. Characteristics of original papers about plants properties in Peruvian medical journals. Rev Peru Med ExpSaludPublica 2009; 26(3): 314-23.
3. Hussain J, Rehman NU, Al-Harrasi A, Ali L, Khan AL, Albroumi MA et al. Essential oil composition and nutrient analysis of selected medicinal plants in Sultanate of Oman. Asian Pac J Trop Dis 2013;3(6):421–8.
4. Rani MS, Pippalla RS, Mohan K. *Dodonaea viscose* Linn an overview. Asian J Pharm Res Health Care2009;1(1):97–112.
5. Owolabi J, Omogbai EKI, Obasuyi O. Antifungal and antibacterial activities of the ethanolic and aqueous extract of *Kigelia africana* (Bignoniaceae) stem bark. Afr.J. Biotechnol2007; 6: 882-885.
6. World Health Organization. Summary of WHO guidelines for assessment of herbal medicines. Herbal Gram 1993; 28: 13-14.
7. Blumenthal M. Herbal medicines: expanded commission E monographys .*Boston:* Integrative Medicine Communications 2000; 419-23.

8. Megraud F, Coenen S, Versporten A, Kist M, Lopez-Brea M, Hirschl AM, Andersen LP, Goossens H, Glupczynski Y, et al. Helicobacter pylori resistance to antibiotics in Europe and its relationship to antibiotic consumption. *Gut*2013; 62:34–42.
9. Ahmad I, Mehmood Z, Mohammad F. Screening of some Indian medicinal plants for their antimicrobial properties. *Journal of Ethnopharmacol* 1998; 62(2): 183-193.
10. Lin CC, Chen YL, Lin JM. Evaluation of the antioxidant and hepatoprotective activity of *Terminalia catappa*. *Am J Chin Med* 1997; 25:153-161.
11. Masuda T, Yonemori S, Oyama Y. Evaluation of the antioxidant activity of environmental plants: activity of the leaf extracts from seashore plants.*J Agric Food Chem* 1999; 47: 1749-54.
12. Lin CC, Hsu YF, Lin TC. Effects of punicalagin and punicalin on carrageen an-induced inflammation in rats. *Am J Chin Med* 1999; 27: 371-376.
13. Chen PS, Li JH, Liu TY. Folk medicine *Terminalia catappa* and its major tannin component, punicalagin, are effective against bleomycin-induced genotoxicity in Chinese hamster ovary cells. *Cancer Lett* 2000;152: 115-22.
14. Fan YM, Xu LZ, Gao J. Phytochemical and anti-inflammatory studies on *Terminalia catappa. Fitoterapia* 2004;75: 253-60.
15. Pati D, Halle P, Bade A. Determination of anthelmintic activity of *Terminalia catappa* extract (red leaves). *World J Pharm Pharm Sci* 2014;4:615–21.
16. Wadood A, Ghufran M, Jamal SB, Naeem M, Khan A, Ghaffar R et al. Phytochemical analysis of medicinal plants occurring in local area of Mardan. *Biochem Anal Biochem* 2013; 2: 144.
17. Vlietinck AJ, Van N, Hoof L, Tott J. Screening of hundred Rwandese medicinal plants for antimicrobial and antiviral properties. *J Ethnopaharmacol*1995; 46: 31-47.
18. NCCLS (2002). Reference method for broth dilution antifungal susceptibility testing of filamentous fungi. Approved standard M38-A. Wayne, Pa: National Committee for Clinical Laboratory Standards.
19. Babayi H, Kolo I, Okogun JI, Ijah, U.J. J. The antimicrobial activities of methanolic extracts of *Eucalyptus camaldulensis* and *Terminalia catappa* against some pathogenic microorganisms. Biokemistri(2004); 16 (2):106-111.
20. Nair R, Sumitra C. Antimicrobial activity of *Terminalia catappa, Manilkara zapota* and *Piper betel* leaf extract. *Indian J. Pharm Sci* 2008;70 (3): 390-393.
21. Baratelli TG, Candido Gomes AC, Wessjohann LA, Kuster RM, Simas NK. Phytochemical and allelopathic studies of *Terminalia catappa* L. (Combretaceae). *Biochemical Systematics and Ecology*2012; 41:119-25.
22. Cordell GA. Biodiversity and Drug Discovery a Sym-biotic Relationship .*Phytochemistry*, Vol. 55, No. 66, 2000, pp. 463-480. doi:10.1016/S0031-9422(00)00230-2
23. Dulger B, Gonuz A. Antibacterial Activity of the Endemic *Hypericumkazdaghensis*. *Fitoterapia* 2005; 76(2): 237-239. doi:10.1016/j.fitote.2004.12.010
24. Akharaiyi FC, Ilori RM, Adesida JA. Antibacterial effect of *Terminalia catappa* on some selected pathogenic bacteria.*Intjr. Pharm Biomed Res* 2011; 2 (2): 64-67.
25. Xie Y, Yang W, Tang F. Antibacterial activities of flavonoids: structure activity relationship and mechanism. *Curr Med Chem*2015; 22(1): 132–49.
26. Fu L, Lu W, Zhou X. Phenolic compounds and in vitro antibacterial and antioxidant activities of Three Tropic Fruits: Persimmon, Guava, and Sweetsop. *Bio Med research international* 2016:4287461.
27. okeke MI, Iroegbu CU, Eze EN, Okoli AS, Esimone CO. Evaluation of extracts the roots of *Landolphia owerrience* for antibacterial activity.*J Ethanopharmacol* 2001;78:119-27.
28. Goun E, Cunningham G, Chu D, Nguyen C, Miles D. Antibacterial and antifungal activity of Indonesian ethno medical plants .*Fitoterapia* 2003; 74(6): 592-596.
29. Sumitra C, Kalpna R, Ratish N. Antimicrobial Activity of *Terminalia catappa* L leaf extracts against some clinically important pathogenic stains. *Chinese Med*2011; 2: 171-177.

Table 1: Phytochemical screening of the leaves' extracts of *T. catappa*

S. No.	Plant constituent	Aqueous	Ethanol	Methanol	Petroleum ether
1	Alkaloids	+	+	+	+
2	Phenols	+	+	+	+
3	Flavonoids	+	+	+	+
4	Steroids	-	-	+	-
5	Terpenoids	+	-	+	-
6	Saponins	-	-	-	-
7	Carbohydrates	+	+	+	-
8	Proteins	+	+	+	-
9	Tannins	+	+	+	+
10	Oils	+	+	+	-
11	Resins	+	+	+	+
12	Glycosides	-	+	+	-
13	Gums	-	-	-	-
14	Aminoacids	+	+	+	+

(+) = Denotes presence of compound, (-) = Denotes absence of compound

Table: 2 Magnitude of zone of inhibition observed by using three different concentrations of aqueous leaves' extracts of *T. catappa* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean±S.E.			
	100µl	250 µl	500 µl	Control
S. aureus	20.67±0.66	24.33±0.33	25.33±0.33	24±0.00
P. mirabilis	17.67±0.33	16.67±0.66	24.33±0.33	26±0.00
P. aeruginosa	11.67±0.33	16±0.58	20.67±0.67	20±0.00
K. pneumonia	19±0.57	23±0.58	27±1.00	23.67±0.33
S. paratyphi A	10.67±0.33	14.33±0.33	17.67±0.33	20±0.00

Mean ± Standard Error values were obtained from triplicate observations.

Table: 3Propensity of zone of inhibition recorded by using three different concentrations of ethanol leaves' extracts of *T. catappa* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean±S.E.			
	100µl	250 µl	500 µl	Control
S. aureus	18±1.16	18±1.16	21±0.58	25.33±0.33
P. mirabilis	18.67±0.67	17.33±1.33	25.33±0.33	24±0.00
P. aeruginosa	12±0.58	16±0.58	18.67±0.33	20±0.00
K. pneumonia	21±0.58	23±0.58	28.67±0.66	31.33±0.68
S. paratyphi A	15±0.58	12±1.16	20.67±0.33	20±0.00

Mean±Standard Error values were obtained from three observations.

Table: 4 Extent of zone of inhibition recorded by using three different concentrations of methanol leaves' extracts of *T. catappa* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean±S.E.			
	100µl	250 µl	500 µl	Control
S. aureus	19±0.58	23±0.58	25.33±0.33	24±0.00
P. mirabilis	17.33±0.33	19±0.58	25.67±0.33	24±0.00
P. aeruginosa	11.33±0.67	18±0.58	21.33±0.67	30±1.16
K. pneumonia	19±0.58	24±0.58	29±0.57	30±0.00
S. paratyphi A	16.33±0.33	14±1.00	19±0.58	20±0.00

Mean ± Standard Error values were obtained from triplicates observation.

Table: 5 Degree of zone of inhibition recorded by using three different concentrations of petroleum ether leaves' extracts of *T. catappa* and a known antibiotic (control) against five bacterial pathogens.

Organisms	Zone of inhibition (mm) Mean±S.E.			
	100µl	250 µl	500 µl	Control
S. aureus	19±0.58	23±0.57	24.33±0.00	24±0.00
P. mirabilis	15.67±0.33	17.67±0.33	20.67±0.33	27.33±0.33
P. aeruginosa	12±0.00	16±0.57	18.67±0.33	20±0.00
K. pneumonia	20.33±0.33	24.33±0.33	25.67±0.33	27.33±0.33
S. paratyphi A	15.67±0.33	17.67±0.33	19±0.58	19.33±0.67

Mean ± Standard Error values were obtained from triplicate observations.

Table: 6 Extent of zone of inhibition recorded by using three different concentrations of aqueous leaves' extracts of *T. catappa* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± S.E.			
	100µl	250 µl	500 µl	Control
C. albicans	10±0.58	12.33±0.33	14.33±0.33	9.67±0.33
A. niger	9.67±0.33	10±0.58	12.33±0.33	14.67±0.33
A. flavus	9±0.58	16±0.58	18±0.58	25.67±0.67
A. fumigatus	10±0.58	12.33±0.33	15±0.58	10.33±0.33

Mean ± Standard Error values were obtained from three observations.

Table: 7 Magnitude of zone of inhibition observed by using three different concentrations of ethanol leaves' extracts of *T. catappa* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± S.E.			
	100µl	250 µl	500 µl	Control
C. albicans	8.33±0.33	10.67±0.67	14±0.58	18.33±0.33
A. niger	11±0.58	12.67±0.67	15.67±0.33	11.67±0.33
A. flavus	10±0.58	17.67±0.33	21±0.58	22.33±0.33
A. fumigatus	9.67±0.88	12.33±0.33	15.33±0.33	10.33±0.33

Mean ± Standard Error values were obtained from three observations.

Table: 8 Degree of zone of inhibition recorded by using three different concentrations of methanol leaves' extracts of *T. catappa* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± S.E.			
	100µl	250 µl	500 µl	Control
C. albicans	14.33±0.67	18.33±0.33	23.67±0.33	9.67±0.33
A. niger	11.67±0.33	14.33±0.67	21±0.58	12±0.00
A. flavus	12±0.58	23±0.58	28.67±0.66	25±0.00
A. fumigatus	10.67±0.67	20.33±0.33	24.33±0.67	11.67±0.33

Mean ± Standard Error values were obtained from triplicates.

Table: 9 Propensity of zone of inhibition recorded by using three different concentrations of petroleum ether leaves' extracts of *T. catappa* and a known antibiotic (control) against four fungal pathogens.

Organisms	Zone of inhibition (cm) Mean ± S.E.			
	100µl	250 µl	500 µl	Control
C. albicans	-	9.33±0.67	12.33±0.33	14.33±0.33
A. niger	10±0.58	12±0.58	14.33±0.33	10.67±0.33
A. flavus	15±0.58	18.33±0.88	20.67±0.67	17.33±0.33
A. fumigatus	9.67±0.88	10.33±0.33	12.67±0.33	9.67±0.67

Mean ± Standard Error values were obtained from three observations.

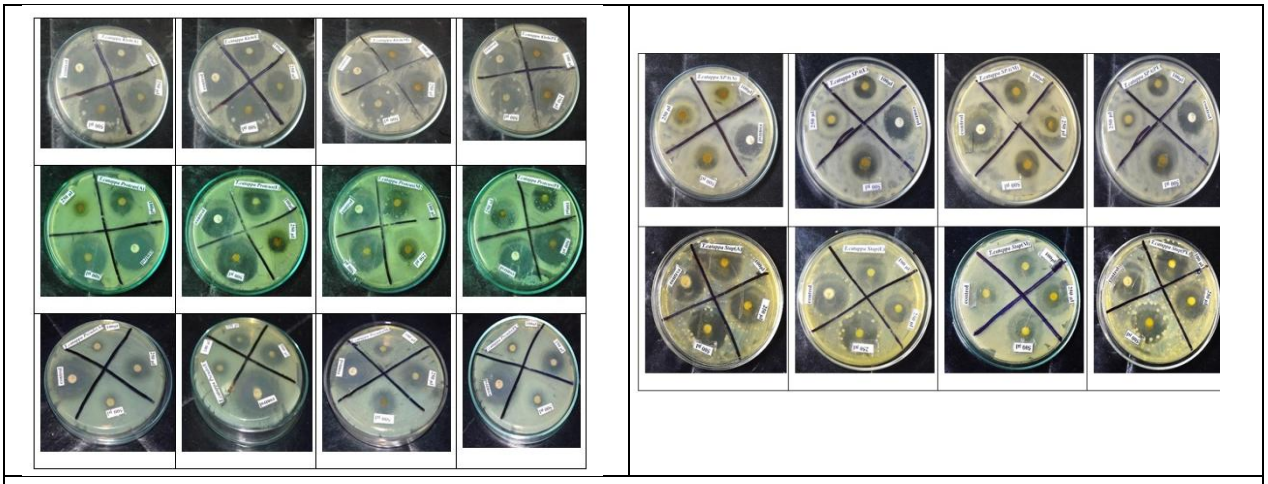


Fig.1.Disc Diffusion Assay results of (A) Aqueous, (E) Ethanol, (M) Methanol and (PE) Petroleum Ether leaves' extracts of *T. catappa* and a known antibiotic (control) against five bacterial pathogens.

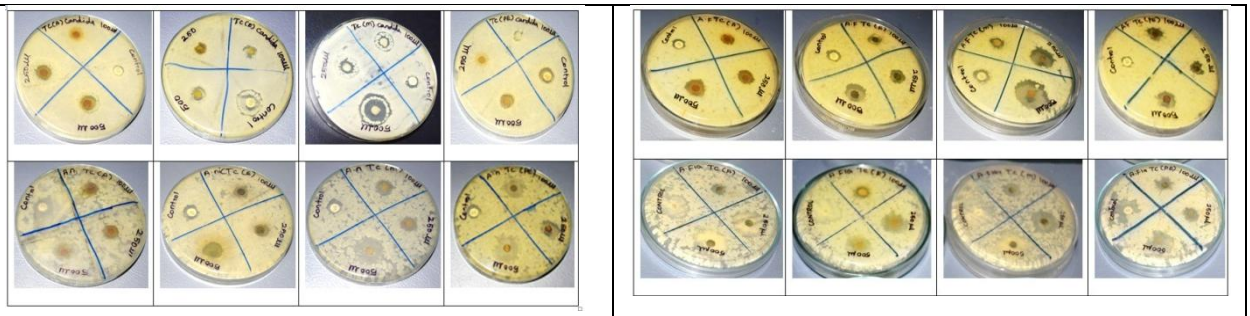


Fig. 2. Disc Diffusion Assay results of (A) Aqueous, (E) Ethanol, (M) Methanol and (PE) Petroleum Ether leaves' extracts of *T. catappa* and a known antibiotic (control) against four fungal pathogens.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Species of the Genus *Cladonia* P. Browne in the Lichen Flora of Azerbaijan

Sevda Alverdiyeva*

Institute of Botany of Azerbaijan National Academy of Sciences, 40 Badamdar hwy., Baku, AZ1004, Republic of Azerbaijan

Received: 18 Dec 2020 Revised: 25 Dec 2020 Accepted: 29 Dec 2020

***Address for Correspondence**
Sevda Alverdiyeva
Institute of Botany of Azerbaijan National Academy of Sciences,
40 Badamdar hwy., Baku, AZ1004,
Republic of Azerbaijan
Email: sevdaalv@gmail.com

ABSTRACT

The purpose of the work was to specify and update the species composition of the lichens of the genus *Cladonia* found in the territory of Azerbaijan. Most of the *Cladonia* species collected in the study region are widespread species mainly in the taiga zone, but also found in broadleaved forests. Based on all the excising in-hand lichenological material, it has been determined 56 taxa, where five of species are new for the lichen flora of Azerbaijan: *Cladonia caespiticia* (Pers.) Flörke, *C. carneola* (Fr.) Fr., *C.glauca* Flörke, *C.grayi* G.Merr. ex Sandst. and *C. macroceras*. (Delise) Hav. The developed list of taxa contains information for each species indicating their dissemination area, location, substrata and literature sources.

Keywords: lichens, flora, diversity, *Cladonia*, Azerbaijan

INTRODUCTION

Lichens of the genus *Cladonia* are widespread in all the vegetable climate zones ranging from polar deserts to tropics. On the globe it is known over 400 species [1], where of over 100 species are known in Russia, about 60 species in the Caucasus [2]. In the lichen flora of Azerbaijan, the genus *Cladonia* comprises 51 species and is one of the leading lichen genera in the region as a whole. In the study area, they grow on sandy and limestone soil, on plant residues, on the base of tree trunks, mossy rocks, on rotten wood in wet and dry habitats. Most of the species are vegetating in the deciduous forests of Azerbaijan. Specimens of original collections and literary sources were used as the material for this article. Identification of species of the genus *Cladonia* was held according to the common methodology [3]. And some questionable specimens were analyzed by the thin-layer chromatography method (TLC) by Finnish lichenologist Prof. Teuvo Ahti. At the present time a collection of 770 specimens of lichens of the genus *Cladonia* is preserved in the lichen bryological herbarium of the Institute of Botany of Azerbaijan National Academy of Sciences

(BAK). And duplicates of some specimens were given and stored at the Herbarium of Botanical Museum of University of Helsinki (Finland) (H). Our researches revealed that the genus *Cladonia* is one of the leading genera in the lichen flora of Azerbaijan, ranking the first in the number of species (6.04%) from the total number of species. This article gives an account of 56 taxa of the genus *Cladonia* which was collected in the territory of four large physical and geographic regions of the country (Azerbaijani part of the Greater Caucasus, Minor Caucasus, Talish and Kura-Araz Lowland). Most of them belong to fruticose lichens by their morphological characters. A lot of species of this genus are common in temperate and northern zones of the studied area. The greatest species diversity of *Cladonia* was observed in deciduous forests growing on forest soil. They were marked also on mossy rocks - *Cladonia strepsilis*, *C. decorticata*; on rotting wood, rotting stumps - *C. pyxidata*, *C.ochrochlora*; on soil among moss - *C.pocillum*, *C. crispata*; at the base of tree trunks - *C. digitata*. On the territory of Azerbaijan *Cladonia* grow both in the lowlands - *Cladonia foliacea*, *C. rangiformis*, and in the highlands - *C. borealis*, *C. fimbriata*. Most of the species of this genus are found in the mid-mountain zone. As an example, 13 species of the genus *Cladonia* identified only in the Minor Caucasus (within Azerbaijan); 3 species - *C. borealis, C. parasitica, C. symphycarpa* only in the Greater Caucasus; and 2 species - *C. bacilliformis* and *C. macilenta* only in Talish. Rare species on one-two locations: *C. acuminata*, *C. cenotea*, *C. digitata*, *C. farinacea*, *C. mitis* were also identified on the researched area. One species - *Cladonia* strepsilis is listed in the Red Book of Azerbaijan [4]. When auditing the collection of *Cladonias* stored in the herbarium fund (BAK) Finnish lichenologist T. Ahti identified the species *Cladonia gracilis* (L.) Willd. subsp. *turbinata* (Ach.) Ahti, which previously was identified as *C. gracilis* (L.) Willd. Since the mention of this species on the territory of Azerbaijan [5], is incorrect, then its habitat in the republic should be considered invalid and it should be excluded from the previously cited lists of lichen species.

List of taxa of the genus *Cladonia*

The list of taxa was compiled based on examination of specimens of original collections and literary data. Names of taxa are given according to the "List of lichen flora of Russia" [2]. The species are arranged in alphabetical order. Each species is provided with the data on its location (place of collection, literary source, collector and substrata). Following designations were used: GC — Greater Caucasus, MC — Minor Caucasus, K.-Ar.L.— Kura-Araz Lowland.

1. *Cladonia acuminata* (Ach.) Norrl. – MC: Nakhchivan, on soil [6].
2. *C. alpina* (Asahina) Yoshim. – MC: Tovuz and Gadabay regions, on soil [7].
3. *C. arbuscula* (Wallr.) Flot. – GC: Zagatala region, territory of the State Preserve, 1400-1600m [5], Shabran region, vicinity of fortress Chiraggala, 1232 m [5]; MC: Nakhchivan: Julfa, Babek regions, on soil, rotting stubs [6].
4. *C. bacilliformis* (Nyl.) Glück – Talish: Astara region, on rotting stubs [8].
5. *C. borelalis* S. Stenroos – GC: Zagatala region, along the road, on rocks [9].
6. *C. botrytes* (K.G. Hagen) Willd. – GC: Guba, Gusar regions, on moss- grown stones and rotting wood, 800-1700 m [5]; MC: Nakhchivan: Julfa, Sharur regions, on soil [6], Talish: Lankaran, Astara regions, on rotten stubs [8].
7. *C. caespiticia* (Pers.) Flörke - GC: Gusar region, in forest zone on rotting wood, S. Alverdiyeva, 10.07.2017, BAK. New to Azerbaijan.
   Thallus is horizontal, squamulose, apothecia to pale brown, arising directly from the surfaces of the squamules. Thallus from K and KC does not change in color, from P shows red.
8. *C. cariosa* (Ach.) Spreng.– MC: Nakhchivan: Shahbuz region [6], Talish: Yardimli region, on soil [10].
9. *C. carneola* (Fr.) Fr. - GC: Gusar region, on rotting wood, S. Alverdiyeva, 15.08.2018, BAK. New to Azerbaijan.
   Primary thallus squamulose, squamules, squamules 3-5mm in length, 1-3 mm in width. Color is light-green on top, white from below. Podetia are 3 cm in height, whitish-greyish, covered with powdery soredium. Apothecia are light-brown, along the edges of the scyphas. Thallus from K and P does not change in color, from KC shows yellow on decaying wood.
10. *C. cenotea* (Ach.) Schaer. – MC: Zangilan region, on bulges of roots [11].

11. *C. chlorophaaea* (Flörke ex Sommerf.) Spreng. – GC: Gusar region, on soil [8], Guba, Ismayilli, Gabala regions, on soil, among moss, on stony slopes 500-2000 m [5], Shaki region [8, 5]; MC: Tovuz region [8], Talish: Masalli, Yardimli region, 880 m, on rotten plant remnants [8].
12. *C. coccifera* (L.) Willd. – MC: Nakhchivan: Babek, Julfa, Sharur regions, on soil [6].
13. *C. coniocraea* (Flörke) Spreng. – GC: Gusar, Zagatala regions [8]; MC: Lachin, Kalbadjar, Goygol regions, on moss-covered earth and old stubs [8], Dashkasan region, on moss-grown cover [11], Talish: Astara, Lankaran, Yardimli regions, on moss-grown cover [8].
14. *C. cornuta* (L.) Hoffm. – GC: Zagatala region, in forest zone, on rotten wood, 2000-2700 m [5]; MC: Kalbadjar region, along the valley of the Terterchay river, on soil [8], Talish: Yardimli, Lerik region, on soil, 970-1957 m [8].
15. *C. crispata* (Ach.) Flot. – Talish: Astara region, in forest, on *Parrotia persica* (DC.) C.A. Mey [8].
16. *C. decorticata* (Flörke) Spreng.– MC: Kalbadjar region, on moss covered rocks [11].
17. *C. deformis* (L.) Hoffm. – GC: Guba, Zagatala regions, on soil, rotting wood, 1900-2100 m [5]; MC: Garayazi State Preserve [12], Dashkasan region, on soil [8].
18. *C. digitata* (L.) Hoffm. – GC: Guba region, on rotting stubs and at the base of tree trunks, 700-900 m [5].
19. *C. farinacea* (Vain.) A. Evans – MC: Goygol region, on soil [9].
20. *C. fimbriata* (L.) Fr. – GC: Gusar, Guba, Shamakhi, Gabala, Ismayilli regions, on soil, moss grown cover [8], Oguz, Zagatala, Balakan regions, 1000- 2400 m, on soil, rotting stubs [8,5]; MC: Gandja, Goygol region [8], Garayazi plain [12], Dashkasan region [11], Gadabay region [8,11], Kalbadjar, Khankandi, Shusha, Lachin, Khodjavand regions [8], Talish: Yardimli, Lankaran, Lerik, Astara regions, on soil and at the basis of tree trunks, stubs, moss-covered rocks [8].
21. *C. floerkeana* (Fr.) Flörke – MC: Shamkir region, on soil [13].
22. *C. foliacea* (Huds.) Willd.– GC: Gusar, Guba, Gabala, Oguz, Balakan region, on moss-grown cover of soil [8], Shaki region, on soil, in foothill and high-mountain steppes, 650-2500 m [8; 5], Zagatala region [8], Akhsu region, on soil [11], Shabran region, Absheron, Sumgait city, on limestone soil [8], Absheron: Gobustan, on soil [11]; MC: Tovuz region [8, 13], Gandja, Gazakh, Goygoln, Gadabay, region on soil [11], Kalbadjar, Khankandi, Shusha, Lachin, Gubadli, Zangilan region [8], Lachin, Khankandi, Shusha regions [13], Khodjavand region [11],Talish: Yardimli, Lerik region, on moss-grown cover, 700-2100 m [8]; Nakhchivan: Ordubad region, on sandy soil [6]; K-Ar.L.: Yevlakh region [11], Salyan, Shirvan, Agdash region [8], Mil steppe [14].
23. *C. foliacea* (Huds.) Willd *f. firma* (Nyl.) Vain. – MC: Tovuz region, on moss-grown cover [8].
24. *C. furcata* (Huds.) Schrad. – GC: Guba, Gusar, Zagatala regions, on soil [8], Balakan region, on moss covered rocks, 3200 m [5]; MC: Tovuz region [8], Gazakh, Agstafa regions [12], Dashkasan region, Khankandi region [11].
25. *C. glauca* Flörke - GC: Guba region, at the basis of trees, S. Alverdiyeva, 05.06.2016, BAK. New to Azerbaijan.
    Primary thallus squamulose, squamules up to 5 mm in length, whitish-greenish on top, white from below. Podetia are 3-5 cm in height, upright, pointed on the edges, covered with powdery soredium layer. Apothecia are brown, at the edges of podetia. Thallus from K, KC and P does not change in color.
26. *C. gracilis* subsp. *turbinata* (Ach.) Ahti – GC: Zagatala State Preserve, on rotting stubs; MC: Zangilan region, on bulges of roots [9].
27. *Cladonia grayi* G.Merr. ex Sandst. – GC: Guba region, on soil, S. Alverdiyeva, 05.06.2016, BAK. New to Azerbaijan.
    Primary thallus squamulose, squamules 2-6 mm in length, greenish-brown, white from below. Podetia are 0,5-4 cm in height, with scyphas. Apothecia are brown, along the edges of the scyphas. Thallus from K, KC and P does not change in color.
28. *C. lepidota* Nyl.– MC: Nakhchivan: Ordubad region, on moss-grown soil [6].
29. *C. macilenta* Hoffm. – Talish: Astara region, on rotting stubs [8].
30. *C. macroceras* (Delise) Hav. – MC: Gadabay region, on soil among moss, S. Alverdiyeva, 03.08.2017, BAK. New to Azerbaijan.
    Primary thallus squamulose, squamules 2-5 mm in diameter, glaucescent-greyish on top, white from below. Podetia are high, 3 cm in height, 2-3 mm in diameter, glaucous, with apices pointed. Apothecia are brown, along the edges of the scyphas. Thallome from K and KC does not change in color, from P shows red.

31. *C. macrophylla* (Schaer.) Stenh. – GC: Ismayilli, Shaki, Balakan regions, on soil and mossy stones, in mountains, 2500-3200 m [5]; MC: Nakhchivan: Babak, Kangarli regions, on soil [15].
32. *C. macrophyllodes* Nyl.– GC: Guba, Ismayilli regions, on limestone soil and on rocks, in mountains, 1600-2600 m [8], Shaki, Zagatala, Balakan regions, on soil and rocks [5]; MC: Nakhchivan: Ordubad region, on limestone soil [6].
33. *C. mitis* Sandst. – MC: Zangilan region, on moss-grown cover [9].
34. *C. ochrochlora* Flörke – GC: Gusar, Khachmaz, Gabala regions, on sandy soil and rotten stubs, 200-700 m [8]; MC: Goygol, Dashkasan regions [11], Gadabay region [11], Agdara, Kalbadjar regions [8], Lachin region [8,13], Talish: Yardimli, Lerik regions, on rotten stubs and soil, under 1900 m [10].
35. *C. parasitica* (Hoffm.) Hoffm. – GC: Guba region, on mossy bark of trees [5].
36. *C. peziziformis* (With.) J. R. Laundon – MC: Nakhchivan: Kangarli, Sharur regions, on soil [6].
37. *C. phyllophora* Hoffm. – MC: Nakhchivan: Shahbuz region, on soil [15].
38. *C. pleurota* (Flörke) Schaer. – MC: Nakhchivan: Shahbuz region, on soil. [6, 15].
39. *C. pocillum* (Ach.) Grognot – GC: Gusar, Guba, Shamakhi, Ismayilli, Gabala, Shaki regions [8]; MC: Shamkir, Tovuz regions [13], Goygol State Preserve [8], Kalbadjar, Agdara, Khodjavand, Khankandi, Shusha, Lachin region, on moss-grown cover of soil [11].
40. *C polycarpoides* Nyl.– GC: Gabala region, on soil [5], Balakan region, on sandy soil [8]; MC: Nakhchivan: Babek, Ordubad, Sharur regions [15].
41. *C. pyxidata* (L.) Hoffm. – GC: Zagatala, Shaki, Ismayilli, Guba, Gusar, Shamakhi, Gabala regions, on soil, rotten stubs, 500-3210 m [10, 5]; MC: Tovuz region [13], Gazakh region [7], Goygol region [8] Dashkasan, Khodjavand, Shusha regions, 1150-2700 m [10], Dashkasan, Kalbadjar regions [13], Lachin region [8], Nakhchivan: Ordubad region, on soil [8], Talish: Yardimli, Lankaran, Lerik regions, on rotten stubs and mossy cover, 1000-2000 m [10],
42. *C. pyxidata* (L.) Hoffm. f. *neglecta* (Flk.) A. Massal. – MC: Goygol, on soil [8].
43. *C. rangiferina* (L.) F.H. Wigg. – GC: Shaki region, Zagatala region, territory of the State Preserve, on soil, mossy rocks and on stubs, 2500-2800 [5]; MC: Shamkir region, on soil [7], Goygol State Preserve, on soil [10], the Kura river bank, riparian woodland, on soil [12], Nakhchivan: Julfa region, Ordubad region, on soil [15].
44. *C. rangiformis* Hoffm. – Southern slopes of the Greater Caucasus, Balakan region, on the ground, along the road to Akkmal mount, 1000-1500 m, Zagatala region, vicinity, on the ground, Punchilov, 2400 m, Gusar, Guba, Shabran, Gabala, Oguz, Shamakhi, Shaki, Zagatala, Balakan regions, on the ground [10]; MC: vicinity of Gandja city [8], Gazakh region [13], Agstafa region [12], Goygol region, vicinity of the Lake Goygol, Dashkasan region, along the bank of the Koshkarchay river, Gadabay region, 1570 m [10], Kalbadjar, Khodjavand, Khankandi, Shusha, Lachin regions, in forests [13], Gubadli, Zangilan region, in forest, on the ground [8, 13], Nakhchivan: Julfa region [6], Babak region [15], Talish: Yardimli, Lankaran, Lerik, Astara regions, on sandy and limestone soil, under 2100 m [10]; K-Ar.L.: Fizuli region [8].
45. *C. rangiformis* Hoffm. f. *variolosa* Sandst. – MC: Tovuz region, on the ground [8].
46. *C. rei* Schaer. – MC: Talish: Lankaran region, on soil [10].
47. *C. scabriuscula* (Delise) Nyl. – GC: Ismayilli region; MC: Nakhchivan: Shahbuz region, on soil among moss [11].
48. *C. squamosa* Hoffm. – MC: Nakhchivan: Babak, Ordubad, Sharur regions, on sandy soil [15].
49. *C. stellaris* (Opiz) Pouzar and Vezda – GC: Zagatala region, south-east slopes, 2550 m, in forests of subalpine belt, 2000- 2600 m [10, 5]; MC: Garayazi State Preserve [12], Goygol State Preserve, Kalbadjar region, Nakhchivan: Ordubad, Shahbuz , Babak regions, on soil [11].
50. *C. strepsilis* (Ach.) Grognot– GC: Gabala region [8]; MC: Shamkir region [13], Nakhchivan: Julfa, Ordubad regions [15], Talish: Yardimli, Lerik regions, on moss-grown cover [10].
51. *C. subrangiformis* Sandst. – GC: Gusar, Guba regions, on sandy and limestone soil [11], Absheron: near Altiaghadj Preserve, on limestone soil, 250 m [10], Shaki region, on sandy soil in steppes, Zagatala region, in the vicinity of mount Rochigel, Gabala region, 2100 m [13]; MC: Shamkir, Gazakh regions, on a soil [13], Goygol, Agdara regions, in forest, 1100 m [10, 13], Gadabay region, in forest [10], Khankandi region, in forest, 1250-1450 m, Lachin region [10, 13], Kalbadjar, Khodjavand, Shusha regions, 2000-2600 m [10], Gubadli region [13],

Nakhchivan: Babak, Julfa regions [8], Talish: Yardimli, Lankaran, Lerik regions, on sandy and limestone soil [8], K-Ar.L.: Agdash region, on soil [11].

52. *C. subulata* (L.) F.H. Wigg. – GC: Gakh, Balakan regions, on soil, mossy stones and stubs, in meadows and forests, 2200-3000m [5]; MC: Nakhchivan: Babak region, on the ground [6].
53. *C. symphycarpa* (Flörke) Fr. – GC: Zagatala region, on sandy soil [10], Balakan region, on the ground [10, 5].
54. *C. turgida* (Ehrh. ex Hoffm. – MC: Nakhchivan: Ordubad, Julfa regions, on the ground [6].
55. *C. uncialis* (L.) F.H. Wigg. – MC: Shusha region, in forest, on soil [11].
56. *C.uncialis* (L.) F.H. Wigg. subsp. *uncialis* (L.) Weber ex F.H. Wigg — MC: Shusha region, in the forest, on soil [9].

Before our research started, it was known 51 taxa of the genus *Cladonia* in the lichen flora of Azerbaijan and the new species were published earlier by Ahti T. [9]. Thus, as the result of conducted researches, we have identified five more new species: *Cladonia caespiticia*, *C. carneola*, *C. glauca*, *C. grayi* and *C. macroceras* and developed a list comprising 56 taxa of the genus *Cladonia*. The species *C. gracilis*, previously mentioned in the literature, should be excluded from the lists of lichen species of Azerbaijan.

ACKNOWLEDGEMENT

The author would like to express her sincere gratitude to Prof. Teuvo Ahti for his support in identification and examination of some questionable specimens.

REFERENCES

1. Ahti T and Samuel H. *Cladonia*. In: Nash THIII, Ryan BD, Gries C, Bungartz F, editors. Lichen Flora of the Greater Sonoran Desert Region. Arizona State University: Tempe, 2002; 1: 131-158.
2. Urbanavichus GP. A checklist of the lichen flora of Russia. St Petersbourg: Nauka Press; 2010.
3. Oxner AN. Handbook of the lichens of the USSR. Morphology, systematics and geobotanical distribution; 1974.
4. Red Data Book of Azerbaijan Republic. Rear and endangered species of plants and fungi. Eds. Bagirov HS, Aliyev JA. Baku; 2014.
5. Novruyov VS. Florogenetic analysis of the Greater Caucasus lichens and issues for their protection. Baku: Elm Press; 1990.
6. Ganbarov DSh. Ground lichens of Nakhchivan Autonomous Republic and their tracer features. Baku; 2007.
7. Bayramova AA. The flora of lichens of the north-eastern part of the Minor Caucasus and protection of gene pool. PhD thesis, Baku; 2007.
8. Barkhalov ShO. Flora lichens of Caucasus. Baku: Elm Press; 1983.
9. Ahti T, Alverdiyeva SM and Agayeva DN. New records of lichens of the genus *Cladonia* for Azerbaijan. Novitates System€aticae Plantarum non Vascularium: 2016; 50: 203-209.
10. Barkhalov ShO. Foliose and fruticose lichens of Azerbaijan. Baku: Elm Press; 1969.
11. Alverdiyeva SM and Novruzov VS. Check list of lichens of Azerbaidzhan. Baku: Elm Press; 2014.
12. Novruzov EA. Epiphytic synusia of the Garayaz State Preserve and agro ecosystems issues of use in ecological monitoring. PhD thesis, Baku; 2004.
13. Alverdiyeva SM. Species composition of lichens of the south- western part of the Minor Caucasus. Proceedings of the Institute of Botany of Azerbaijan National Academy of Sciences: 2007; 27: 142-145.
14. Pakhunova VG.. Lichens from the Mil steppe (Azerbaijan). News of Tbilisi botanic garden: 1926–1927; 3-4: 203-207.
15. Pashayev TY. Lichen flora of Nakhchivan Autonomous Republic. PhD thesis, Baku; 2008.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Production of Vermicompost from Organic Waste using Epigenic Earthworms *Eudrilus eugeniae* and Evaluation for Field Trial on Vegetable Crops

Hanumantappa Bherigi Nayaka* and U. Sivagamsundari

Assistant Professor, Department of Life Sciences, Kristu Jayanti College, Autonomous, Bengalur, Karnataka, India.

Received: 26 Nov 2020 Revised: 07 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
Hanumantappa Bherigi Nayaka
Assistant Professor,
Department of Life Sciences,
Kristu Jayanti College, Autonomous,
Bengalur, Karnataka, India.
Email: hbnayaka3@gmail.com

ABSTRACT

The present research was conducted with the objective to manage the solid organic waste by converting into compost by using earth worms and also evaluation of nutritional value of the vermicompost so produced such as potassium, nitrogen phosphorus and C:N ratio which are essential for soil fertility and plant growth and the compost also subjected to field trial to evaluate its potential to improve soil fertility and product yield. An experimental study was conducted to obtain the vermicompost using organic solid waste such as fruit, vegetable, paper wastes. The earthworm species used for this process was *Eudrilus eugeniae.* Moisture content was maintained by spraying proper percentage of water. The partially decomposed organic waste was converted into castings by earthworms. The castings was obtained on the top surface of the bin were in the range from 60 to 90 days depending on the type of solid waste used. The castings obtained were sieved, dried, tested and used as Bio-fertilizer. Thus the vermicomposting process helps in the management and disposal of organic solid waste in a safe, economic and useful manner. In this study we also analyzed cost effectiveness of this biofertilizer production and it shows promising profit with low cost investment so one can start small scale baseness on this as Biofertilizer Company. The present study covers environment cleaning, Biofertilizer production business, Soil fertility improvement for formers, Enhancement of crop product yield.

Keywords: Vermicompost, Plant debris, Earth worms, Bio-fertilizer.

INTRODUCTION

The increase in population (India) causes an increase in the quantity and type of urban and rural organic solid wastes. Such wastes are undesirable pollutants to the environment and time could even be a health menace. As far as rural wastes are concerned, there are enormous quantities of organic materials that are not utilized [1], Vermicomposting is a green technology that converts organic wastes into plant available nutrient rich organic fertilizer, Vermicompost, when used as fertilizer, not only bears positive impact on soil quality, plant growth and yield but also enhances nutritional value of crops produced. Vermicomposting is the process of producing compost by utilizing earthworms to turn the organic waste into high-quality compost that consists mainly of worm cast in addition to decayed organic matter [2, 3]. Today vermicompost is an important component of organic farming systems, because it is easy to prepare, has excellent properties and is harmless to plants. Vermicompost improves the physical, chemical and biological properties of the soil as well contribute to organic enrichment [5, 6]. Vermiculture is ecofriendly since earthworms feed on anything that is biodegradable, vermicomposting then partially aids in the garbage disposal problems. No imported inputs required, worms are now locally available and the materials for feeding are abundant in the locality as market wastes, grasses, used papers and farm wastes. It is also highly profitable, both the worms and castings are saleable [6]. Nowadays chemical fertilizers are being used in high quantities which degrade soil quality in long run [7]. Many researchers have reported positive changes in soil quality and soil productivity by application of VC compared to chemical fertilizers [8]. Many have testified significantly greater crop production through Vermicompost amendment. The present research was conducted with the objective to manage the solid organic waste by converting into compost by using earth worms and also evaluation of nutritional value of the vermicompost so produced. As far as wastes are concerned, there are enormous quantities of organic materials that are not utilized. "Vermicomposting technology" is a fast growing one with its pollution free, cost effective and efficient nature.

MATERIALS AND METHODS

Vermiculture compost production

General

This study, carried out at Kristu Jayanti College (Autonomous) Bangalore Karnataka (2019–2020), consisted of different stages, viz. building of a vermicompost unit at the College compound; import of a composting Epigeic earthworm, *Eisenia fetida* and production of vermicompost using organic solid waste, dry green leaf and cow manure.

Construction of the vermicompost unit

A vermicompost unit of 3 × 4 × 3 feet (w × h) was built in a shaded area, following [2]. The vermicomposting units were set up at the vermicompost unit using the Vermitech Pattern reported by [2]. Concrete unit of 3 × 4 × 3 feet (w × h) was built as containers for culturing the earthworms. The concrete units had the drainage holes (2 × 2 cm) to facilitate the effective water drainage as shown in the Fig 1. The roof of the station was made of sheets with underneath isolation paper to ensure a cool environment. The walls of the vermicompost station were built of wired mesh to facilitate air flow.

Preparation of Culture bed

The culture bed was prepared as described by [2]. as shown in Fig. 2: 1st layera basal layer of vermin-bed comprising broken bricks, then a layer of sand to the thickness of 6–7.5 cm was set up to ensure proper drainage. 2nd layer was Loamy soil up to the height of 15 cm, which was moistened. The earthworms, *Eisenia fetida*, were inoculated into this layer. 3rd layer: Lumps of fresh/dry cattle dung were scattered over the soil. 4th layer: The soil was then covered with organic solid waste, dry green leaf up to 10 cm thickness. The entire unit was covered with banana leaves to protect

the earthworms from sunlight and birds. It was kept moist by sprinkling of water twice a week and turned once a week, up to the harvest of the vermicompost.

Import of *Eisenia fetida*

In this, two hundred (200) composting earthworms, *Eisenia fetida* (Epigeic species), were imported from the University of Agriculture Science Bangalore. The earthworms were cultured for 120 days in one unit and were used for the production of vermicompost from solid waste and cow manure as shown in Fig 2. The solid waste was collected from the College garden after the lawn was mowed and stored and the cow manure was procured. The organic waste consisted of 5 kg cow manure and 2 kg dry solid waste on a weekly basis.

Compost analysis

After a hundred and twenty (120) days, the following parameters of the vermicompost were analyzed: 1. the total population of earthworms by passive method [9]: physically separating the earthworms from the vermicompost; 2. The total amount of the vermicompost produced (weight in kg); 3. Chemical analyses using the methods applied in the laboratory: p^H-H_2O using a p^H meter, total organic carbon (TOC in %) by Titrimetry using the Walkley– Black method; total nitrogen (N in %) using the Kjeldahl method; C: N ratio; total phosphorus (P in %) determined by the colorimetric method using a spectrophotometer.

Microbial analysis

To guarantee food safety, the cow manure and vermicompost were analyzed for the presence of *Salmonella* and *E. coli* bacteria in the Diagnostic Laboratory. The presence of *Salmonella* in various matrices was detected using the Modified Semi-solid Rappaport- Vassiliadis medium (MRSV) method (draft Annex D of the ISO 6579:2002 (E); the presence of *E. coli* bacteria was determined by the plate count method (NEN-EN-ISO 4833-1:2013).

Compost field trial on Vegetable crops

After successful production of vermicompost from vermicompost unit established in Kristu Jayanti College campus, was analyzed for physicochemical and biological properties to enriching the soil fertility, the compost so formed was also tested on field trial study on some vegetable plants such as Chill and Brinjal to evaluate its efficacy to improve crop yield by improving the soil fertility, this test was done by taking some small land area in association with one NGO under the departmental extension activity names as Lab to Land program. The 3 feet x 3 feet land space is used for each verity of vegetable crop and also one 3 feet x 3 feet land space used as control without adding this biofertilizer all of these setup as shown in Fig. 3. This experiment was carried out for the period of 3 months until the crops give rise to product to analyze growth parameters and product yield.

Statistical analysis

All the reported data are the Mean values of three replicates. Two way analysis of variance (ANOVA) was done to determine any significant difference among the parameters analyzed in vermicompost.

RESULT AND DISSCUSSION

Compost formation

The process of vermicomposting activity showed significantly changes in the physical and chemical properties of plant debris, cattle dung and paper waste material that can be an important tool for organic compost farming. The compost formed can be identified by seeing the color; it changes from brown to black this is one of most important physical characteristic used to confirm the compost formation by seeing in naked eyes and the compost so formed is as shown in the Fig. 4.

Compost Analysis

The compost so formed in the last step was subjected to in details analysis weight loss, color change, degradation, odour, microbial growth, earth worms multiplication etc. in addition to this some very important nutritional components of the compost was analyzed to know there role in improving soil fertility and to enhance crop yield so Table.1 showed clearly indicates that vermicompost technology reduces the amount of waste and also increased the nutrient content of the product (vermicompost) to be used as a biofertilizer in agricultural practices. Weight loss was found in three material of plant debris, cattle dung and paper waste by the degrading activity of earth worm *Eisenia fetida.* The content of N-total was increased as the process of composting days increases the significantly different from the previous initial days. This is caused by the weathering process of mineralization of organic matter involving the performance of enzymes that hydrolyze the protein complex that will increase the nitrogen content in the vermicompost [10], on the other hand, there is a simultaneous addition of nitrogen by worms in the form of mucus and excretory material, this process occurs in a high intensity so that the content of nitrogen will increase [11]. The content of C-organic was decreased as the days go on increased it indicates that the decomposition is took place. The decomposition process is the release of carbon from complex into more simple bonds due to the use of C elements by the organism to get their life energy through respiration and biosynthetic processes, releasing CO_2 to organic material so that the organic materials that have undergone the decomposition process will have lower levels of Carbon [12]. The C/N ratio was decreased as the days go on increased it indicates that the decomposition took place well. The decline of C/N ratio was caused by the decreasing number of carbon as well as the increasing number of nitrogen. The decomposition process is the release of carbon from complex into more simple bonds due to the use of C elements by the organism to get their life energy through respiration and biosynthetic processes, releasing CO_2 to organic material so that the organic materials that have undergone the decomposition process will have lower levels of C, compared to the C levels of raw materials [12]. The decreasing amount of C/N ratio is also due to the increasing content as the result of mineralization, a process that changes the organic nitrogen into inorganic nitrogen through the weathering process which involves the work of enzymes that hydrolyzes protein complexes, as well as the nitrogen that was produced from the worms' excrement [13].

Microbial analyses of compost

The vermicompost so formed was also subjected to microbial analysis by serial dilution method, as a result both fungi and bacterial colonies are got and they were partially identified as 1. *Klebsiella,* 2. *Pseudomonas,* are bacterial colonies and 3. *Aspergilla's, 4. Fusirium,* are fungi colonies. The colony morphology of all these 4 microorganisms on petri plate is as shown in the Fig. 5.

Economic analyses

The cost–benefit analysis of vermicomposting is as follows the total cost of setting a vermicompost station of 2 units is INR 15000, whereas the total fixed and variable costs are INR 5000. With an half annual production of 200 kg and sale revenue of INR 250 per kg, the estimated profit will be INR 30000 for 6 months and 60000 per year from only two units.

Compost field trial on vegetable crop growth parameters

The ANOVA analysis as shows that the effects of soil amendments was significant for all roots and shoot vegetative growth parameters and crop yield at the three growth stages. In general, plant growth was better in farmyard manure than in control where the soil not receiving the compost and also the vegetable crop yield has increased 50% as compared to control plants all of these Growth morphology parameters and product yield as shown in Table 2., and Fig. 6. The vermicomposting of dry grass clippings, rice straw and cow manure using *Eisenia fetida* was successful. The produced vermicompost had a dark color, a mull-like soil odor and was homogeneous. It had all the essential macro- and micro-plant nutrients like N, P, K, Ca, Mg, Mn, Cu, Zn and Fe, indicating the achievement of getting an environment friendly nutrient-rich Biofertilizer for the agriculture sector. The enhancement of nutrients and beneficial microbial population in the vermicompost is yet another important evolving trend where the vermicompost is value added with nutrients and or microorganisms resulting in improved growth and yield of crop

plants. The economic analysis revealed that this method is low cost investment and high yield of more profit and also this compost has subjected to field trial analysis on some selected vegetable plants has shown promising enhancement in growth permeates and product yield, so from this research we can conclude that Biofertilizer production can be started as small business and it proved that it can improve soil fertility and vegetable crop yield.

ACKNOWLEDGEMENT

The authors are thankful to The Centre for Research, Kristu Jayanti College (Autonomous), Bengaluru-560077, Karnataka (India) for providing lab facility and financial support to carry out this research work under the Scheme of "Shodh Pravartan Research Grant".

CONFLICT OF INTEREST

Conflict of interest declared none.

REFERENCES

1. Entre Pinoys. Retrieved on September 29, 2010 from http:/www.mixph.com/2006/12/ vermiculture-the-management-of-worms.htm.
2. Ismail SA 2005, the earthworm book. Other India Press, Mapusa, pp 101
3. Devi J, Prakash M 2015, Microbial Population dynamics during vermicomposting of three different substrates amended with cowdung. Int J Curr Microbiol Appl Sci 4(2):1086–1092
4. Ansari AA, Jaikishun S, Islam SK, Kuri KF, Nandwani D (2016) Principles of vermitechnology in sustainable organic farming with special reference to Bangladesh. In: Nandwani D (eds) Organic farming for sustainable agriculture. Sustainable development and biodiversity, vol 9. Springer International Publishing, Switzerland, pp 213–229.
5. Chauhan HK, Singh K (2013) Effect of tertiary combinations of animal dung with agrowastes on the growth and development of earthworm *Eisenia fetida* during organic waste management. Int J Recy Org Agric 2:11.
6. Bureau of Jail Management and Penology (2010). Retrieved on October 2, 2010 from http://www.bjmp.gov.ph/pdf%20and%20html/verm/vermiculture.htm.
7. Abu A, May C, Zalina N, and Abdullah N, 2015,"Effect on heavy metals concentration from vermiconversion of agro-waste mixed with landfill leachate," WASTE MANAGEMENT, pp. 1–5.
8. Ansari AA and K. Sukhraj, 2010, "Effect of vermiwash and vermicompost on soil parameters and productivity of okra (*Abelmoschus esculentus*) in Guyana," African Journal of Agricultural Research Vol., vol. 5, no. 14, pp. 1794–1798.
9. Edwards CA and Bohlen, PJ. 1996, Biology and Ecology of Earthworms. 3rd Edition, Chapman & Hall, London.
10. Anwar EK, 2009, Earthworm effectiveness *Pheretimahupiensis*, *Edrellus* sp. and *Lumbricus* sp.
11. Pattnaik Swati and M. Vikram Reddy. 2009. Nutrient status of Vermicompost of Urban Green Waste Processed by Three Earthworm Species *Eisenia fetida*, *Eudrilus eugeniae*, and *Perionyx excavatus.*
12. Nagavallemma KP, Wani SP, Stephane Lacroix, Padmaja VV, Vineela C, Babu Rao M and Sahrawat KL, 2004, Vermicomposting: Recycling Wastes into Valuable Organic Fertilizer. Journal ICRISAT. Vol.2 / Issue 1. India.
13. Decomposition of Organic Matter in the Process. Journal Soil Trop. Vol.14:149-158. Bogor. Indonesia.

Table 1: Showing analysis of compost with different parameters

Sl. No.	Parameters	30 days	60 days	120 days	Comments
1	pH	7.2	7.2	7.1	Excellent
2	Temperature	32	35	37	Good
3	Nitrogen	0.22	0.45	0.74	Excellent
4	Phosphorus	0.70	1.38	2.25	Good
5	Potassium	0.11	0.23	1.35	Excellent
6	Carbon	12.65	7.88	5:10	Moderate
7	Carbon: Nitrogen ratio	21.12	11.23	5.91	Good

Table 2: Showing growth parameters of vegetable crops for vermicompost analysis

Sl. No.	Plants type	30 days	60 days	90 days
1.	**Brinjal plants**			
Control	Shoot	+	++	+++
	Root	+	++	+++
	Yield	00	00	2Kg
Compost	Shoot	+	++	+++
	Root	+++	+++	+++
	Yield	00	00	4Kg
2.	**Chilli plants**			
Control	Shoot	+	++	+++
	Root	+	++	+++
	Yield	00	00	0.5Kg
Compost	Shoot	+	++	+++
	Root	+	++	+++
	Yield	00	00	1Kg

Note: +: Good Growth, ++: Very Good Growth, +++: Excellent Growth.

Fig. 1. Showing Vermiculture Composting tanks

Fig. 2. Inoculation of earth worms in to composting tank

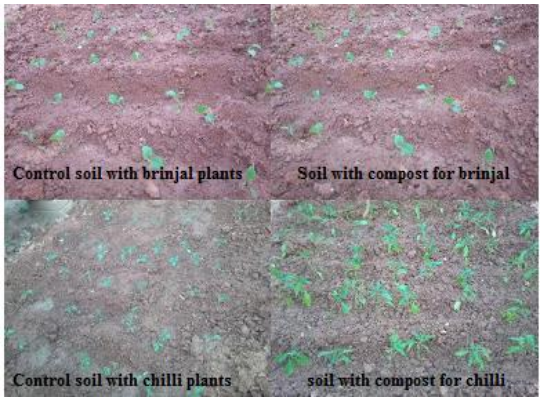


Fig. 3. Showing Plantation
1. Control of Brinjal, 2. Brinjal with Biofertilizer, 3. Control of Chilli, 4. Chilli with Biofertilizer

Fig. 4. Showing Vermicompost (Biofertilizer)

Fig. 5. Petri Plates with bacterial isolates from Vermicompost

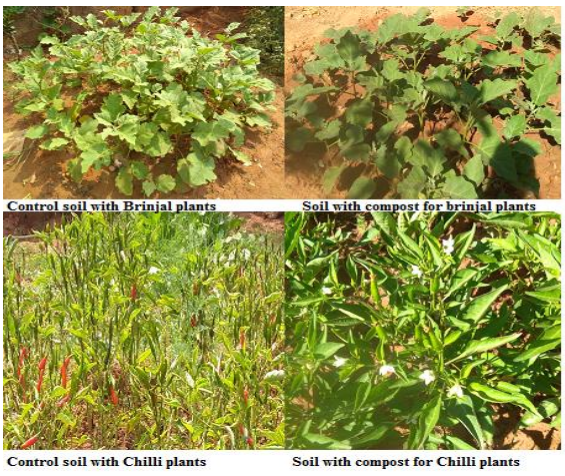


Fig. 6. Showing Plants after 3 months of plantation
1 Control of Brinjal, 2. Brinjal with Biofertilizer, 3.Control of Chilli, 4.Chilli with Biofertilizer

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect on the Physical and Thermo Dynamical Properties of Silver Doped As-Se Chalcogenide Systems

Nirupma Saxena[1], Dinesh Saxena[1] and Saurabh Kumar Tiwari[2*]

[1]Department of Physics, DBS College Kanpur (U.P.), India.
[2]Department of Physics, Sri Ram Kumar Bhartiya Gyan Devi PG College Phaphund Auraiya (U.P.), India.

Received: 28 Nov 2020 Revised: 15 Dec 2020 Accepted: 21 Dec 2020

***Address for Correspondence**
Saurabh Kumar Tiwari
Department of Physics,
Sri Ram Kumar Bhartiya Gyan Devi PG College Phaphund Auraiya
(U.P.), India.
Email: saurabht60@gmail.com

ABSTRACT

The difference in the compositional variations on the physical and thermo- dynamical properties via mean coordination number (<Z>), glass transition temperature (T_g), Urbach tail slope (Δ), mean bond energy (<E>) and thermal relaxation with the variation of Ag content has been studied theoretically for silver doped As-Se glass systems. In the present work we have analyzed comparative study of the concentration effect on the various physical and thermal properties on the basis of different theoretical models.

Keywords: Glass transition temperature, mean bond energy, Urbach tail slope, Thermal- relaxation.

INTRODUCTION

The term chalcogen means "ore former "is as characteristic name that was proposed by Fischer[1] in 1932 to refer to the group of elements O, S, Se, and Te."Chalcogenide" is used to address chalcogenide compounds with elements such as Arsenic (As), Silver (Ag), Bismuth (Bi), Germanium (Ge), Indium (In), Antimony (Sb) etc. These varieties of elements entering in the formation of chalcogenide alloy, chalcogenides offer wide range of variations in their properties. Selenium (Se) is a promising material for a large number of applications in xerography, switching and memory devices and photocells. In case of pure Se, the carrier life time is short and sensitivity is low. Selenium is often allowed with Germanium (Ge), Antimony (Sb) or Arsenic (As) in order to achieve higher sensitivity [2-5]. Arsenic based chalcogenides are semiconductors with applications in the production of micro-circuits e.g. as resist in photo- and electron beam lithography due to the effects of photo -doping and photo- darkening [6-8].(AsSe) and (As_2Se_3) are predominantly covalent solids and they are among the most studied binary systems [9-12]. The introduction of metal atoms like Ag in to the chalcogenide matrix is after used to vary the glass properties in a desired direction. Chalcogenide arsenic – based glasses are important functional materials for the present day science

and engineering. Specially the As-Se-Ag system is characterized by the presence of wide regions of stable glasses with interacting semiconductor and photo-electric properties. The silver – enriched As-Se-Ag glasses features high ion conductivity by Ag^+ cations, which opens the possibility of using them as solid electrolytes in sensors, monitors etc [13-15]. Some specific properties of As, Se and Ag are listed in Table-1. The aim of our work is to is to study and compare the modification in the structure and behaviors due to the introduction of silver (Ag) in to the chalcogenide matrix of the three sets of As-Se-Ag glass systems as $(AsSe)_{100-x}Ag_x$, $(As_2Se_3)_{100-x}Ag_x$ and $(As_{50}Se_{50})_{100-x}Ag_x$ with x = 0, 5, 10, 15, 20, 25.

Analysis of Various theoretical parameters

Mean coordination number and Number of Constraints

For amorphous chalcogenide materials the mean coordination number is as important characteristic, indicating the average number of bond per atom, which must be broken to the obtained fluidity. The mean coordination number Z for the three sets of As-Se-Ag glass systems is calculated using the expression-

$$\langle Z \rangle = X_{As}Z_{As} + X_{Se}Z_{Se} + X_{Ag}Z_{Ag} \quad \text{.....(1)}$$

Where $Z_{As} = 3$, $Z_{Se} = 2$ and $Z_{Ag} = 4$ are the coordination number of As, Se and Ag and X_{As}, X_{Se} and X_{Ag} are the mole fractions of As, Se and Ag respectively, the mean coordination number <Z> being studied in the range $2.375 \leq Z \geq 2.625$ for the three sets of As-Se-Ag glass systems. On the basis on topological arguments counting constraints and the degree of freedom. Phillips and Thorpe [16] suggested that glass having Z<2.40 consist of rigid regions whose volume fraction is too small to be fully connected. In this case rigid regions are immersed in the 'floppy' matrix. In a glassy system covalent networks can be mechanically constraints by inter atomic valence force such as bond stretching and bond bending. Optimal glass formation is attained when the network is at a mechanically critical point. This point is reached when the number of constraints (N_{CO}) per atom is equal to the degrees of freedom (N_d) per atom i.e. for ideal glass $N_{CO} = N_d$. When Z >2.40, the solid has continuously connected rigid regions with floppy regions inter-dispersed and may be termed as 'amorphous solids'. It is obvious that N_{CO} increases with the addition of silver for the $(AsSe)_{100-x}Ag_x$, $(As_2Se_3)_{100-x}Ag_x$ while decrease with addition of silver for the $(As_{50}Se_{50})_{100-x}Ag_x$ glassy system. For the ideal glass N_{CO} equal to N_d, ($N_d = 3$), where the mechanical stability of the network is optimized. The enumeration of mechanical constraints in the glass system gives <Z>/2 bond stretching constraints (N_α) and 2<Z>-3 bond bending constraints (N_β) [17]. The mean coordination number Z and the average number of constraints, given by $N_{CO} = N_\alpha + N_\beta$ for various compositions of the three sets of As-Se-Ag glass systems are listed in Table -2. The variation of mean coordination number <Z> with Ag content for the three glass systems of As-Se-Ag is illustrated in Fig.1.

The glass transition temperature and mean bond energy

The glass transition temperature (T_g) of a multi-component glass is known to depends on several independent parameters such as band gap, coordination number, effective molecular weight and type and fraction of various structural units formed. In the present work result obtained obeys the well known equation followed by most of alloys including chalcogenides [18-19] i.e.-

$$\ln T_g = 1.6\langle Z \rangle + 2.3 \quad \text{.....(2)}$$

The variation of T_g with Ag content for the three sets of As-Se-Ag glass systems are listed in Table-3. The variation of the glass transition temperature as a function of Ag content for the three glassy systems of As-Se-Ag is illustrated in Fig.:-2.The covalent bond approach of Tichy and Ticha [20] may be considered as a first approximation in the case of chalcogenide glass. The glass transition temperature is considered to be proportional to mean bond energy <E>, which depends on factors like mean coordination number, degree of cross linking, bond energy and the nature of

bonds. Taking account all these factors they have examined 186 chalcogenide glasses with T_g ranging from 320K to 760K, and obtained a good correlation between T_g and <E> in the form-

$T_g = 311[<E> - 0.9]$ ……..(3)

which satisfied the Arrhenius relation for viscosity [21]. Applying this model in our problem, we have evaluated mean bond energies for various components of $(AsSe)_{100-x}Ag_x$, $(As_2Se_3)_{100-x}Ag_x$ and $(As_{50}Se_{50})_{100-x}Ag_x$ glass systems. The calculated values of <E> for the three glass systems of As-Se-Ag are listed in Table-3. The variation of the mean bond energy <E> as a function of Ag content for the three glassy systems of As-Se-Ag is illustrated in Fig.3.

Urbach tail slope

The dependence of glass transition temperature on the Urbach tail slope describe the temperature dependence of the absorption coefficient .The spectral dependence of temperature is given by the Boltzmann factor. The relation between Urbach tail slope (Δ) and the glass transition temperature (T_g) is given by a simple relation [22-23].

$\Delta = 1.3k_BT_g$ ……(4)

Where k_B , the Boltzmann constant(1.38×10^{-23}J/K). The calculated values of Urbach tail slope (Δ) for the three glass systems of As-Se-Ag are listed in Table -4. The variation of the Urbach tail slope (Δ) as a function of Ag content for the three glassy systems of As-Se-Ag is illustrated in Fig.4.

Thermal Relaxation

The obtained values of <E> were found to be obeying the equation (3), this behavior means that the value of the T_g in chalcogenide glasses is mainly determined through bonding arrangement. This does not mean that, intermolecular interactions have no effect on Tg as indicated by many authors. Furthermore this interaction plays a role in relaxation processes in the glass transition region. This correlation is expressed as an Arrhenian relation [24-25] for the viscosity of the form –

$\mu (T_g) = \mu_0 e^{E\mu/k_BTg}$ …….. (5)

where $\mu_0 = 10^{-3}$ Pa, E_μ = <E> - 0.9 and k_B is the Boltzmann constant (1.38×10^{-23} J/K). The obtained values of $\mu(T_g)$ are listed in Table-4 and it is found to be in the order of 10^{13}Pa for the studied glasses. The variation of thermal relaxation as a function of Ag content for the three glassy systems of As-Se-Ag is illustrated in Fig.5.Also the variation of $\mu(T_g)$ in terms of glass transition temperature (T_g) and mean bond energy (<E>) are illustrated in Fig.6 and in Fig.7 for $(AsSe)_{100-x}Ag_x$, $(As_2Se_3)_{100-x}Ag_x$ and $(As_{50}Se_{50})_{100-x}Ag_x$ glassy systems.

CONCLUSION

As seen from the calculated values of different parameters we find that the values of mean coordination number (<Z>), glass transition temperature (Tg), mean bond energy (<E>), Urbach tail slope (Δ) and the thermal relaxation parameter $\mu(T_g)$increases with increase of Ag content in the $(AsSe)_{100-x}Ag_x$ and $(As_2Se_3)_{100-x}Ag_x$ glassy systems. In $(AsSe)_{100-x}Ag_x$ and $(As_2Se_3)_{100-x}Ag_x$ glassy systems the increase of parameters is consistent with the higher metallicity of Ag atoms as compared with the As atoms also overall tendency is the increase of with silver content over the entire studied range is related to the large atomic radius of Ag atoms as compared to Se atoms. The increase in T_g, <E> and $\mu(T_g)$ upon Ag deposition is a consequence of local structural modification that brings to the Ag atoms close to the As and Se atoms, so there will be change in the bond length due to matrix modification. On the other hand in the $(As_{50}Se_{50})_{100-x}Ag_x$ glassy system, the atomic substitution of As by Ag slightly stiffens the network cohesion. In addition to the calculation of Tg, <E>, Δ, and $\mu(T_g)$of the semiconducting materials are important parameters can be

determined by using the value of <Z>. The decrement of these parameters with addition of Ag could be associated with the structural transformation in the material as a result of Ag incorporation. It has been reported that the basic structural units in As-Se glasses are $AsSe_3$ pyramids and accordingly the increment of Ag in the As-Se matrix might leads to a decrease of such types of pyramids, which are replaced by Ag_3AsSe_3 structural units. The appearance of these new structural units could cause an increase of disorder and number of defect states in material structure. This may in turn, results in an increase is the density of localized states and consequently decrease in the T_g, <E>, Δ and $\mu(T_g)$ of the $(As_{50}Se_{50})_{100-x}Ag_x$ glassy system.

REFERENCES

1. Fischer W.: A Second note on the term"Chalcogen".J.Chem.Educ. 2001;7(10): 1333.
2. Sharda Sunanda, Sharma Neha, Sharma Pankaj, Sharma Vineet: Physical analysis of structural transformation in Ge incorporated a- Sb_xSe_{100-x} system. Defect and Diffusion Forum 2011; 316-317:45-53.
3. Wang T, Gulbiten O, Wang R, Yang Z, Smith A, Davies B.L, Lucas P: Relative contribution of stiochiometry and mean coordination to the fragility of Ge-As-Se glass forming liquids. J.Phys.Chem. B, 2014; 118: 1436-1442.
4. Wang T, Wei W.H, Shen X, Wang R.P, Davies B.L, Jackson I :Elastic Transition thresholds in Se-As(Sb)-Se glasses. J. of Physics D: Applied Physics 2013;46: 165302(1-6).
5. Prasad Amba, Saxena Ashish Kumar, Tiwari Saurabh Kumar: Compositional effects on the thermodynamical properties of $Ge_{40-x}Se_{60}In_x$ chalcogenide glasses. IOSR-Journal of Appl.Phys. 2017; 9(3): 81-84.
6. Weidenhof W, Friedrich I, Ziegler S, Wutting M: Laser induced crystallization of amorphous $Ge_2Sb_2Te_5$ films. J. Appl. Phy.2001; 89: 3168.
7. Mitkova M, Kozicki M.N: Silver incorporation in Ge-Se glasses used in programmable metallization cell devices. J. Non-Cryst. Solids 2002; 1023:299-302.
8. Mietzsch K, Fitzgerald A.G: Electron beam induced patterning of thin film arsenic based chalcogenide, Appl.Surf.Sci. 2000; 464:162-163.
9. Ilcheva V, Petkova T, Petkov P, Bove V, Socol G, Sima F, Ristoscu C, Mihailescu C.N, Mihailescu L.N, Popov C, Reithmaier J.P : Spectroscopic studies of $(AsSe)_{100-x}Ag_x$ thin films. Appl. Surf. Sci. 2009; 255: 9691-9694.
10. Aparimita Adyasha, Sripan C, Ganrsan R, Naik Ramakant: Photo- and thermally induced property change in Ag diffusion in to Ag/As_2Se_3 thin films. Appl. Phys. A 2018; 124(267): 1-10.
11. Yoshikawa A, Ochi O, Nagai H et. al.: A novel inorganic photo resists utilizing Ag photodoping in Se-Ge glass films. Appl.Phys. Lett. 1976; 29: 161.
12. Marquez E, Pefia J.F, Gonzhlez-Leal J.M. et.al. Optical reflectivity monitoring of the Ag photo dissolution kinetics in $As_{30}S_{70}$ chalcogenide glass films. Mater. Lett. 1995; 25:143-146.
13. Kalyva M, Siokou A, Yannopoulos S.N, Wanger T, Orava J, Frumar M: Ag diffusion in amorphous $As_{50}Se_{50}$ films studies by XPS: J.Non-Cryst. Solids 2009; 355: 1844-1848.
14. Kovalskiy A, Miller A.C, Jain H, Mitkova M: In Situ Measurements of X-ray-Induced silver diffusion in to a $Ge_{30}Se_{70}$ thin film. J.Am.Ceram.Soc. 2008; 91:760.
15. Shaaban E.R, Abdel-Rahim M.A, Abd-El Salam M,N, Mohamed Mansour, Abdel-Latief A.Y, Yousef El Sayed: Structural and optical constants of annealed $As_{47.5}Se_{47.5}Ag_5$ film using DSC transformation curve. Acta Physica Polonica A, 2019; 135(3): 401-408.
16. Phillips J.P, Thorpe M.F: Constraint theory, vector percolation and glass formation. Solid Stat. Commun. 1985; 52: 699.
17. Bushnach-Haruvi I, Dror J, Croituro N.J: Chalcogenide glasses Ge-Sn-Se, Ge-Se-Te and Se-Sn-Te for infrared optical fibers. Mater.Res. 1990; 5: 1215.
18. Freitas R.J, Shimakawa K, Kuglar S: Some remark on the glass transition temperature in chalcogenide glasses: a correlation with the microhardness. Chalcogenide Letters 2013; 10(1): 39.
19. Tanaka K: Glass transition of covalent glasses. Solid Stat. Commun. 1985; 54(10): 867.
20. Tichy L, Ticha H: On the chemical threshold in chalcogenide glasses. Matter. Lett. 1994; 21: 313.

21. George A, Sushamma D, Predeep P: Effect in indium content on the optical and physical characteristics of As-Te glass systems. Chalcogenide Letters 2006;3: 33-39.
22. Chan C.T, Louie S.G, Phillips J.C: Potential fluctuations and density of gap states in amorphous semiconductors. Phys.Rev.B 1987; 35: 2744.
23. Tichy L, Ticha H, Smrcka V: Compositional trends of the glass transition temperature, Urbach tail clope and optical gap in $As_{0.5}Se_{0.5-x}Te_x$ glasses. Matter. Lett. 1992; 15: 202-206.
24. Matsuishi K, Kasamura H, Onari S, Arai T: Intermediate range correlation in a $Se_{1-x}Te_x$ and $(As_2S_3)_{1-x}Ag_x$ systems. J.Non-Cryst. Solids 1989; 114: 46.
25. Aly K.A, Abd-Elnaeim A.M, Uosif M.A.M et. al.: Optical properties of Ge-As-Te thin films.Phys. B: Condenced Matter 2011; 406: 4227-4232.

Table 1. Some basic properties of the elements As, Se and Ag

Elements	Density (g/cm^3)	Molar mass (g/mol)	Molar Volume (cm^3/mol)	Atomic density (atom/ cm^3)	Coordination number (Z)	Atomic radius(pm)
As	5.72	74.92	12.96	4.59×10^{22}	3	119
Se	4.97	78.97	16.42	3.66×10^{22}	2	120
Ag	10.49	107.86	10.27	5.86×10^{22}	4	145

Table 2. The values of <Z> and N_{CO} for the three glass systems of As-Se-Ag

Composition/X	$(AsSe)_{100-x}Ag_x$		$(As_2Se_3)_{100x}Ag_x$		$(As_{50}Se_{50})_{100x}Ag_x$	
	<Z>	N_{CO}	<Z>	N_{CO}	<Z>	N_{CO}
X =0	2.500	3.250	2.400	3.000	2.500	3.250
X =5	2.525	3.312	2.430	3.075	2.475	3.187
X =10	2.550	3.375	2.460	3.150	2.450	3.125
X = 15	2.575	3.437	2.490	3.225	2.425	3.062
X =20	2.600	3.500	2.520	3.300	2.400	3.000
X =25	2.625	3.565	2.550	3.375	2.375	2.937

Table 3. The values of T_g(K) and <E>(eV) for the three glass systems of As-Se-Ag

Composition/X	$(AsSe)_{100-x}Ag_x$		$(As_2Se_3)_{100-x}Ag_x$		$(As_{50}Se_{50})_{100x}Ag_x$	
	T_g(K)	<E>(eV)	T_g(K)	<E>(eV)	T_g(K)	<E>(eV)
X =0	544.57	2.65	464.05	2.39	544.57	2.65
X =5	566.80	2.72	468.87	2.40	523.12	2.58
X =10	589.92	2.79	510.81	2.54	502.70	2.51
X = 15	614.03	2.87	535.92	2.62	482.99	2.45
X =20	639.06	2.95	562.28	2.70	464.05	2.39
X =25	665.14	3.03	589.93	2.79	445.85	2.33

Table 4. The values of Urbach slope Δ (eV) and μ (T_g) for the three glass systems of As-Se-Ag

Composition/X	$(AsSe)_{100-x}Ag_x$		$(As_2Se_3)_{100-x}Ag_x$		$(As_{50}Se_{50})_{100-x}Ag_x$	
	Δ(eV)	$\mu(T_g)$ x10^{13} (Pa)	Δ(eV)	$\mu(T_g)$ x10^{13} (Pa)	Δ(eV)	$\mu(T_g)$x10^{13} (Pa)
X =0	0.0610	1.516	0.0520	1.470	0.0610	1.516
X =5	0.0635	1.437	0.0545	1.522	0.0586	1.482
X =10	0.0661	1.356	0.0572	1.317	0.0563	1.338
X = 15	0.0688	1.429	0.0600	1.447	0.0541	1.441
X =20	0.0716	1.425	0.0630	1.316	0.0520	1.476
X =25	0.0745	1.342	0.0662	1.354	0.0499	1.414

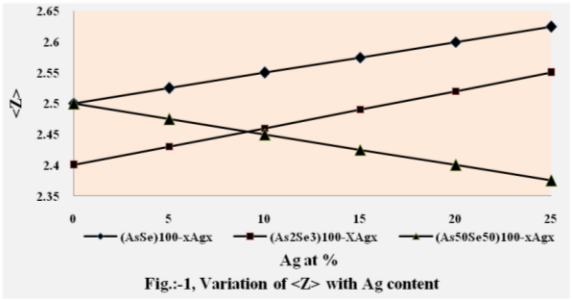


Fig.1. Variation of <Z> with Ag content

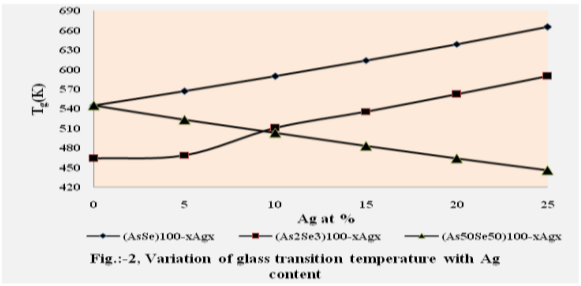


Fig.2. Variation of glass transition temperature with Ag content

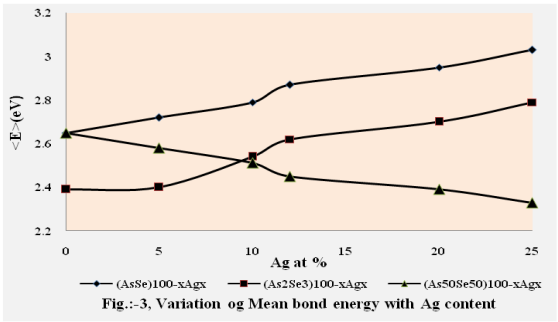


Fig.3. Variation og Mean bond energy with Ag content

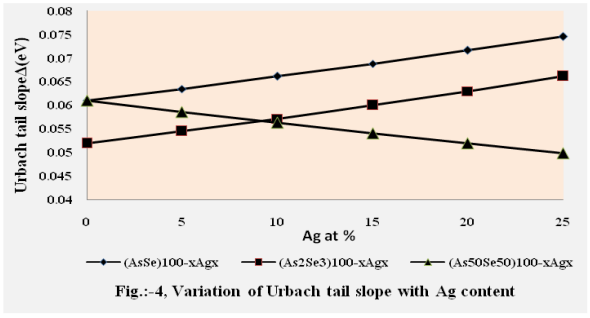


Fig.4 Variation of Urbach tail slope with Ag content

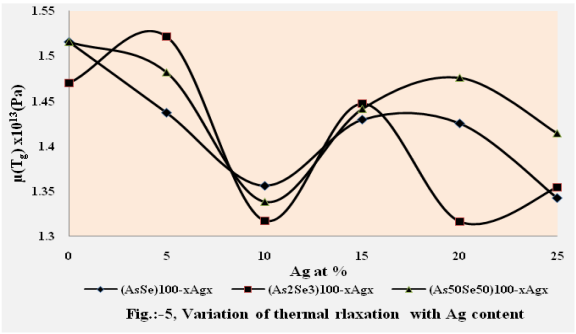


Fig.5. Variation of thermal relaxation with Ag content

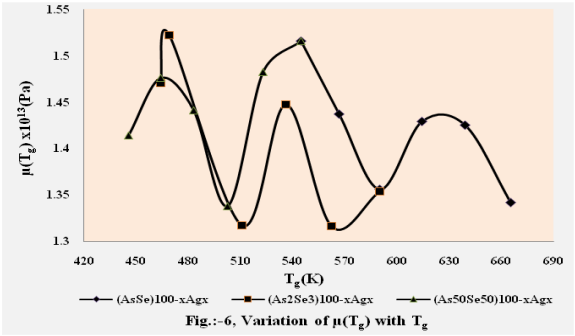


Fig.6. Variation of $\mu(T_g)$ with T_g

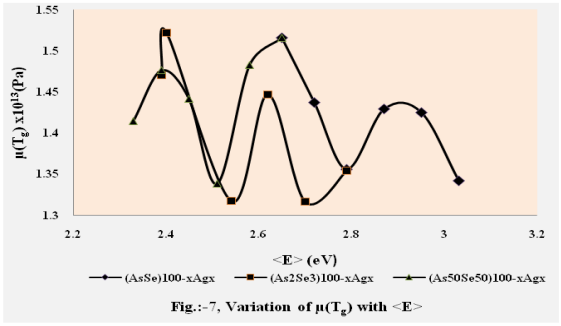


Fig.7. Variation of $\mu(T_g)$ with <E>

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Solar Cell Efficiency: A Mathematical Approach

M. Srinivas*

Associate Professor, Department of H&S (Physics), Keshav Memorial Institute of Technology, Hyderabad, Telangana, India.

Received: 23 Nov 2020 Revised: 07 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
M. Srinivas
Associate Professor, Department of H&S (Physics),
Keshav Memorial Institute of Technology,
Hyderabad, India.
Email: msrinivas901@gmail.com

ABSTRACT

The need for energy in India has increased over the years. As the possibilities of non-renewable energy sources have not been successful with this increase, there has been an increasing focus on renewable energy sources. Photovoltaic solar energy could be one of these substitute sources because solar energy is completely available in India. It is also renewable and non-polluting. In this work the study of Photovoltaic Solar Energy under the influence of various physical conditions was considered. The analysis of the material characteristics of solar cells considering various factors such as material properties, form factors, specific heat, area ratio and other environmental parameters is described in this article with a numerical equation An equation based on some assumptions is formulated to replicate the actual operating conditions. Logarithmic graphs of energy and time are obtained for different permutations and combinations to show actual operating conditions.

Keywords: Photovoltaic cell, Shape factor, Area ratio, Specific heat, Temperature, Wavelength

INTRODUCTION

Photovoltaic solar energy is effective, quiet, easy to set up and requires minimal maintenance. In a appropriate location, generating power using photovoltaic systems can be cheaper than conventional electricity generation from fossil fuels [1], [2].In order to make solar cells cost effective; the worldwide R&D efforts are directed to improve the effectiveness of solar cells to achieve substantial cost reduction. Further, R&D is as well being taken up on non-silicon based solar cell modules and other features of PV systems [1]. In recent years the difficulties of Solar Photovoltaics under the influence of numerous physical conditions have attracted the attention of a number of researchers because of their possible applications in many branches of science and technology [3]. Mathematical modeling discussed in this paper describes the material characteristics of solar cells by considering various factors such as material properties, geometric parameters, form factors and other environmental variables. An equation has

been formulated to examine the energy transfer taking place in it. The mathematical equation formulated is centered on specific assumptions to simulate the actual working situations. The solution achieved indicates certain governing factors on the performance of a solar cell. Other aspects revealed interesting outcomes which are interpreted to be in tune with real performance quoted elsewhere for actual field conditions. Study of these graphs show the details for actual operating conditions and some of the principal factors amongst the principal environmental conditions and the material properties of a solar cell. Actual form factors and area numbers indicate the optimal use of any solar cell. Elements such as silicon and some compound semiconductors such as Gallium Arsenide, Cadmium Telluride, Zinc Selenide act as Solar cell materials [4]. The properties like band gap energy level, effective doping in case of compound semiconductors determine the utilization of these materials [6]. Also the geometrical parameters like size, shape and thickness do play a role in its performance. The thickness values of the wafer like pellets were in the range of 0.1 to 0.2 mm. For solar cells, the preferred thickness range is of the order of a few microns. Tooling methodologies as of now are mostly of circular shape. Other factors like temperature and specific heat are also considered, for a given material. Based on these parameters, the problem formulation and its solution obtained are discussed next.

Formulation of the Determinant Problem Equation

With the help of digital image processing for the energy discharge from a photon the following mathematical modeling is formulated. Visible light is characterized by its wavelength λ in the range from about 400 nm to 700nm. A functional relationship is considered for the different combinations of several factors and complexity of the actual conditions. A complex function can also be chosen for the same. The backup material, soldering leads to connect an array of solar cells for a panel and the corresponding resistance with heat losses, are not considered. These may be negligible for a good processing with controlled environmental conditions and a quality production system.

The energy in a photon in electron volts is considered a function F (p)

$$F(p) = S_f e^{-[(\lambda \gamma / \delta).(Ac/A) Cp T t]}$$

Where

λ	→	Wavelength of incoming light, m
γ	→	Frequency of incoming light, Hertz
δ (delta)	→	Thickness of Solar Cell (water), m
Ac / A	→	Area ratio
Ac	→	Effective Area, m^2
A	→	Actual Area, m^2
Cp	→	Specific Heat at constant pressure, KJ/Kg ^{o} K
T	→	Temperature ^{o} K
t	→	Time, sec
S_f	→	Shape factor

Solutions

The values imprinted in the equation are encompassing the real conditions to cater for different temperature conditions and production methodologies adopted across the length and breadth of the country.

Hence the ranges of values selected are as follows

δ	=	0.1 to 1 mm
Ac/A	=	0.1 to 1mm
T	=	293 ^{o} K to 333 ^{o} K
Cp	=	0.01 to 0.1 KJ / Kg ^{o} K
t	=	$0X10^{-6}$ to $200X10^{-6}$ nanosecs
c	=	$\gamma\lambda$ (valid for the visible range of spectrum of Wavelength and frequency).

DISCUSSION

The graphical plots for various combinations of factors show an asymptotic behavior of a solar cell. For values of Cp (property number) close to 0.05 and below the asymptotic behavior signifies the optimum performance. For increased values, beyond $Cp = 0.05$ the curve merges with the X-axis. Effective area ratio indicates the performance considering the shadowing losses for any given shape and area. This probably indicates a solar cell should have low property number (*i.e.*, low specific heat for efficient absorption of light and delivery of dc current). The tabulated results and trend of the shape of the curves reveal some interesting findings: the peak efficiency does not exceed 27% for any system. This mathematically supports the Carnot efficiency which is the maximum efficiency that can be obtained for any ideal system including the solar cycle. The maximum efficiency obtainable can be used as a standard to be achieved by solar cell manufacturing companies by optimizing the cell thickness, effective area ratio for a given temperature (place which can be defined by latitude and longitude)[5]. For the given operating temperatures at 273,313 ^{o}Kelvin respectively it is seen that the cell performance is not much affected. From the equation and graphical output, the maximum efficiency should not increase beyond e = 2.718 i.e., 27% efficiency. This can be linked to the maximum Carnot efficiency of 30% of any heat engine. The shape factor should indicate the relative merits of having maximum area utilization (minimum shadowing losses) for which ideally Ac/A should be equal to unity.

REFERENCES

1. Zentrum für Sonnenenergie-und Wasserstoff-Forschung Baden- Württemberg, Stuggart (2015) "White paper for CIGS thin film solar cell technology",
2. Mayer J.N., Philipps S., Hussein N.S., Schlegl T., Senkpiel C.,"Current and future cost of photovoltaics: Long-term scenarios for market development, system prices and LCOE of utility-scale PV systems ", Fraunhofer Institute for Solar Energy Systems, Freiburg (2015),
3. Almosni S, Delamarre A, Jehl Z, et al. Material challenges for solar cells in the twenty-first century: directions in emerging technologies. Sci Technol Adv Mater. 2018;19(1):336–369
4. Rajendra Singh and Akhlesh Lakhtakia: Making Solar Cells a Reality in Every Home: Opportunities and Challenges for Photovoltaic Device Design, IEEE JOURNAL OF THE ELECTRON DEVICES SOCIETY, VOL. 1, NO. 6, JUNE 2013
5. Won Y. Lee, Sang S. Kim, Maximum power-conversion efficiency for the utilization of solar energy, International Journal of Energy Research, May1991
6. Reference: Electronic Properties of Materials Rolf E. Hummel, Nervosa Publishing House (1994).

Graphical Plots

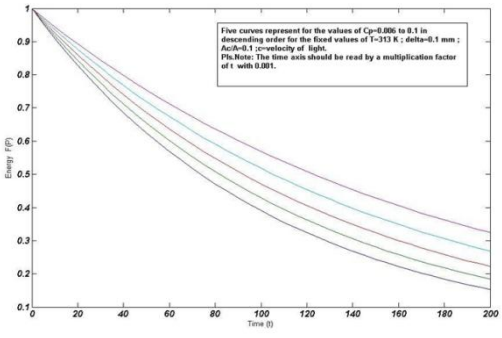


Fig. 1. Plot of Energy Vs. Time

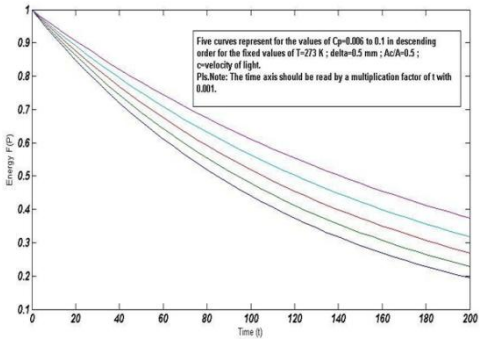


Fig. 2. Plot of Energy Vs. Time

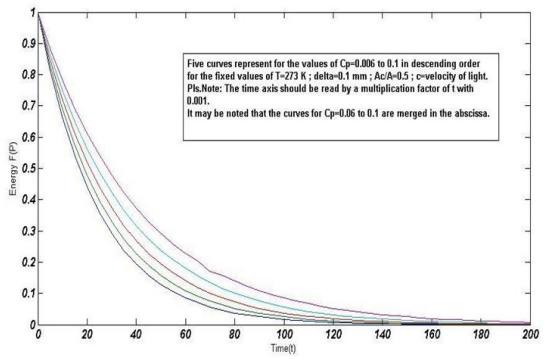


Fig. 3. Plot of Energy Vs. Time

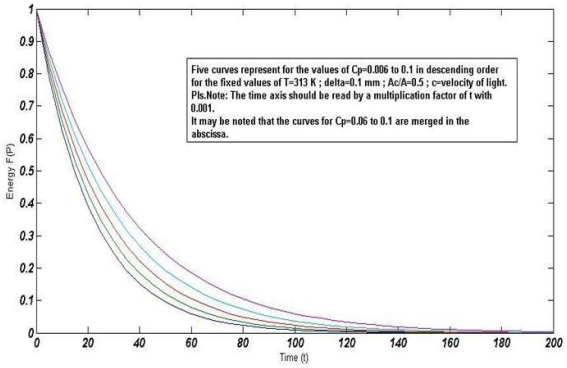


Fig. 4. Plot of Energy Vs. Time

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Preparation and Evaluation of Etodolac Loaded Nanoparticles for Modified Drug Delivery

Saminathan C*, Pavankumar Gangavarapu, Venkatesan.P and Madhusudhan S

Department of Pharmacy, Faculty of Engineering and Technology, Annamalai University, Annamalainagar, Tamil Nadu, India.

Received: 26 Nov 2020 Revised: 12 Dec 2020 Accepted: 21 Dec 2020

***Address for Correspondence**
Saminathan C
Department of Pharmacy,
Faculty of Engineering and Technology,
Annamalai University, Annamalainagar, Tamil Nadu, India.
Email: samicdm@gmail.com

ABSTRACT

The aim of present study was to formulate and evaluate the Etodolac loaded Sustained release nanoparticles by emulsion solvent evaporation technique. Eudragit RS 100is used as coating material. The effects of process conditions such as drug loading, polymer type and solvent type on the characteristics of nanoparticles were investigated. The prepared nanoparticles were characterized for their particle size, drug loading and drug release. The *in-vitro* release studies were carried out in phosphate buffer at pH 7.4. The prepared nanoparticles have showed good entrapment efficiency and the *in-vitro* release studies showed that Etodolac nanoparticles of 1:3 ratios exhibited better sustained effect over a period of 12 hours.

Keywords: Nanoparticles, Etodolac, Eutragit RS 100, Solvent evaporation technique.

INTRODUCTION

Drug delivery systems (DDS) that can precisely control the release rates or target drug to a specific body site have had an enormous impact on the health care system. The last two decades have witnessed a remarkable improvement in the field of novel drug delivery systems. Carrier technology offers an intelligent approach for drug delivery by coupling the drug to a carrier particle such as microspheres, nanoparticles, liposomes, etc, which modulates the release and absorption characteristics of the drug. Nanoparticles constitute an important part of these particulate DDS by virtue of their small size and efficient carrier characteristics [1]. Solvent evaporation technique (SET) is one of the several methods that are used for production of nanoparticles. Although (SET method) may not be a prominent method, but it is the one of the simplest, versatile technique and by controlling certain variables, desired outcome can be predicted and obtained. as well. Kilicarsan and Baykara (2003) investigated the effect of the drug/polymer ratio on properties of the verapamil loaded nanoparticles prepared by solvent evaporation method and found that

the drug release profile could be sustained by increasing polymer ratio and the particle size and surface characterization of microsphere could be modified through the variation of drug/polymer ratio. Diaminopyridine microparticles by solvent evaporation method were prepared by Gibaud et al. (2002) [2-4]. Rheumatoid arthritis is a chronic autoimmune disease that causes continuous articular devastation and bone deterioration. It is associated with chronic inflammation and tissue damage [9]. The activation of the immune inflammatory reactions during night forces the symptoms to worsen in the early morning resulting in sleep disturbances related to quality and discontinuity [10]. Symptoms continue over the morning time and they are commonly characterised by joint stiffness and functional disability [11]. Etodolac (ETD), a non-steroidal anti-inflammatory drug, is used to manage rheumatoid arthritis associated symptoms via inhibition of cyclooxygenase pathways and other inflammatory mediators [12]. ETD is a selective COX-2 inhibitor, which inhibits only cyclooxygenase- 2 mediators. It causes less gastrointestinal complication compared to the majority of other NSAIDs [13].

Conventional delivery systems of ETD were found to engender stomach complications, such as nausea, epigastric pain, heartburn, and indigestion [14]. Delayed drug release formulation would be a suitable solution especially for chronic patients. In a patent assigned to Michelucci and Sherman [15], a sustained release dosage form of etodolac was provided in the form of matrix tablets with a release rate modifying agents. Although controlled release medication decreases the frequency of administration and diminishes the sleeping problems, yet the morning complications are not completely exterminated. Thus, a specialized drug delivery device is considered to be helpful in delivering a loading dose in the early morning and a maintenance dose over the day time. Therefore, few researches have focussed towards designing a bilayer tablet to include a fast release layer for rapid onset of action, beside a sustained release layer for drug level maintenance [16]. Nevertheless, the rapid drug release in the stomach prevents the success of the system, due to manifested side effects on gastric mucosa. Recently, another sigmoid release profile has attracted many workers interested in the field of pharmaceutical formulation, the so-called pulsatile drug delivery system. Multiple benefits could be acquired through the new design as the delivery device was capable of releasing the drug in a controlled programmable strategy after a precisely calculated lag phase [17]. Different formulation approaches could be applied with the new design, either single or multiunit systems supplied with controlled release coating materials. Multicoating of tablets with time dependent polymers providing a lag-time prior to drug release initiation could attain the goal for pulsatile release [18].

Sustained release nanoparticles may be produced by several methods utilizing emulsion system (oil-in-water, oil-in-oil, water-in-oil-in-water), as well as by spray drying. The common emulsion system used oil-in-water (o/w), with nanoparticles being produced by the emulsion solvent evaporation method. This relatively simple method enables the entrapment of a wide range of hydrophobic drugs [12]. Eudragit RS 100 is bio-compatible, non-toxic polymer and widely used in oral and topical formulation [13,14]. The main objective of this work was to investigate the possibility of obtaining a sustained release formulation of Etodolac nanoparticles by using Eudragit RS 100 in various drugs, polymer ratios (1:1, 1:3, and 1:5). The various physicochemical characteristics and the *in-vitro* release profile from these nanoparticles were investigated.

MATERIALS AND METHODS

Etodolac(99.79%) donated by M/S Shasun pharmaceuticals, Puducherry; Eudragit RS 100 and PVA was procured from Sigma Aldrich. Dichloromethane was of analytical-reagent grade supplied by M/s SD Fine chemicals, Mumbai, India. All the reagents and solvents used were of analytical grade satisfying pharmacopoeial standards.

Preparation of Etodolac Nanoparticles

Nanoparticles were prepared by solvent evaporation method reported by previous authors [19-25] with some modifications. The method involves preparation of o/w emulsion between organic phase (OP) consisting of Etodolac and Eudragit RS100 in dichloromethane (DCM) and aqueous phase (AP), containing 1% w/v PVA. Etodolac and

Eudragit RS100 was dissolved in dichloromethane by sonication (Ampere 60%, 6 minutes) using probe sonicator (Vibra Cell Sonics, India) and the organic phase was emulsified in aqueous phase containing 1% w/v PVA. The emulsion obtained was stirred overnight (12 to 16 hrs) at 25±2^{0}C using magnetic stirrer to ensure complete evaporation of dichloromethane. The nanoparticles thus formed were recovered by centrifugation (12,000 rpm, 20 mins, -10^{0}C) using Remi centrifuge and the precipitate was washed repeatedly (at least 3 times) with ice cold MilliQ (MQ) water to ensure complete removal of traces of polyvinyl alcohol (Madhusudhan *et al.* 2010). Finally, the product was dispersed in cold water and recovered by lyophilisation (Benchtop Pro, SP Scientifics, India). The critical parameters involved are duration of sonication, volume of organic solvent and polymer to drug ratio etc. Finally, three different batches of EtodolacEudragitRS100 nanoparticles were prepared

Physicochemical Characterization of the Nanoparticles

Percentage yield: The dried nanoparticles were weighed and percentage yield of the prepared nanoparticles was calculated by using the following formula [26,27].

$$\text{Percentage yield} = \left(\frac{\text{Weight of nanoparticles}}{\text{Weight of polymer + drug}}\right) \times 100$$

Drug content: The various batches of the nanoparticles were subjected for drug content analysis. Accurately weighed nanoparticles samples were mechanically powdered. The powdered nanoparticles were dissolved in adequate quantity of phosphate buffer PH 7.4 then filtered. The UV absorbance of the filtrate was measured using a UV spectrometer at 272nm.

Drug loading and encapsulation efficiency: Drug loading and encapsulation efficiency was determined for all batches using the following formulas.[11,13] Values are expressed as percentage.

$$\text{Drug loading} = \left(\frac{\text{Weight of drug in nanoparticles}}{\text{Nanoparticles Sample Weight}}\right) \times 100$$

$$\text{Encapsulation efficiency} = \left(\frac{\text{Actual weight of drug in sample}}{\text{Nanoparticles Sample Weight}}\right) \times 100$$

Particle Size Analysis of Nanoparticles

Average particle diameter and size distribution of nanoparticles were determined by laser diffractometry using a Mastersizer 2000 (Malvern Instruments, Malvern, UK). Approximately 10 mg of nanoparticles were dispersed in 2 to 3 ml distilled water containing 0.1% Nonidet P40 for several minutes using an ultrasonic bath. Then, an aliquot of the nanoparticle suspension was added into the small volume recirculation unit [28-32], which was subsequently circulated 3500 times per minute. Each sample was measured in triplicate for the analysis. Particle size was expressed as the weighted mean of the volume distribution.

In-vitro drug release

The USP XXIV dissolution rate testing apparatus was employed to study the release of Etodolac using phosphate buffer PH7.4 as a dissolution medium.100mg equivalent of Etodolac containing Eutragit RS 100nanoparticles was filled in hard gelatin capsule and dissolution test was being carried out at 50 rpm maintained at 37^{o}C±0.5^{o}C. 5ml of sample were withdrawn at specific time interval for 12 hours. The sample volume was replaced by an equal volume of fresh medium. The concentration was determined spectrophotometrically at 272 nm. The same procedure was repeated for other formulations also. The percentage of drug release at various time intervals was calculated and plotted against time [33-38].

RESULTS AND DISCUSSION

Percentage yield

The percentage yield of three formulations were ranging from 87.14to 92.81% respectively (Table 2). The higher percentage yield indicates that this method can be successfully incorporated in the formulation of Etodolac nanoparticles.

Determination of drug content

The percentage drug content was determined for nanoparticles prepared with various polymers: drug ratios and the results were depicted in table 2.Among the three formulationsENF1 has the highest percentage (39.15%) of the drug content followed by other formulations. The reason may be due to higher percentage of loading of drug content with respect to polymer concentration (1:1) as compared with other formulations (1:3 & 1:5) and the formulation ENF1 have shown greater yield.

Drug loading and encapsulation efficiency

The results of the variation in drug loading and encapsulation efficiency among formulation prepared with various polymer: Etodolac ratio is shown in table 3. Higher percentage of loading was obtained by increasing the amount of Etodolac with respect to Eutragit RS 100. The encapsulation process was found to be good and ranges from 78.31% to 85.23%and the drug was successfully employed in the process.

Particle size

Particle size distribution of drug has influence on many bulk properties of pharmaceutical interest such as flow properties, packing, packing densities, compressibility segregation characteristics etc. Hence, particle size distribution is one of the aim of pharmaceutical technologist. All the batches of nanoparticles shows uniform size distribution. The average particle size of Etodolac loaded nanoparticles was found to be in the range of 165 to 192 nm. As the polymer: drug ratio was increased, the size of nanoparticles also increases considerably (table 4). The particle size distribution of etodolac nanoparticles was shown in Figure 1.

In-vitro drug release studies

Nanoparticles of all batches had faster initial drug release approximately 25 percentages within 15 minutes. Then the release was slow and sustained over 12 hours, depending upon the polymer: drug ratio. By the end of 12th hour the percentage of drug release was found to 92.47%, 81.89% and 89.72% for F1, F2 and F3 formulation respectively (figure 1). The formulation F2 showed better sustained release (89.17%) at the end of the 12th hour as compared to other batches. This may be due to better loading, encapsulation efficiency and increased particle size as compared to other batches.

SUMMARY AND CONCLUSION

The Eutragit RS 100nanoparticles of Etodolac were successfully prepared using solvent evaporation technique and confirmed that it is a best method for preparing Etodolac loaded nanoparticles from its higher percentage yield. The formulation ENF2 has highest milligram of drug content followed by other formulations. The percentage of encapsulation of three formulations was found to be in the range of 78.31% to 85.23%. Higher percentage of loading was obtained by increasing the amount of Etodolac with respect to polymer. The particle size of nanoparticles was determined by optical microscopy and Malvern particle size analyser and majority of batches of nanoparticles have shown uniform size distribution. The prepared nanoparticles had good spherical geometry. The *in-vitro* dissolution studies showed that Etodolac loaded nanoparticles formulation ENF3 showed better sustained effect (81.89%) over a period of 12 hours than other formulations.

REFERENCES

1. Vasir JK, Tambwekar K, Garg S (2003). Bioadhesive microspheres as a controlled drug delivery system. Int. J. Pharm. 255: 13-32.
2. Kilicarslan M, Baykara T (2003). The effect of the drug/polymer ratio on the properties of the verapamil HCl loaded microspheres. Int. J.Pharm. 252: 99-109.
3. Gibaud S, Bonneville A, Astier A (2002). Preparation of 3, 4- diaminopyridine microparticles by solvent-evaporation methods. Int. J. Pharm. 242: 197-201.
4. P. Venkatesan, C. Muralidharan, R. Manavalan and K. Valliappan, Selection of better method for the preparation of microspheres by applying Analytic Hierarchy Process. J. Pharm. Sci. & Res. Vol.1(3), 2009, 64-78.
5. Vane, J.R. (1971) Nature New boil, pp 231-235
6. Vane JR, YS Bakhle, and RM Botting. (1998) Annu. Rev. Pharmacol. Toxicol. 38: 97-120
7. Matsuda K, Ohnishi K, Sha T, Yamazaki M, TanakaY, Tanaka K, Jpn. (1982) J Inflam 2: 263
8. Yamaguti T, Kojima T, Kobayashi K, Endo Y, Misawa Y, Nakajima E, Misaka E, Tanaka K, Jpn. (1983) J Inflam 3: 63
9. Naganuma. H, Mochizuki. Y, Kawahara. Y, (1986) J Clin Therap Med 2: 1219
10. Tohru Araki, Teruhiko Yokoyama, Motoo Araki and Seiji Furuya. (2008) J Acta Med Okayama 62: 373-378
11. Shinichi Yoshikawa, Ryo Murata, Shigenari Shida, Koji Uwai, Tsuneyoshi Suzuki, Shunji Katsumata and Mitsuhiro Takeshita. (2010) J Chem Pharm Bull 58: 34-37
12. Thompson.C.J, Hansford. D, Higgins. S, Rostron. C, Hutcheon, Munday. D. L, Evaluation of ibuprofen-loaded microspheres prepared from novel copolyesters, Int.J.Pharm, 329, 2007, 53-61.
13. Ainley wade & Paul J Weller, Handbook of pharmaceutical excipients, second edition, The Pharmaceutical Press, London, 1994, 186-190.
14. T. Sudhamani, K. Noveenkumar reddy, V.R. Ravi Kumar, R. Revathi, V. Ganesan, preparation and evaluation of ethyl cellulose microspheres of ibuprofen for sustained drug delivery, Int.J.Pharma Research and Development. 2010, 119-125.
15. Saravanan. M., Dhanaraju. D, Sridhar. S.K, Ramachandran. S, kishore Gran sam. S, Anand. P, Bhaskar. K & Srinivasarao. G, Preparation, Characterization and *in-vitro* release kinetics of ibuprofen polystyrene microspheres, Ind.J.Pharm.Sci, 66(3), 2004,287-292.
16. Sengel CT, Hascicek C, Gonul N, Development and *In-vitro* evaluation of modified release tablets enclosing ethyl cellulose microspheres loaded with diltiazem hydrochloride, Microencapsule, *2005, 23(2), 135-152*.
17. Das MK and Rao K.R., Evaluation of Zidovudine encapsulated ethyl cellulose microspheres prepared by water in oil in oil (w/o/o) double emulsion solvent diffusion technique. *Acta Pol Pharm 2006 Mar- Apr, 63(2) , 141-148.*
18. Zinutti C, Kedzierewicz F, Hoffman M, Maincent P preparation and Characterisation of ethyl cellulose microspheres containing 5- fluorouracil Microencapsule, *1994 Sep- Oct, 11 (5), 555- 563.*
19. Y. Kawashima, T. Niwa, et al., Journal of Pharmaceutical Sciences, 1992, 81: 135-140.
20. Katare YK, Panda AK. Influence of excipients on *in-vitro* release and *in-vivo* performance of tetanus toxoid loaded polymeric particles. *Eur J Pharm Sci.* 2006 28:179 – 188.
21. Katare YK, Muthukumaran T, Panda AK .Influence of particle size, antigen load, dose and additional adjuvant on the immune response from antigen loaded PLA microparticles, *Int J. Pharm.*2005; 301:149-160.
22. Shaji and Kumbhar, Formulation and characterization of linezolid loaded eudragit RS 100 polymeric nanoparticles, International Journal of Pharmaceutical Sciences and Research IJPSR, 2019; 10(4): 1944-1952
23. C. Karuppusamy, and P. Venkatesan. Role of Nanoparticles in Drug Delivery System: A Comprehensive review, J. Pharm. Sci. & Res. Vol. 9(3), 2017, 318-325
24. Jaganathan Kowshik, Ramesh Nivetha, Singaraj Ranjani, Palanivel Venkatesan, Subramanian Selvamuthukumar, Veeran Veeravarmal Siddavaram Nagini. Astaxanthin inhibits hallmarks of cancer by targeting the PI3K/NF-κB/STAT3 signalling axis in oral squamous cell carcinoma models. International Union of Biochemistry and Molecular Biology,2019:71:1595-1610.

25. S. Madhusudhan, Amulya K. Panda, S. Parimalakrishnan, R. Manavalan and P.K. Manna P.K. (2010). Design, In-vitro and in vivo evaluation of glipizide Eudragit Microparticles, J. Microencapsul., Vol. 27, No. 4, Pages 281-291.
26. P. Venkatesan, V. Sree Janardhanan, R. Manavalan and K. Valliappan, Preformulation parameters characterization to design, development and formulation of loxoprofen loaded microspheres, *Int. J. Pharm. Bio. Res. (IJPBR).* 2011; 2(3):107-117.
27. P. Velavan and P. Venkatesan. Preformulation Parameters Characterization to Design, Development and Formulation of Pregabalin Loaded Nanoparticles. J. Pharm. Sci. & Res. Vol. 6(12), 2014, 436-440
28. R. Sathiya Sundar, A. Murugesan, P. Venkatesan, and R. Manavalan, Formulation Development and Evaluation of Carprofen Microspheres. Int.J. Pharm Tech Research. 2010 Vol.2, No.3, 1674-1676.
29. P. Venkatesan, R. Manavalan and K. Valliappan Microencapsulation: A Vital Technique In Novel Drug Delivery System. J. Pharm. Sci. & Res. Vol.1(4), 2009, 26-35.
30. Trivedi P., Verma A.M.L., Garud N., Preparation and Characterization of Acclofenac Microspheres, Asian Journal of pharmaceutics. 2008;2(2): 110-115.
31. Alfred Martine, physical Pharmacy, 4th edition, BI Waverly pvt Ltd, New Delhi, 1996, 423-431.
32. K.P.R. Chowdry and J. Vijaya Ratna, The Indian Drugs, 1992, 29 : 494
33. Lachman, Liberman, Kaing "Theory and practice of Industrial Pharmacy", 3rd edn: 26-30.
34. R. Rastogi, Y. Sultana, M. Agil, A. Ali, S. Kumar, K. Chuttani, A.K. Mishra, Alginate microspheres of Isonizaid for oral sustained drug delivery. *Int. j. Pharm. 334(2007) page No. 71-77*.
35. Perumal. D, Microencapsulation of ibuprofen & Eudragit RS 100 by the emulsion solvent diffusion technique, Int.J.Pharm, 218, 2001,1-11.
36. Rishibatra, A.K. Madan, Indian Drugs, 1984,23: 691.
37. Sanghvi S.P. and Nain J.G. Journal of Microencapsulation, 1993, 10: 171-180.
38. P. Venkatesan, V. Sree Janardhanan, C Muralidharan, and K. Valliappan, Improved HPLC Method with the Aid of Chemometric Strategy: Determination of Loxoprofen in Pharmaceutical Formulation. *Acta Chim. Slov.* 2012; 59: 242–248.

Table 1. Formulation of Etodolac and Eudragit RS 100 nanopartilces

S. No	Form. Code	D:P	Wt. of Etodolac (mg)	Wt. of Eud. RS100 (mg)	Vol. of DCM (ml)	AP Vol. (1%PVA) (ml)
			Organic Phase			Aq. ph
1	ENF1	1:1	50	50	6	40
2	ENF2	1:3	50	100	6	40
3	ENF3	1;5	50	150	6	40

Table 2. Physicochemical Properties of Etodolac loaded Eudragit RS100 nanoparticles

S. No	Formulation code	Drug: Polymer	Drug content (%)*	Encapsulation Efficiency* (%)	Particle Size (nm)	% of Yield
1	ENF1	1:1	39.15 ± 0.23	78.31 ± 0.55	181	87.14
2	ENF2	1:3	21.17 ± 0.32	84.67 ± 0.89	165	88.51
3	ENF3	1:5	14.21 ± 0.27	85.23 ± 1.19	192	92.81

* The values are represented as Mean ± SD for n = 6.

Note: ENF indicatesEtodolac loaded Eudragit RS100 nanoparticles;

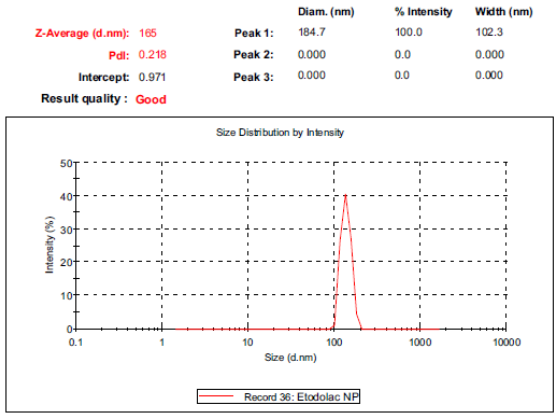


Figure 1. Particle size of Etodolac loaded nanoparticles

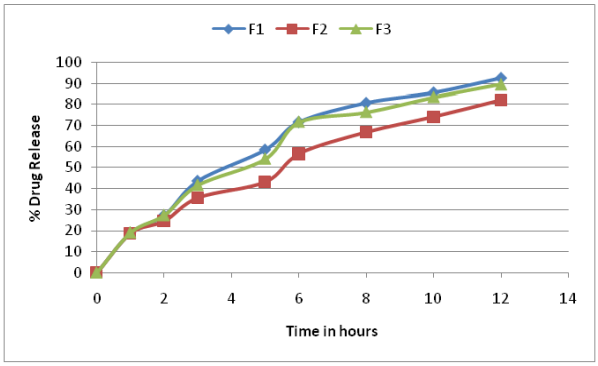


Figure 2. *In-vitro* drug Release Profile of Formulation F1, F2, F3

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Identification of the Existing Feeding Practices of Captive Asian Elephants Reared in Temples of Kerala

Nandana Chandrasekhar[1]*, Ajith K. S. [2], Ally K[3], Deepa A[2] and Rajeev T. S[4]

[1]MVSc Scholar, Department of Animal Nutrition, Kerala Veterinary and Animal Sciences University, Pookode, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India.
[2]Assistant Professor, Department of Animal Nutrition, Kerala Veterinary and Animal Sciences University, Pookode, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India.
[3]Professor and Head, Department of Animal Nutrition, Kerala Veterinary and Animal Sciences University, Pookode, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India.
[4]Associate Professor, Department of Veterinary and AH Extension, Kerala Veterinary and Animal Sciences University, Pookode, College of Veterinary and Animal Sciences, Mannuthy, Thrissur, Kerala, India.

Received: 28 Nov 2020 Revised: 22 Dec 2020 Accepted: 11 Jan 2021

***Address for Correspondence**
Nandana Chandrasekhar
MVSc Scholar,
Department of Animal Nutrition,
Kerala Veterinary and Animal Sciences University,
Pookode, College of Veterinary and Animal Sciences,
Mannuthy, Thrissur, Kerala, India.
Email: nandanachandrasekhar@gmail.com

ABSTRACT

The study was conducted to understand the feeding systems followed among captive Asian elephants in Kerala under Temple ownership. A field survey was conducted to analyse the type of feed given to captive Asian elephants in temple management system in Kerala. Nine animals were selected for the purpose of sampling for the survey. All the animals of temple surveyed were stall fed. The study revealed that in non-festive seasons, the major concentrates offered to captive Asian elephants included rice, rice flakes and dates and the major roughage sources included palm leaves, grass, coconut tree leaves and plantain stem. On an average, elephants of temples in Kerala received 5.44±1.75 kg concentrates and 366±25.35 kg roughages. Proximate composition of the feeds given were analysed. Vitamin and mineral supplements were given to 22.20 per cent of the elephants and 54.54 per cent received special diets.

Keywords: Captive Asian elephants, feeding system, Temple ownership, Survey, Kerala

INTRODUCTION

Kerala has about 482 captive Asian elephants in 2020 (KCEMS, 2020), which accounts for nearly 12 per cent of the elephant population of the state. Captive Asian elephants in Kerala are managed under three different systems such as elephants owned by individuals, elephants owned by temples and elephants of forest camps (Govind *et al*., 2017). Temples in Kerala owns about 122 captive temple elephants (KCEMS, 2020). Temple elephants are mainly used for religious ceremonies, as exhibits and are also leased out. Elephants are mega herbivores animals (McKay, 1973) and are hindgut fermenters (Hatt and Clauss, 2006). Elephants generally spend 60 per cent of daylight hours for feeding, 20 per cent for resting, 14 per cent for moving and six per cent for other activities (Bhaskaran *et al.,* 2010). So proper feeding management is very crucial for elephants in captivity to ensure its health and wellbeing (Stevenson and Walter, 2006). Since elephants in captivity solely depend upon food provided by the management or mahout, it becomes necessary to evaluate whether food and feeding practices followed are similar to their natural food preferences. Hence the study has been undertaken with the objective of identifying the existing feeding practices of captive elephants in temples of Kerala.

MATERIALS AND METHODS

A survey was conducted to study the feeding systems followed among captive Asian elephants in temples of Kerala. The survey included discussion with elephant owners and mahouts and collection of feed samples for nutritive evaluation. Nine elephants maintained by temple trusts in Thrissur district were selected for the purpose of sampling for survey. A detailed questionnaire was prepared incorporating details regarding identity of elephants, quantity and type of concentrate feed and roughage offered to elephants, time of feeding and change in feeding patterns of elephants. The nutrient contents of feeds offered were done as per AOAC (2016). Statistical analysis was done using various statistical techniques like mean, percentage and standard error.

RESULTS AND DISCUSSION

All the elephants were tethered to a point and were stall fed. Concentrate feeds given to Elephants in temples of Thrissur district of Kerala included rice, rice flakes and dates. Roughages offered were palm leaves (*Caryota urens*), grass (*Pennisetum purpureum*), coconut leaves (*Cocos nucifera*) and plantain stem. The concentrate and roughage given differed with each temple management. The amount of feed offered is based on local knowledge and feeding based on body weight is mostly not followed among temple elephants. Elephants are given concentrates mainly in pre-monsoon season and in association with the musth status of the animal. During festive seasons, animals have got access to only roughages. Consolidated data on number of elephants, given different varieties of feed and roughages and their Chemical composition are given in Table 1 and 2 respectively. The feeding habits of elephants in wild changes according to seasons and they feed on predominantly a browse diet during dry season and grass diet during monsoon and winter seasons (Joshi and Singh, 2009) and this may be due to the unavailability of fresh grass. This is in contrast to the feeding pattern followed in captivity in the temples, where they are offered monotonous fodder round the year (Vanitha *et al*., 2008 and Govind *et al*., 2017). The amount of feed and fodder offered were based on mahout's or owner's knowledge, except in case of two elephants, where feeding based on body weight of animal was practised. Elephants of temples are not offered concentrates daily and this is in contrast to ICAR (2013) recommendations which suggests a daily supply of nearly 6 kg concentrates to a 4000 kg bull elephant. ICAR (2013) also recommends to provide 225 kg of green fodder to Asian elephants. The present study revealed that the mean amount of total roughage supplied was about 366.00±25.35 kg. So, the required ICAR recommendation is met in captivity. Feeding roughage in excess has led to feed wastage in field condition which is mainly due to discrepancy in feeding management. Since the body weight of the elephants and quantity of feed residue left behind were not calculated, comparison of intake could not be made with metabolic body weight.

Out of nine elephants surveyed, only two elephants were given vitamin and mineral supplements. Vitamin and mineral supplements especially for elephants are not available in the state and supplements intended for ruminants like Agrimin Forte and sharkoferrol-vet were generally given orally at the rate of 150 g and 150 ml respectively. Temple elephants in Kerala receive special diets like ashta choornam and navadhaanyam and is offered mainly during the pre-monsoon season, as part of restorative treatment followed among elephants in the state. The elephant owners believe that providing these special diets during pre-monsoon season helps the animal to improve its body condition so as to make the animal physically fit for the festive seasons. Ashta choornam, is an ayurvedic preparation, supposed to improve digestion in elephants and increase their appetite. Navadhanyam is a mixture of cereals and pulses, which include, wheat, rice, chick peas, white beans, black sesame, Indian black lentil, horse gram, pigeon pea and green gram. One day prior to feeding, the ingredients are soaked in water and are then boiled and made into a bolus, which is offered to elephants. Navadhanyam is given to improve the body conditions of elephants. Among the surveyed population, 54.54 per cent of the elephants, received ashtachoornam and navadhaanyam. 27.27 per cent of the surveyed elephants were given 50 g of ashtachoornam per day mixed with rice and nearly 5.00 kg of navadhanyam is offered to 27.27 per cent of the elephants. In the absence of grazing, feed and roughages available to the elephants in captivity is limited to those given by mahouts. Roughage is offered throughout the day. Rice is offered to 66.7 per cent of the elephants in the evening, whereas 33.3 per cent of the elephants do not receive rice. Other concentrates like rice flakes and dates are also offered in the evening to 33.3 per cent of the population and 66.7 per cent of the population are not given dates and rice flakes.

CONCLUSION

From the study, it is concluded that, the temple elephants in Kerala are mainly fed monotonous feed and fodder round the year. The quantity of concentrates and roughage offered is sufficient as per ICAR (2013). A suggestion can be made to elephant owners as to provide more varieties of fodder, offer concentrates on daily basis and feed the animal based on its body weight. This study focussed on the feeds offered to captive Asian elephants owned by temples and in order to understand the efficiency of the elephants in utilizing the offered feeds, further studies including digestibility trials are needed.

REFERENCES

1. AOAC. 2016. *Official Methods of Analysis*. (20th Ed.). Association of Official Analytical Chemists, Rockville, Meryland, USA. pp. 1-85.
2. Baskaran, N., Balasubramanian, M., Swaminathan, S. and Desai, A.A. 2010. Feeding ecology of the Asian elephant *Elephas maximus* Linnaeus in the Nilgiri Biosphere Reserve, southern India. *J. Bombay Nat. Hist. Soc.* 107 (1):3-13.
3. Govind, D.K., Deepak, M., Rajeev, T.S., Suraj, P.T. and Anil, K.S. 2017. Comparison of management systems of captive elephants in forest camps of Kerala and Tamil Nadu. *J. Indian Vet. Ass. Kerala (JIVA*). 15 (2):28-33.
4. Hatt, J.M. and Clauss, M. 2006. Feeding Asian and African elephants *Elephas maximus* and *Loxodonta africana* in captivity. *Int Zoo Yearb.* 40 (1):88-95.
5. ICAR [Indian Council of Agricultural Research]. 2013. *Nutrient requirements of companion, laboratory and captive wild animals.* (1stEd.). Indian Council of Agricultural Research, New Delhi, 40p.
6. Joshi, R. and Singh, R. 2009. Movement and ranging behaviour of Asian elephants *Elephas maximus* in and around the Rajaji National Park, North-West India. *Nat. Sci.* 7 (2): 76-94.
7. Kerala Forest and Wildlife Department. Kerala captive elephant management system. http:/www.kcems.in. [2 Dec. 2020]. (KCEMS, 2020).
8. McKay, G.M., 1973. Behavior and ecology of the Asiatic elephant in southeastern Ceylon. *Smithsonian Contrib. Zool.* 125 :1-113.

9. Stevenson, M.F. and Walter, O. 2006. Elephants: *Loxodonta africana*and *Elephas maximus*. In *Management Guidelines for The Welfare of Zoo Animals*. (2nd Ed.). British and Irish Association of Zoos and Aquariums, London, 163p.
10. Vanitha, V., Thiyagesan, K. and Baskaran, N. 2008. Food and feeding of captive Asian Elephants (*Elephas maximus*) in the three management facilities at Tamil Nadu, South India. ***Sci. trans. env. Technovation***. 2 (2):87-97.

Table 1. Concentrates and roughages offered to elephants of temples in Kerala

Feed	Number of elephants given different feeds and roughages	Average quantity of feed and roughage offered (kg/day) *
Rice	6	4.83 ± 0.48
Rice flakes	3	4.67 ± 0.33
Dates	2	3.00 ± 0.00
Palm leaves	9	326.11 ± 32.81
Grass	5	68.00 ± 16.17
Coconut leaves	2	170.09 ± 0.28
Plantain stem	2	45.02 ± 0.56

* Mean values are for the number of elephants mentioned that are offered the particular feed and roughage among the total elephants surveyed.

Table 2. Proximate composition of feed offered to captive Asian elephants (%Dry matter basis)

Ingredients	Dry matter	CP	CF	EE	NFE	TA	NDF	ADF	AIA
Rice flakes	86.16	6.86	0.40	1.61	84.87	6.26	18.93	11.59	1.63
Boiled white rice	31.92	8.57	0.68	0.29	90.10	0.36	48.79	2.28	0.16
Dates	86.08	4.11	1.60	0.70	91.75	1.84	8.03	6.27	0.08
Hybrid Napier	23.25	10.23	28.73	2.19	49.4	9.45	64.41	42.53	1.46
Palm leaves	31.12	7.69	37.06	1.18	45.52	8.55	63.28	46.51	6.8
Plantain stem	8.32	2.54	21.77	1.56	67.48	6.65	51.92	30.95	0.29
Coconut leaves	33.01	6.98	28.86	2.18	54.26	7.72	27.98	41.79	1.13

CP – crude protein, CF- Crude fibre, EE – Ether extract, NFE- Nitrogen free extract, TA – Total ash, NDF- Neutral detergent fibre, ADF-Acid detergent fibre, AIA- Acid insoluble ash

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Analysis of School Students' Mathematical Innovative Thinking Abilities

Ashim Bora*

Associate Professor and Head, Department of Mathematics, Diphu Government College, Diphu, Assam, India.

Received: 23 Nov 2020 Revised: 25 Dec 2020 Accepted: 27 Jan 2021

***Address for Correspondence**
Ashim Bora
Associate Professor and Head,
Department of Mathematics,
Diphu Government College,
Diphu, Assam, India.

ABSTRACT

Mathematics is a complex topic involving high-level thinking ability. The ability to think innovatively is one of this mastery, so students need to develop their mathematical creative thinking skills so that they can help students overcome challenges in mathematics learning in schools as well as problems relating to everyday life. This study targets to determine the strength of school learners' innovative mathematical thinking skills in terms of gender. With a comparable quantitative approach, the tool used in the analysis is descriptive. The methodology used in the sampling was the deliberate sampling of 122 students enrolled in class VIII. Data retrieval is done with a test instrument in the form of a description of 6 questions by analyzing the results of student answers to indicators of innovative mathematical thinking abilities. The results of descriptive statistics show that the average mathematical innovative thinking ability of male and female students belongs to the low category, the total score of male learners is 29.5 with mean value of 65.3 and the total outcome of female learners is 24.7 with mean value of 59.8. The findings of the analysis indicate that male students displayed a 51.7 percent intensity of creative mathematical skill in the information predictor.

Keywords: Mathematical Thinking, Innovative Thinking Ability, Secondary Schools.

INTRODUCTION

Starting from elementary school level to college, Mathematics is always treated as a standout amongst the most significant subjects. One of the subjects underlying human life is mathematics. From the beginning of its discovery, mathematics continued to improve dynamically along with change of times. The improvement of mathematics has never stopped because mathematics will continue to be needed in various aspects of human day to day life. If we

examine the education curriculum, mathematics is a subject that influences the improvement of science as well as technology. The headway of present science just as technology is evidence of the development of thinking skills. This ability to think is inseparable from eminent thinking power (Higher order thinking power). One of these excellent thinking qualities is the ability to think innovatively. The proficiency to think innovatively is one of the targets of "The National Curriculum Framework-2005", concerning content standards for elementary as well as secondary education in India. Based on the NCF-2005, mathematics subject is very closely associated to the potentiality to think creatively.

Suciu (2014), Alvonco (2013), Alghafri and Ismail (2014) suggested several benefits of creative thinking, namely generating new ideas and concepts, developing new ways, finding new innovations, carrying out continuous improvement processes, developing problem solving skills by finding creative solutions to problems in complex life, ability positive thinking in seeing things and so on. One of the benefits of creative thinking skills is the discovery of Braille for blind people. So many benefits that we can get with the capacity to think innovatively, it needs to be improved and developed that power, especially in learning mathematics. It is expected that with the capacity to think creatively students can easily solve mathematical problems with various possible solutions. As expressed by Edward and David (1982), creative and basic reasoning ought to be incorporated into the educational programs through the unique circumstance, exercises and questions in the school mathematics. Regardless of whether scientific research has demonstrated the significance of innovation, educators are not urged to utilize innovative reasoning aptitudes on their students (Smith, 2006).

In fact, innovative thinking skills are still an education issue in India. The level of potential of students to think innovatively is still limited. India is ranked 99th out of 139 countries, according to Global Creativity Index (GCI) info. GCI of the first ranking Australia is 0.95 and that of India is only 0.292. This demonstrates that the capacity to think creatively in India is still very low. The low dimension of learners' innovative reasoning aptitudes in learning mathematics is impacted by a few elements. One of the prime factors that impact learners' innovative reasoning abilities is gender difference (Kyunghwa and Hyejin, 2016; Al-sulaiman, 2009; Baer and Kaufman, 2008). Psychological and physiological influences can be influenced by gender differences and this can contribute to differences in the manners and academic outcomes of male and female pupils. Sex gaps manifest in differences in mathematical capabilities and in the learning of mathematical expertise. Sex, social, and cultural aspects interact very closely with the conceptualization of education in mathematics. Sex, social and cultural factors thus affect mathematics education. Compared to female students, male students were more interested in studying mathematics, because female students were more worried about mathematics than male students (Mann, 2005; Manohara and Ramganesh, 2009; Johny, 2011). But some researchers (Middents, 1970; Desai, 1987) found that there is no gender difference in solving algebra and geometry problems. Reddy (2008) suggested that in terms of verbal, non-verbal and composite creativity, male and female teacher learners do not vary significantly. Based on the conditions above, the researcher considers the need for an analysis of mathematical innovative reasoning abilities to have the capacity to know and uncover any phenomena that affect mathematical creative thinking capacity as one of the answers to the challenges of the times.

Mathematical Innovative Thinking Abilities (MITA)

Inventive thinking in mathematics alludes to the thought of imaginative thinking when all is said in done. An individual requires to correlative models of various reasoning in science, in particular the inventive reasoning that is both instinctive and explanatory reasoning that is legitimate. This understanding demonstrates that innovative thinking is not based on logical thinking, but rather as an idea that is suddenly manifest, unexpected and unusual. Creative thinking can be seen in the form of a combination of logical thinking and different thinking which is based on intuition but is still in consciousness. Now when an individual applies imaginative rationale to the act of taking care of an issue, natural reasoning is connected to produce new thoughts. Finding an answer for an issue is valuable. This interpretation explains that the concepts theoretically contribute to constructive reasoning and intuitive thought. That is, in order for the equilibrium of reasoning and imagination to be very important, the brain would

need two pieces of imaginative thought. Logical deduction positioning would neglect so many imaginative concepts. A person who is free thinking means thinking not under the control or pressure. The freedom of thought needed to bring innovative thinking. Creative thinking, according to Krulik and Rudnick (1999), is the original, reflective thought that creates a complex object. Gotoh (2004) portrayed three phases of advancement of mathematical reasoning in critical thinking. They are the empirical action, the algorithmic movement and the constructive action. The beginning phase is informal, second one if formal and the third phase is innovative. Basic reasoning believes that looks at, relates, and assesses all parts of a circumstance or issue. Inventive reasoning is imagining that is unique and intelligent and that delivers an unpredictable item. Viewpoint on mathematical innovative thinking alludes to the blend of consistent and divergent thinking which depends on instinct yet is a conscious goal. In line with Haylock (1997), the contrasting thinking is centered on flexibility, articulacy, and novelty. The integration of thoughts, the development of new ideas and the assessment of their usefulness are involved in thinking. In addition, it also requires the freedom to make choices and create new items.

Purpose of the study

The motivation behind this examination is to break down learners' mathematical inventive thinking abilities dependent on gender perspectives. This investigation focuses to portray the intensity of inventive thinking capacities of male and female learners studying in VIII in solving mathematical problems. The outcomes of this study are expected to provide benefits for several parties, including:

i) Answering the curiosity of researchers towards students' mathematical creative thinking abilities based on gender.
ii) As a reference for teachers in making improvements to the learning process by providing special attention to gender factors.
iii) As a reference material for researchers who are interested in conducting further research regarding the analysis of students' mathematical creative thinking skills in terms of gender.

RESEARCH METHODOLOGY

Population and Sample

All high school students enrolled in class 8 in schools situated in a hilly area of Assam, India, were the focus population for this research. A selection of 122 students from secondary schools located in urban and rural areas was calculated using purposeful sampling. 60 students are taken from rural schools and 62 from urban schools. 61 students are male and 61 are female students.

Research Technique

The technique employed in this study is to focus more on indicators of students' mathematical innovative thinking skills. Four indicators are applied in the study. These indicators are (1) Fluency: the number of ideas produced, (2) Flexibility: the diverse classifications of thoughts delivered, (3) Elaboration: adornment and improvement of a thought, (4) Originality: the unusualness or irregularity of a perception or its uncommonness. The table 1 shows indicators of Innovative Thinking Ability and Sub indicators of Innovative Thinking Ability.

Research Instrument

Analysts built up an exploration device called MITA (Mathematical Innovative Thinking Ability) to test the innovative thinking ability of secondary school students. It included 28 self-reported items on five points Likart-scale format. The instrument indicated four mathematical innovative abilities, fluency, flexibility, amplification and originality.

Data Analysis

Throughout this report, researcher uses descriptive statistics in data processing techniques as percentage, mode and mean. Researchers compiled several tools in the form of a description test before the research was conducted. This test aims to determine the innovative mathematical thinking abilities of students. To collect data, the results of the test are used. The data obtained is the result of student answers that have been gathered based on the indicators of mathematical innovative thinking ability tests with the results found in tables 2 and 3. It appears to be shown in the table above that the consistency of the mathematical inventive thinking capacity of male learners is superior to female students in the flexibility variable (5.5) and originality variable (8.8), while female students are the strength of numerical innovative thinking prevailing in two other points, viz fluency (4.1), elaboration (6.9). Based on the results of the total score and the average value, it is shown that male students are dominant in students' creative mathematical reasoning skills with the attainment of an overall total score of 29.5 with a mean value of 65.33. The results of these data are in the table 3. However, the mathematical creative thinking capacity of students on fluency and elaboration indicators is categorized into low categories based on the outcomes of the percentages and categorization of indicators, while versatility indicators and originality indicators are categorized into medium categories. The categorization of data is adapted based on three levels of categories namely high, medium, and low. The percentage results and categorization of data are in tables 4 and 5

DISCUSSION AND CONCLUSION

The motivation behind this investigation is to examine learners' numerical innovative reasoning aptitudes dependent on gender orientation viewpoints. The specialist estimated the numerical imaginative reasoning capacities of learners by utilizing MITA (Mathematical Innovative Thinking Ability) instrument, test tools rely on measures of innovative mathematical thinking skills that combine fluency, versatility, elaboration, and originality. In view of the consequences of the information that have been analyzed, it is found several facts about the strength of students' innovative mathematical thinking abilities. The analysts found that the quality of MITA of male students was superior to female learners in the indicator of flexibility which was equal to 50.4% and in the indicator originality with percentage 68, while the indicators of fluency and elaboration were superior to male students with percentage of 34.4% and 56% respectively. The indicator fluency was of low category as both male learner and female learners' posses less than 40%. Flexibility and elaborator showed moderate categories and originality indicator was of high category of both the innovative thinking abilities of male learners and female learners.

SUGGESTIONS

From the outcomes of the present study, the researchers may suggest the followings

i) Secondary school teachers are expected to provide more opportunities and chances for their students to develop innovative thinking skills, especially in mathematics
ii) Teachers are expected to pay more consideration regarding the elements that can influence the ability of students to develop mathematical innovative thinking skills, one of which is gender.
iii) It is expected that the next researcher will be able to perform a more in-depth study of the innovative mathematical reasoning abilities of students, both in terms of gender and other factors.

REFERENCES

1. Alghafri, A. R. and Ismail, H. N. (2014). The Effects of Integrating Creative and Critical Thinking on Schools Students' Thinking. *International Journal of Social Science and Humanity*, 4(6), 518-525.
2. Al-sulaiman, N. (2009). Cross- Cultural Studies and Creative Thinking Abilities. *Umm Al-Qura University Journal of Educational & Psychological Sciences*, 1(1), 42-92.

3. Alvonco, J. (2013). *The Way of Thinking*. Jakarta: Elex Media Komputindo.
4. Baer, J. and Kaufman, J. C. Gender Differences in Creativity. *Journal of Creative Behavior*, 42(2), 75-105.
5. Desai, N. N. (1987). *An Investigation into the Creative Thinking Ability of Students of Higher Secondary of Gujarat State in the Context of some Psycho-socio Factors*. PhD Thesis, Dept of Education, Sardar Patel University, Gujarat.
6. Edward, L. D. and David, E. K. (1982). *American Educators Encyclopedia*, Westport, CT: Green Wood Press.
7. Gotoh, G. (2004). *The Quality of the Reasoning in Problem Solving Processes*. The 10th International Congress on Mathematical Education, July 4-11, 2004. Copenhagen, Denmark.
8. Haylock, D.(1997). Recognizing Mathematical Creativity in Schoolchildren. *ZDM-Mathematics Education*, 29(3), 68-74.
9. Johny, S. J. (2011). *Attitude, Gender and Socio-Economic Status as correlates of mathematical creativity of secondary school students in Uganda*. PhD Thesis, Makerere University.
10. Krulik, S. and Rudnick, J. A. (1999). Innovative Tasks to Improve Critical and Creative Thinking Skills. In V. Lee and F. R. Curcio (Eds.), *Developing Mathematical reasoning in Grades K-12* (pp.138-145). Reston, Virginia: The National Council of Teachers of Mathematics, Inc.
11. Kyunghwal, L. and Hyejin, Y. (2016). Cross-cultural Research on the Creativity of Elementary School Students in Korea and Australia. *Universal Journal of Educational Research*, 4(11), 2618-2626.
12. Manohara, J. L. and Ramganesh, E. (2009). Creative problem solving ability of standard XI students. *Edutracks*, 9(4), 29-31.
13. Mann, E.L. (2005). *Mathematical Creativity and School Mathematics: Indicators of Mathematical Creativity in Middle School Students*. PhD Thesis, University of Connecticut.
14. Middents, G. J. (1970). The Relation of Creativity and Anxiety *Journal of Religion and Health*, 9(3), 258-275.
15. Reddy, B. S.V. (2008). Creativity of Student Teachers of College of Education. *Edutracks*, 7(12).
16. Haring-Smith, T. (2006). Creativity research review: Some Lessons for Higher Education". *Peer Review*, 8(2), 23-28.
17. Suciu, T. (2014). The Importance of Creativity in Education. *Bulletin of the Transilvania University of Braşov Series V: Economic Sciences*, 7(56), 151-158.

Table 1. Indicators of Innovative Thinking Ability and Sub indicators of Innovative Thinking Ability

Indicators	Sub Indicator
Fluency	Students can answer mathematics problems with relevant answers and smooth flow of thoughts
Elaboration	Students can provide detailed information and expand the problem area given
Flexibility	Students can use several approaches to solve issues or provide some correct answers
Originality	Students can answer math problems by using their own language, method or idea

Table 2: Average and modal Scores of Students' MITA

Indicator	Average Score		Modal Score
	Male	Female	
Fluency	3.7	4.1	10
Flexibility	5.5	5.0	12
Elaboration	6.8	6.9	11
Originality	8.8	8.0	12
Total	6.2	6.0	-

Table 3. Descriptive Statistics of Students' MITA

Gender	Average Total Score	Mean
Male	29.5	65.3
Female	24.7	59.8

Table 4. Percentage of Indicators of MITA

Gender	Percentage of MITA Measures				
	Fluency	Flexibility	Elaboration	Originality	Total
Male	33.0%	50.4%	55.2%	68.0%	51.7%
Female	34.4%	48.0%	56.0%	63.3%	50.4%

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antifungal Activity of Turmeric (*Curcuma longa*) Rhizome against Different Fungi

Kalpa Oza[1], B. K. Jain[2]* and Bharat Maitreya[3]

[1]Ph.D. Research Scholar, Department of Botany, Bioinformatics, Climate Change Impacts Management, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.
[2]Principal, M. G. Science Institute, Ahmedabad, Gujarat, India.
[3]Professor, Department of Botany, Bioinformatics, Climate Change Impacts Management, University School of Sciences, Gujarat University, Ahmedabad, Gujarat, India.

Received: 05 Nov 2020 Revised: 27 Dec 2020 Accepted: 13 Jan 2021

***Address for Correspondence**
B. K. Jain
Principal,
M. G. Science Institute,
Ahmedabad, Gujarat, India.
Email: bkjain_mgsc@yahoo.com

ABSTRACT

Haldi (Turmeric) scientifically known as *Curcuma longa* belongs to family *Zingiberaceae*. Its polyphenolic compound curcumin has been variety as of antifungal investigations due to extensive traditional uses and very low side effects. In present work antifungal activity for row turmeric powder against *Aspergillus sp*. And *Fusarium sp.* And different solvent has been reported. The promising results for antifungal activity of *curcuma longa* made itself a good candidate to enhance the inhibitory effect of existing antifungal agents. Basic aim of study is to prof that turmeric can use as natural antifungal agent.

Keywords: Antifungal, Curcumin, Fungi, Haldi, Turmeric.

INTRODUCTION

Herbal plant extracts used as medicine also used as a replacement for synthetic drugs. Plant play the significant role in remedy and many drugs which are used the derivatives from plants. In Ayurveda turmeric is used for medicine, it also used in Unani and Siddha medicines. Turmeric, scientific name *Curcuma longa* belongs to *Zingiberaceae* or ginger family. Turmeric is known as Indian golden spice [1]. *Curcuma longa* is tall and perennial plant which have underground rhizomes and those rhizomes were mostly ovate, ablong, pyriform and short branched plant. It is act as a scavenger of oxygen free radicals and also helps to protect the oxidations of hemoglobin. Turmeric will help to destroy the growth of cancer cell and helps to cure prostate and breast cancer [2]. *Curcuma longa* is commonly known as amba haldar. It is used to relieve hiccups in infusion and the most common recipe for it is a pickle [3]. It is also used for base of some perfumes. Low concentration of curcumin, volatile oil from wild turmeric exhibit anti-

inflammatory property and also has wound healing property. It is used as cream for its healing property. It also helps in skin irritation, bruise and quickly healing sprains. It has antifungal action against pathogens causing infections in the body. It is highly used in beautification specially for pimples and dark spots [4]. The present study based on antifungal activity of row turmeric powder with methanol and aqueous extract against *Aspergillus sp.* and *Fusarium sp.* both.

MATERIALS AND METHODS

Collection of plant material

Collecting the rhizome of turmeric from Ahmedabad dist. and air dried for 10days.

Extract preparation method

Two conical flasks were selected and weighed powder transformed into these conical flasks. Solvent methanol and distil water were added. Cover the flask with aluminum foil. Both sets were kept on shaker for 24 hrs. after that both extracts separately filtered with help of whatman filter paper number one. After filtration transfer it into petri plates and allow it open for few hours for solvent evaporation. After some days both extracts were ready [5].

Preparation of liquid extract series for antifungal test

Crude extracts were weighed with help of weighing balance (mg) and solvent were added in appropriate proportion like 5mg extract in 2ml solvent, 10mg/2ml, 15 mg/2 ml, 20 mg/2 ml solvent (Table 1 and 2). Here two solvent named Methanol and Aqueous (distil water) were used for liquid extract preparation [6].

Antifungal Activity

In this study agar well diffusion method selected for antifungal activity. 20 to 25 ml of potato dextrose agar medium was poured in sterilized petri-plates and allowed it to solidify at 38°C. after fully set PDA medium plates become set fungal culture will striking on plate under aseptic condition. Several wells of 2mm were punched by using cock borer.100µl of extract was poured. The petri-plates were incubated for growth at room temperature. Inhibition zone was measured with transparent zone scale, one mm or more was considered as positive results [7].

RESULT

The results obtained shows methanol extract against *Aspergillus sp.* that 5 mg concentration of extract result in 2.0 mm zone of inhibition, 10 mg concentration of extract result in 2.5 mm zone of inhibition, 15 mg concentration of extract result in 7.5 mm zone of inhibition and 20 mg concentration of extract result in 8.0 mm zone of inhibition. In Aqueous extract against *Aspergillus sp.* that 5 mg concentration of extract result in 1.5 mm zone of inhibition, 10 mg concentration of extract result in 2.5 mm zone of inhibition, 15 mg concentration of extract result in 3.0 mm zone of inhibition and 20 mg concentration of extract result in 4.5 mm zone of inhibition recorded. The results obtained shows methanol extract against *Fusarium sp.* that 5 mg concentration of extract result in 2.0 mm zone of inhibition, 10 mg concentration of extract result in 2.5 mm zone of inhibition, 15 mg concentration of extract result in 3.0 mm zone of inhibition and 20 mg concentration of extract result in 3.5 mm zone of inhibition. In Aqueous extract against *Aspergillus sp.* that 5 mg concentration of extract result in 1.5 mm zone of inhibition, 10 mg concentration of extract result in 2.0 mm zone of inhibition, 15 mg concentration of extract result in 3.0 mm zone of inhibition and 20 mg concentration of extract result in 4.0 mm zone of inhibition recorded.

CONCLUSION

The results obtained in present study indicates that *Curcuma longa* is rich in different phytochemicals. *Curcuma longa* shows the antifungal activity against *Aspergillus sp.* And *Fusarium sp*. *Curcuma longa* having more antifungal potential as compare to other plants. Some researcher observes that the purified *C. longa* lectin at a dose of 47 and 94 µg/0.3 cm2 disc showed antifungal activity against the 3 tested phytopathogenic fungal species, *E. turicicum, F. oxysporum,* and *C. cassiicola.* While the lectin dose of 47 µg/0.3 cm2 disc slightly inhibited the growth of these 3 fungi, that at 94 µg/0.3 cm2 disc showed a higher and significant degree of antifungal activity on all3 isolates [8].The antifungal activity with methanol, chloroform, n-hexane and water at room temperature and 121°C extracted turmeric against *Candida albicans* [3].

ACKNOWLEDGMENT

The authors are thankful to Professor and Head of the Department of Botany, Bioinformatics, Climate Change Impacts Management; Dr. Archana Mankad for providing all the facilities for this work.

REFERENCES

1. Joshi B, Pandya D, Mankad A. Comparative study of Phytochemical Screening and Antibacterial Activity of *Curcuma longa* (L.) and *Curcuma aromatic* (Salib.). Journal of Medicinal Plant Studies. 2018; 6:145-48.
2. Akter J, Md. Hossain A, Sano A, Takara K, Md. Islam Z, and De-Xing Hou. antifungal activity of various species and strains of turmeric (curcuma spp.) against fusarium solani sensu lato. Pharmaceutical Chemistry Journal. 2018; 52.
3. Gul P& Bakht J. Antimicrobial activity of turmeric extract and its potential use in food industry. J Food Sci Technol. 2013.
4. Sharma R, Sharma G and Sharma M. Additive and inhibitory effect of antifungal activity of Curcuma longa (Turmeric) and *Zingiber officinale* (Ginger) essential oils against Pityriasis versicolor infections. Journal of Medicinal Plants Research 2011; 532: 6987-90.
5. Prajapati M, Pandya D, Maitrey B. Comparative Phytochemical screening and Antibacterial activity of leaf and flowering bud of *Syzygiumaromaticum*L. International Journal of Botany Studies. 2018; 3:56-60.
6. Thalian N, Pandya D, Pandya H. Comparative Phytochemical Screening and Antibacterial Studies of *Cordia sebestena*(L.) leaves and flowers. International Journal of Research and Analytical Reviews 2018; 4: 1634-42.
7. Jungharea A, Nasareb P. N, Bhowl M. Fungal Diversity in Fruits and their Impact on Human Health and Businessman with Reference to Losses. Online International Interdisciplinary Research Journal. 2015; 5:166-72.
8. Petnual P, Sangvanich P, Karnchanatat A. A Lectin from the Rhizomes of Turmeric (*Curcuma longa* L.) and Its Antifungal, Antibacterial, and α-Glucosidase Inhibitory Activities. Food Sci. Biotechnol. 2010; 4: 907-16.

Table 1: Shows zone of inhibition of turmeric against *Aspergillus sp.*

Extracts	Extract concentration (Mg/2 ml)				
	Control	5mg/2ml	10mg/2ml	15mg/2ml	20mg/2ml
Methanol	0.0 mm	2.0 mm	2.5 mm	7.5 mm	8.0 mm
Aqueous	0.0 mm	1.5 mm	2.5 mm	3.0 mm	4.5 mm

Table 2: Shows zone of inhibition of turmeric against *Fusarium sp*

Extracts	Extract concentration (Mg/2 ml)				
	Control	**5mg/2ml**	**10mg/2ml**	**15mg/2ml**	**20mg/2ml**
Methanol	0.0 mm	2.0 mm	2.5 mm	3.0 mm	3.5 mm
Aqueous	0.0 mm	1.5 mm	2.0 mm	3.0 mm	4.0 mm

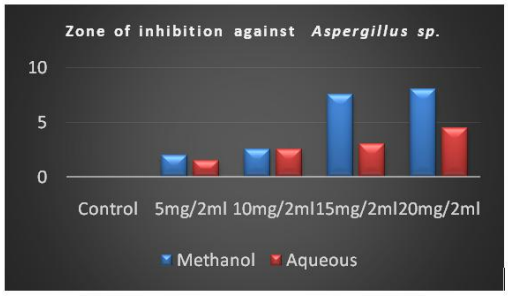


Chart 1: Shows zone of inhibition of turmeric against *Aspergillus sp.*

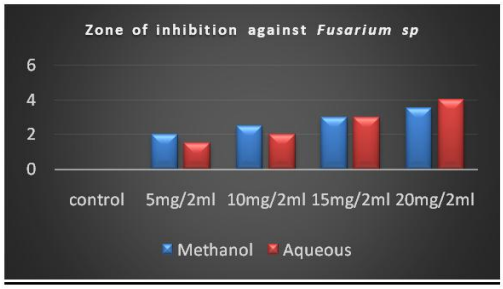


Chart 2: Shows zone of inhibition of turmeric against *Fusarium sp.*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Bipolar Intuitionistic Anti Fuzzy HX Group

M. Sridharan[1]*, M.S. Muthuraman[1], G. Sabarinathan[1], K.H. Manikandan[1] and Muthuraj[2]

[1]Department of Mathematics, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.
[2]PG and Research Department of Mathematics, H.H.The Rajah's College, Pudukkottai, Tamil Nadu, India.

Received: 14 Nov 2020 Revised: 16 Dec 2020 Accepted: 18 Jan 2021

***Address for Correspondence**
M. Sridharan
Department of Mathematics,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: msridharan1972@gmail.com

ABSTRACT

In this paper, we define a new algebraic structure of a bipolar intuitionistic anti fuzzy HX group and some related properties are investigated. The purpose of this study is to implement the fuzzy set theory and group theory in bipolar intuitionistic anti fuzzy HX subgroup of a HX group.

Keywords: HX group, fuzzy set, bipolar intutionistic fuzzy set, bipolar intuitionistic anti fuzzy group, bipolar intuitionistic anti fuzzy HX group.

INTRODUCTION

The concept of fuzzy sets was initiated by Zadeh [10]. Rosenfeld [9] gave the idea of fuzzy subgroups. Li Hongxing [3],[4] initiated the concept of HX group and fuzzy HX group. W.R. Zhang [11] commenced the concept of bipolar fuzzy sets as a generalization of fuzzy sets in 1994. In fuzzy sets the membership degree of elements range over the interval [0, 1]. The membership degree expresses the degree of belongingness of elements to a fuzzy set. The membership degree 1 indicates that an element completely belongs to its corresponding fuzzy set and membership degree 0 indicates that an element does not belong to fuzzy set. The membership degrees on the interval (0, 1) indicate the partial membership to the fuzzy set. Sometimes, the membership degree means the satisfaction degree of elements to some property or constraint corresponding to a fuzzy set .In case of Bipolar-valued fuzzy sets membership degree range is enlarged from the interval [0, 1] to [−1, 1]. In a bipolar-valued fuzzy set, the membership degree 0 means that the elements are irrelevant to the corresponding property, the membership degree (0,1] indicates that elements somewhat satisfy the property and the membership degree [-1,0) indicates that elements somewhat satisfy the implicit counter-property. R. Muthuraj [5], et.al redefined an algebraic structure of Bipolar fuzzy subgroups and Bipolar anti-fuzzy subgroups in the year 2012.They also introduced a new algebraic structure of Bipolar fuzzy HX subgroup and Bipolar anti-fuzzy HX subgroup and discussed some of its properties. The concept of bipolar intuitionistic fuzzy subset was discussed by D. Elilmaran [8] . In this paper we define a new algebraic structure of a bipolar intuitionistic anti fuzzy HX group of a HX group and investigate some related

properties. We define the necessity and possibility operators of bipolar intuitionistic fuzzy subset of a bipolar intuitionistic anti fuzzy HX group and discuss some of its properties.

PRELIMINARIES

In this section, we site the fundamental definitions that will be used in the sequel. Throughout this paper, G = (G , *) is a group, e is the identity element of G, and xy, we mean x * y.

2.1 Definition

Let G be a finite group. In $2^G - \{\phi\}$, a nonempty set $\vartheta \subset 2^G - \{\phi\}$ is called a HX group on G, if ϑ is a group with respect to the algebraic operation defined by AB = { ab /a ∈ A and b ∈ B}, which its unit element is denoted by E.

2.2 Definition

Let G be any non-empty set. A fuzzy subset μ of G is a function $\mu : G \to [0,1]$.

2.3 Definition

Let μ be a fuzzy subset defined on G. Let $\vartheta \subset 2^G - \{\phi\}$ be a HX group on G. A fuzzy set λ_μ defined on ϑ is said to be a anti fuzzy subgroup induced by μ on ϑ or a anti fuzzy HX subgroup on ϑ if for any A, B ∈ ϑ,

i. $\lambda_\mu(AB) \le \max\{\lambda_\mu(A), \lambda_\mu(B)\}$ ii. $\lambda_\mu(A^{-1}) = \lambda_\mu(A)$

2.4 Definition

Let G be a non-empty set. A bipolar intuitionistic fuzzy set Ω in G is an object having the form $\Omega = \{\langle x, \mu^+(x), \mu^-(x), \gamma^+(x), \gamma^-(x)\rangle : x \in G\}$ where $\mu^+ : G \to [0,1]$, $\gamma^+ : G \to [0,1]$ and $\mu^- : G \to [-1,0]$, $\gamma^- : G \to [-1,0]$ are mappings such that $0 \le \mu^+(x) + \gamma^+(x) \le 1$, $-1 \le \mu^-(x) + \gamma^-(x) \le 0$. The positive membership degree $\mu^+(x)$ denotes the satisfaction degree of an element x to the property corresponding to a bipolar intuitionistic fuzzy set μ and the negative membership degree $\mu^-(x)$ denotes the satisfaction degree of an element x to some implicit counter property corresponding to a bipolar intuitionistic fuzzy set. Similarly we use the positive non membership degree $\gamma^+(x)$ denote the satisfaction degree of an element x to the property corresponding to a bipolar intuitionistic fuzzy set and the negative non membership degree $\gamma^-(x)$ denote the satisfaction degree of an element x to some implicit counter property corresponding to a bipolar intuitionistic fuzzy set.

If $\mu^+(x) \neq 0$, $\mu^-(x) = 0$, $\gamma^+(x) = 0$, $\gamma^-(x) = 0$ it is the situation that x is regarded as having only positive satisfaction for μ. If $\mu^+(x) = 0$, $\mu^-(x) \neq 0$,$\gamma^+(x) = 0$ and $\gamma^-(x) = 0$ it is the situation that x does not satisfy the property of μ ,but somewhat satisfies the counter property of μ . If $\mu^+(x) = 0$, $\mu^-(x) = 0$, $\gamma^+(x) \neq 0$, $\gamma^-(x) = 0$ it is the situation that x is regarded as having only positive non membership property of μ . If $\mu^+(x) = 0$, $\mu^-(x) = 0$,$\gamma^+(x) = 0$ and $\gamma^-(x) \neq 0$ it is the situation that x does not satisfy the property of μ ,but somewhat satisfies the counter property of μ .

It is possible for an element x to be such that $\mu^+(x) \neq 0$, $\gamma^+(x) \neq 0$ and $\mu^-(x) \neq 0$, $\gamma^-(x) \neq 0$ when the membership and non membership function of property overlaps that its counter property over some portion of G. For the sake of simplicity, we shall use the symbol $\Omega = (\mu^+,\mu^-,\gamma^+,\gamma^-)$ for the bipolar intuitionistic fuzzy set $\Omega = \{\langle x, \mu^+(x), \mu^-(x), \gamma^+(x) , \gamma^-(x)\rangle : x \in G\}$.

2.5 Definition

Let G be a group .Let a bipolar intuitionistic fuzzy subset $\Omega = \{\langle x, \mu^+(x), \mu^-(x), \gamma^+(x) , \gamma^-(x)\rangle : x \in G\}$ is called as a bipolar intuitionistic anti fuzzy subgroup of G if for x,y ∈G,

i. $\mu^+(xy) \le \max\{\mu^+(x) , \mu^+(y)\}$ ii. $\mu^-(xy) \ge \min\{\mu^-(x) , \mu^-(y)\}$

iii. $\mu^+(x^{-1}) = \mu^+(x)$, $\mu^-(x^{-1}) = \mu^-(x)$ iv. $\gamma^+(xy) \ge \min\{\gamma^+(x) , \gamma^+(y)\}$

v. $\gamma^-(xy) \le \max\{\gamma^-(x), \gamma^-(y)\}$ vi. $\gamma^+(x^{-1}) = \gamma^+(x)$, $\gamma^-(x^{-1}) = \gamma^-(x)$.

2.6 Definition

Let Ω be a bipolar intuitionistic fuzzy subset defined on G . Let $\vartheta \subset 2^G - \{\phi\}$ be a HX group of G. A bipolar intuitionistic fuzzy set $\lambda_\Omega = \{\langle A, \lambda_\mu^+(A), \lambda_\mu^-(A), \lambda_\gamma^+(A), \lambda_\gamma^-(A) \rangle : A \in \vartheta\}$ defined on ϑ is said to be a bipolar intuitionistic anti fuzzy subgroup induced by Ω on ϑ or a bipolar intuitionistic anti fuzzy HX subgroup of ϑ if for $A, B \in \vartheta$,

i. $\lambda_\mu^+(AB) \leq \max\{\lambda_\mu^+(A), \lambda_\mu^+(B)\}$ ii. $\lambda_\mu^-(AB) \geq \min\{\lambda_\mu^-(A), \lambda_\mu^-(B)\}$

iii. $\lambda_\mu^+(A^{-1}) = \lambda_\mu^+(A), \lambda_\mu^-(A^{-1}) = \lambda_\mu^-(A)$ iv. $\lambda_\gamma^+(AB) \geq \min\{\lambda_\gamma^+(A), \lambda_\gamma^+(B)\}$

v. $\lambda_\gamma^-(AB) \leq \max\{\lambda_\gamma^-(A), \lambda_\gamma^-(B)\}$ vi. $\lambda_\gamma^+(A^{-1}) = \lambda_\gamma^+(A), \lambda_\gamma^-(A^{-1}) = \lambda_\gamma^-(A)$.

where

$\lambda_\mu^+(A) = \min\{\mu^+(x) / \text{for all } x \in A \subseteq G\}$ and

$\lambda_\mu^-(A) = \max\{\mu^-(x) / \text{for all } x \in A \subseteq G\}$.

$\lambda_\gamma^+(A) = \max\{\mu^+(x) / \text{for all } x \in A \subseteq G\}$ and

$\lambda_\gamma^-(A) = \min\{\mu^-(x) / \text{for all } x \in A \subseteq G\}$.

PROPERTIES OF BIPOLAR INTUITIONISTIC ANTI FUZZY HX SUBGROUP

3.1 Theorem

Let Ω be a bipolar intuitionistic fuzzy subset defined on G. Let $\vartheta \subset 2^G - \{\phi\}$ be a HX group of G. A bipolar intuitionistic fuzzy set λ_Ω defined on ϑ is a bipolar intuitionistic anti fuzzy subgroup induced by Ω on ϑ or a bipolar intuitionistic anti fuzzy HX subgroup of ϑ if and only if

i. $\lambda_\mu^+(XY^{-1}) \leq \max\{\lambda_\mu^+(X), \lambda_\mu^+(Y)\}$ ii. $\lambda_\mu^-(XY^{-1}) \geq \min\{\lambda_\mu^-(X), \lambda_\mu^-(Y)\}$

iii. $\lambda_\gamma^+(XY^{-1}) \geq \min\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y)\}$ iv $\lambda_\gamma^-(XY^{-1}) \leq \max\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y)\}$, for every $X, Y \in \vartheta$, where,

$\lambda_\mu^+(X) = \min\{\mu^+(x) / \text{for all } x \in X \subseteq G\}$ and $\lambda_\mu^-(X) = \max\{\mu^-(x) / \text{for all } x \in X \subseteq G\}$

$\lambda_\gamma^+(X) = \max\{\gamma^+(x) / \text{for all } x \in X \subseteq G\}$ and $\lambda_\gamma^-(X) = \min\{\gamma^-(x) / \text{for all } x \in X \subseteq G\}$

Proof

Let λ_Ω be a bipolar intuitionistic anti fuzzy HX subgroup of a HX group ϑ

for $X, Y \in \vartheta$

i. $\lambda_\mu^+(XY) \leq \max\{\lambda_\mu^+(X), \lambda_\mu^+(Y)\}$ ii. $\lambda_\mu^-(XY) \geq \min\{\lambda_\mu^-(X), \lambda_\mu^-(Y)\}$

iii. $\lambda_\mu^+(X^{-1}) = \lambda_\mu^+(X), \lambda_\mu^-(X^{-1}) = \lambda_\mu^-(X)$ iv. $\lambda_\gamma^+(XY) \geq \min\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y)\}$

v. $\lambda_\gamma^-(XY) \leq \max\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y)\}$ vi. $\lambda_\gamma^+(X^{-1}) = \lambda_\gamma^+(X), \lambda_\gamma^-(X^{-1}) = \lambda_\gamma^-(X)$.

Now,

i. $\lambda_\mu^+(XY^{-1}) \leq \max\{\lambda_\mu^+(X), \lambda_\mu^+(Y^{-1})\}$

$\Leftrightarrow \lambda_\mu^+(XY^{-1}) = \max\{\lambda_\mu^+(X), \lambda_\mu^+(Y)\}$

$\Leftrightarrow \lambda_\mu^+(XY^{-1}) \leq \max\{\lambda_\mu^+(X), \lambda_\mu^+(Y)\}$

ii. $\lambda_\mu^-(XY^{-1}) \geq \min\{\lambda_\mu^-(X), \lambda_\mu^-(Y^{-1})\}$

$\Leftrightarrow \lambda_\mu^-(XY^{-1}) = \min\{\lambda_\mu^-(X), \lambda_\mu^-(Y)\}$

$\Leftrightarrow \lambda_\mu^-(XY^{-1}) \geq \min\{\lambda_\mu^-(X), \lambda_\mu^-(Y)\}$

iii. $\lambda_\gamma^+(XY^{-1}) \geq \min\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y^{-1})\}$

$\Leftrightarrow \lambda_\gamma^+(XY^{-1}) = \min\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y)\}$

$\Leftrightarrow \lambda_\gamma^+(XY^{-1}) \geq \min\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y)\}$

iv. $\lambda_\gamma^-(XY^{-1}) \leq \max\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y^{-1})\}$

$\Leftrightarrow \lambda_\gamma^-(XY^{-1}) = \max\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y)\}$

$\Leftrightarrow \lambda_\gamma^-(XY^{-1}) \leq \max\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y)\}$

3.2 Theorem

Let λ_Ω be a bipolar intuitionistic fuzzy HX subgroup of a HX group ϑ then $(\lambda_\Omega)^c$ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.

Proof

Let λ_Ω be a bipolar fuzzy HX subgroup of a HX group ϑ.For all $X,Y \in \vartheta$,

Now

i. $\lambda_\mu^+(XY) \geq \min\{\lambda_\mu^+(X), \lambda_\mu^+(Y)\}$

$\Leftrightarrow 1-(\lambda_\mu^+)^c(XY) \geq \min\{1-(\lambda_\mu^+)^c(X), 1-(\lambda_\mu^+)^c(Y)\}$

$\Leftrightarrow (\lambda_\mu^+)^c(XY) \leq 1-\min\{1-(\lambda_\mu^+)^c(X), 1-(\lambda_\mu^+)^c(Y)\}$

$\Leftrightarrow (\lambda_\mu^+)^c(XY) \leq \max\{(\lambda_\mu^+)^c(X), (\lambda_\mu^+)^c(Y)\}$

ii. $\lambda_\mu^-(XY) \leq \max\{\lambda_\mu^-(X), \lambda_\mu^-(Y)\}$

$\Leftrightarrow -1-(\lambda_\mu^-)^c(XY) \leq \max\{-1-(\lambda_\mu^-)^c(X), -1-(\lambda_\mu^-)^c(Y)\}$

$\Leftrightarrow (\lambda_\mu^-)^c(XY) \geq -1-\max\{-1-(\lambda_\mu^-)^c(X), -1-(\lambda_\mu^-)^c(Y)\}$

$\Leftrightarrow (\lambda_\mu^-)^c(XY) \geq \min\{(\lambda_\mu^-)^c(X), (\lambda_\mu^-)^c(Y)\}$

iii. $\lambda_\mu^+(X^{-1}) = \lambda_\mu^+(X)$

$\Leftrightarrow 1-(\lambda_\mu^+)^c(X^{-1}) = 1-(\lambda_\mu^+)^c(X)$

$\Leftrightarrow (\lambda_\mu^+)^c(X^{-1}) = (\lambda_\mu^+)^c(X)$ and

$\lambda_\mu^-(X^{-1}) = \lambda_\mu^-(X)$

$\Leftrightarrow -1-(\lambda_\mu^-)^c(X^{-1}) = -1-(\lambda_\mu^-)^c(X)$

$\Leftrightarrow (\lambda_\mu^-)^c(X^{-1}) = (\lambda_\mu^-)^c(X)$

Therefore, $(\lambda_\mu^+)^c(X^{-1}) = (\lambda_\mu^+)^c(X)$, $(\lambda_\mu^-)^c(X^{-1}) = (\lambda_\mu^-)^c(X)$.

iv. $\lambda_\gamma^+(XY) \leq \max\{\lambda_\gamma^+(X), \lambda_\gamma^+(Y)\}$

$\Leftrightarrow 1-(\lambda_\gamma^+)^c(XY) \leq \max\{1-(\lambda_\gamma^+)^c(X), 1-(\lambda_\gamma^+)^c(Y)\}$

$\Leftrightarrow (\lambda_\gamma^+)^c(XY) \geq 1-\max\{1-(\lambda_\gamma^+)^c(X), 1-(\lambda_\gamma^+)^c(Y)\}$

$\Leftrightarrow (\lambda_\gamma^+)^c(XY) \geq \min\{(\lambda_\gamma^+)^c(X), (\lambda_\gamma^+)^c(Y)\}$

v. $\lambda_\gamma^-(XY) \geq \min\{\lambda_\gamma^-(X), \lambda_\gamma^-(Y)\}$

$\Leftrightarrow -1-(\lambda_\gamma^-)^c(XY) \geq \min\{-1-(\lambda_\gamma^-)^c(X), -1-(\lambda_\gamma^-)^c(Y)\}$

$\Leftrightarrow (\lambda_\gamma^-)^c(XY) \leq -1-\max\{-1-(\lambda_\gamma^-)^c(X), -1-(\lambda_\gamma^-)^c(Y)\}$

$\Leftrightarrow (\lambda_\gamma^-)^c(XY) \leq \max\{(\lambda_\gamma^-)^c(X), (\lambda_\gamma^-)^c(Y)\}$

vi. $\lambda_\gamma^+(X^{-1}) = \lambda_\gamma^+(X)$

$\Leftrightarrow 1-(\lambda_\gamma^+)^c(X^{-1}) = 1-(\lambda_\gamma^+)^c(X)$

$\Leftrightarrow (\lambda_\gamma^+)^c(X^{-1}) = (\lambda_\gamma^+)^c(X)$ and

$\lambda_\gamma^-(X^{-1}) = \lambda_\gamma^-(X)$

$\Leftrightarrow -1-(\lambda_\gamma^-)^c(X^{-1}) = -1-(\lambda_\gamma^-)^c(X)$

$\Leftrightarrow (\lambda_\gamma^-)^c(X^{-1}) = (\lambda_\gamma^-)^c(X)$

Therefore, $(\lambda_\gamma^+)^c(X^{-1}) = (\lambda_\gamma^+)^c(X)$, $(\lambda_\gamma^-)^c(X^{-1}) = (\lambda_\gamma^-)^c(X)$

So, $(\lambda_\Omega)^c$ is a bipolar intuitionistic anti-fuzzy HX subgroup of HX group ϑ.

Hence, λ_Ω is a bipolar intuitionistic fuzzy HX subgroup of a HX group ϑ if and only if $(\lambda_\Omega)^c$ is a bipolar intuitionistic anti-fuzzy HX subgroup of HX group ϑ.

3.3 Theorem

Let Ω be a bipolar intuitionistic anti fuzzy subgroup of G then the bipolar intuitionistic fuzzy set λ_Ω is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.

Proof: Obvious

3.4 Theorem

Let Ω , Ψ and $\Omega \cup \Psi$ be bipolar intuitionistic anti fuzzy subgroups of G. Let $\vartheta \subset 2^G - \{\phi\}$ be a HX group of G. Let λ_Ω and σ_Ψ be bipolar intuitionistic anti fuzzy HX subgroups of ϑ then $\lambda_\Omega \cup \sigma_\Psi$ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.

Proof: Obvious.

3.5 Theorem

Let λ_Ω , σ_Ψ , $\omega_{\Omega \cup \Psi}$ be bipolar intuitionistic anti fuzzy HX subgroups of ϑ induced by bipolar intuitionistic fuzzy subsets Ω , Ψ and $\Omega \cup \Psi$ of G respectively then $\omega_{\Omega \cup \Psi} = \lambda_\Omega \cup \sigma_\Psi$.

Proof

Let $\lambda_\Omega = (\lambda_\mu^+, \lambda_\mu^-, \lambda_\gamma^+, \lambda_\gamma^-)$ and $\sigma_\Psi = (\sigma_\phi^+, \sigma_\phi^-, \sigma_\eta^+, \sigma_\eta^-)$ be two bipolar intuitionistic anti fuzzy HX subgroups of ϑ.

By Theorem , $\lambda_\Omega \cup \sigma_\Psi$ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.

Let $\omega_{\Omega \cup \Psi}$ be a bipolar intuitionistic anti fuzzy HX subgroup of ϑ induced by bipolar intuitionistic fuzzy subset $\Omega \cup \Psi$ of G.

i. $(\omega_{\mu \cup \phi})^+(X) = \min \{(\mu \cup \phi)^+ (x) / x \in X \subseteq G\}$
$= \min \{\max \{\mu^+ (x), \phi^+ (x)\} / x \in X \subseteq G\}$
$= \max \{\min \{\mu^+ (x) / x \in X \subseteq G\}, \min \{\phi^+ (x) / x \in X \subseteq G\}\}$
$= \max \{\lambda_\mu^+ (X), \sigma_\phi^+ (X)\}$

Therefore, $(\omega_{\mu \cup \phi})^+(X) = (\lambda_\mu \cup \sigma_\phi)^+ (X)$.

ii. $(\omega_{\mu \cup \phi})^-(X) = \max \{(\mu \cup \phi)^- (x) / x \in X \subseteq G\}$
$= \max \{\min \{\mu^- (x), \phi^- (x)\} / x \in X \subseteq G\}$
$= \min \{\max \{\mu^- (x) / x \in X \subseteq G\}, \max \{\phi^- (x) / x \in X \subseteq G\}\}$
$= \min \{\lambda_\mu^- (X), \sigma_\phi^- (X)\}$

Therefore, $(\omega_{\mu \cup \phi})^-(X) = (\lambda_\mu \cup \sigma_\phi)^- (X)$.

iii. $(\omega_{\gamma \cup \eta})^+(X) = \max \{(\gamma \cup \eta)^+ (x) / x \in X \subseteq G\}$
$= \max \{\min \{\gamma^+ (x), \eta^+ (x)\} / x \in X \subseteq G\}$
$= \min \{\max \{\gamma^+ (x) / x \in X \subseteq G\}, \max \{\eta^+ (x) / x \in X \subseteq G\}\}$
$= \min \{\lambda_\gamma^+ (X), \sigma_\eta^+ (X)\}$

Therefore, $(\omega_{\gamma \cup \eta})^+(X) = (\lambda_\gamma \cup \sigma_\eta)^+ (X)$.

iv.$(\omega_{\gamma \cup \eta})^-(X) = \min \{(\gamma \cup \eta)^- (x) / x \in X \subseteq G\}$
$= \min \{\max \{\gamma^- (x), \eta^- (x)\} / x \in X \subseteq G\}$
$= \max \{\min \{\gamma^- (x) / x \in X \subseteq G\}, \min \{\eta^- (x) / x \in X \subseteq G\}\}$
$= \max \{\lambda_\gamma^- (X), \sigma_\eta^- (X)\}$

Therefore, $(\omega_{\gamma \cup \eta})^-(X) = (\lambda_\gamma \cup \sigma_\eta)^- (X)$.

Hence, $\omega_{\Omega \cup \Psi} = \lambda_\Omega \cup \sigma_\Psi$.

3.6 Theorem

If λ_Ω and σ_Ψ are any two bipolar intuitionistic anti fuzzy HX subgroups of ϑ then $\lambda_\Omega \cap \sigma_\Psi$ is also a bipolar intuitionistic anti fuzzy HX subgroup of ϑ .

Proof

Let $\lambda_\Omega = (\lambda_\mu^+, \lambda_\mu^-, \lambda_\gamma^+, \lambda_\gamma^-)$ and $\sigma_\Psi = (\sigma_\phi^+, \sigma_\phi^-, \sigma_\eta^+, \sigma_\eta^-)$ be bipolar intuitionistic anti fuzzy HX subgroups of ϑ.
For every $X,Y \in \vartheta$,

i. $(\lambda_\mu \cap \sigma_\phi)^+ (XY^{-1}) = \min \{\lambda_\mu^+ (XY^{-1}) , \sigma_\phi^+(XY^{-1})\}$
$\leq \min \{\max \{\lambda_\mu^+ (X) , \lambda_\mu^+ (Y)\} , \max \{\sigma_\phi^+ (X) , \sigma_\phi^+ (Y)\}\}$
$= \max \{\min \{\lambda_\mu^+ (X) , \sigma_\phi^+ (X)\} , \min \{\lambda_\mu^+ (Y) , \sigma_\phi^+ (Y)\}\}$
$= \max \{(\lambda_\mu \cap \sigma_\phi)^+ (X), (\lambda_\mu \cap \sigma_\phi)^+ (Y)\}$
Therefore, $(\lambda_\mu \cap \sigma_\phi)^+ (XY^{-1}) \leq \max \{(\lambda_\mu \cap \sigma_\phi)^+ (X), (\lambda_\mu \cap \sigma_\phi)^+ (Y)\}$.

ii. $(\lambda_\mu \cap \sigma_\phi)^- (XY^{-1}) = \max \{\lambda_\mu^- (XY^{-1}) , \sigma_\phi^- (XY^{-1})\}$
$\geq \max \{\min \{\lambda_\mu^- (X), \lambda_\mu^- (Y)\}, \min \{\sigma_\phi^- (X), \sigma_\phi^- (Y)\}\}$
$= \min \{\max \{\lambda_\mu^- (X), \sigma_\phi^- (X)\}, \max \{\lambda_\mu^- (Y), \sigma_\phi^- (Y)\}\}$
$= \min \{(\lambda_\mu \cap \sigma_\phi)^- (X), (\lambda_\mu \cap \sigma_\phi)^- (Y)\}$
Therefore, $(\lambda_\mu \cap \sigma_\phi)^- (XY^{-1}) \geq \min \{(\lambda_\mu \cap \sigma_\phi)^- (X), (\lambda_\mu \cap \sigma_\phi)^- (Y)\}$.

iii. $(\lambda_\gamma \cap \sigma_\eta)^+ (XY^{-1}) = \max \{ \lambda_\gamma^+ (XY^{-1}) , \sigma_\eta^+ (XY^{-1})\}$
$\geq \max \{\min \{ \lambda_\gamma^+ (X), \lambda_\gamma^+ (Y)\}, \max \{\sigma_\eta^+ (X), \sigma_\eta^+ (Y)\}\}$
$= \min \{\max \{ \lambda_\gamma^+ (X), \sigma_\eta^+ (X)\}, \max \{ \lambda_\gamma^+ (Y), \sigma_\eta^+ (Y)\}\}$
$= \min \{(\lambda_\gamma \cap \sigma_\eta)^+ (X), (\lambda_\mu \cap \sigma_\eta)^+ (Y)\}$
Therefore, $(\lambda_\gamma \cap \sigma_\eta)^+ (XY^{-1}) \geq \min \{(\lambda_\gamma \cap \sigma_\eta)^+ (X), (\lambda_\gamma \cap \sigma_\eta)^+ (Y)\}$.

iv. $(\lambda_\gamma \cap \sigma_\eta)^- (XY^{-1}) = \min \{ \lambda_\gamma^- (XY^{-1}) , \sigma_\eta^- (XY^{-1})\}$
$\leq \min \{\max \{ \lambda_\gamma^- (X) , \lambda_\gamma^- (Y)\} , \max \{\sigma_\eta^- (X) , \sigma_\eta^- (Y)\}\}$
$= \max \{\min \{ \lambda_\gamma^- (X) , \sigma_\eta^- (X)\} , \min \{ \lambda_\gamma^- (Y) , \sigma_\eta^- (Y)\}\}$
$= \max \{(\lambda_\gamma \cap \sigma_\eta)^- (X), (\lambda_\gamma \cap \sigma_\eta)^- (Y)\}$
Therefore, $(\lambda_\gamma \cap \sigma_\eta)^- (XY^{-1}) \leq \max \{(\lambda_\gamma \cap \sigma_\eta)^- (X), (\lambda_\gamma \cap \sigma_\eta)^- (Y)\}$.

Hence, $\lambda_\Omega \cap \sigma_\Psi$ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.

3.7 Remark

Let G be a group. Let Ω and Ψ be two bipolar intuitionistic anti fuzzy subgroups of G and $\Omega \cap \Psi$ is also a bipolar intuitionistic anti fuzzy subgroup of G.
By Theorem, a bipolar intuitionistic fuzzy subset $\omega_{\Omega\cap\Psi} = ((\omega_{\Omega\cap\Psi})^+,(\omega_{\Omega\cap\Psi})^-)$ of ϑ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ induced by $\Omega \cap \Psi$ of G.

3.8 Theorem

If λ_Ω, σ_Ψ , $\omega_{\Omega\cap\Psi}$ are bipolar intuitionistic anti fuzzy HX subgroups of ϑ induced by bipolar intuitionistic anti fuzzy subgroups Ω , Ψ and $\Omega \cap \Psi$ of G then $\omega_{\Omega\cap\Psi} = \lambda_\Omega \cap \sigma_\Psi$.

Proof

Let $\lambda_\Omega = (\lambda_\mu^+, \lambda_\mu^-, \lambda_\gamma^+, \lambda_\gamma^-)$ and $\sigma_\Psi = (\sigma_\phi^+, \sigma_\phi^-, \sigma_\eta^+, \sigma_\eta^-)$ be two bipolar intuitionistic anti fuzzy HX subgroups of ϑ. By Theorem $\lambda_\Omega \cap \sigma_\Psi$ is a bipolar intuitionistic anti fuzzy HX subgroup of ϑ.
Let $\omega_{\Omega\cap\Psi}$ be a bipolar intuitionistic anti fuzzy HX subgroup of ϑ induced by $\Omega \cap \Psi$ of G. For all $X \in \vartheta$,

i. $(\omega_{\mu\cap\phi})^+ (X) = \min \{(\mu \cap \phi)^+ (x) / x \in X \subseteq G\}$
$= \min \{\min \{\mu^+ (x) , \phi^+ (x) \} / x \in X \subseteq G\}$
$= \min \{\min \{\mu^+ (x) / x \in X \subseteq G\}, \min \{\phi^+ (x) / x \in X \subseteq G\}\}$
$= \min \{\lambda_\mu^+ (X), \sigma_\phi^+ (X)\}$

Therefore, $(\omega_{\mu\cap\phi})^+(X) = (\lambda_\mu \cap \sigma_\phi)^+(X)$.

ii. $(\omega_{\mu\cap\phi})^-(X) = \max\{(\mu\cap\phi)^-(x) / x \in X \subseteq G\}$

$= \max\{\max\{\mu^-(x), \phi^-(x)\} / x \in X \subseteq G\}$

$= \max\{\max\{\mu^-(x) / x \in X \subseteq G\}, \max\{\phi^-(x) / x \in X \subseteq G\}\}$

$= \max\{\lambda_\mu^-(X), \sigma_\phi^-(X)\}$

Therefore, $(\omega_{\mu\cap\phi})^-(X) = (\lambda_\mu \cap \sigma_\phi)^-(X)$.

iii. $(\omega_{\gamma\cap\eta})^+(X) = \max\{(\gamma\cap\eta)^+(x) / x \in X \subseteq G\}$

$= \max\{\max\{\gamma^+(x), \eta^+(x)\} / x \in X \subseteq G\}$

$= \max\{\max\{\gamma^+(x) / x \in X \subseteq G\}, \max\{\eta^+(x) / x \in X \subseteq G\}\}$

$= \max\{\lambda_\gamma^+(X), \sigma_\eta^+(X)\}$

Therefore, $(\omega_{\gamma\cap\eta})^+(X) = (\lambda_\gamma \cap \sigma_\eta)^+(X)$.

iv. $(\omega_{\gamma\cap\eta})^-(X) = \min\{(\gamma\cap\eta)^-(x) / x \in X \subseteq G\}$

$= \min\{\min\{\gamma^-(x), \eta^-(x)\} / x \in X \subseteq G\}$

$= \min\{\min\{\gamma^-(x) / x \in X \subseteq G\}, \min\{\eta^-(x) / x \in X \subseteq G\}\}$

$= \min\{\lambda_\gamma^-(X), \sigma_\eta^-(X)\}$

Therefore, $(\omega_{\gamma\cap\eta})^-(X) = (\lambda_\gamma \cap \sigma_\eta)^-(X)$.

Hence, $\omega_{\Omega\cap\Psi} = \lambda_\Omega \cap \sigma_\Psi$

3.9 Definition

Let G be a group .Let $\Omega = (\Omega^+, \Omega^-)$ be a bipolar intuitionistic fuzzy subset of G then the "necessity" operator $\Box$ is defined as

$\Box\,\Omega^+ = \{x, \mu^+(x), 1-\mu^+(x) / x \in G\}$ & $\Box\,\Omega^- = \{x, \mu^-(x), -1-\mu^-(x) / x \in G\}$.

3.10 Definition

Let G be a group .Let $\Omega = (\Omega^+, \Omega^-)$ be a bipolar intuitionistic fuzzy subset of G then the "possibility" operator $\Diamond$ is defined as

$\Diamond\,\Omega^+ = \{x, \gamma^+(x), 1-\gamma^+(x) / x \in G\}$ & $\Diamond\,\Omega^- = \{x, \gamma^-(x), -1-\gamma^-(x) / x \in G\}$.

3.11 Theorem

Let Ω be a bipolar intuitionistic fuzzy subset on G .Let λ_Ω be a bipolar intuitionistic anti fuzzy HX subgroup on ϑ then $\Box\lambda_\Omega$ is a bipolar intuitionistic anti fuzzy HX subgroup on ϑ.

Proof

Let λ_Ω be a bipolar intuitionistic anti fuzzy HX subgroup on ϑ. Then

Now $\lambda_\mu^+(AB) \le \max\{\lambda_\mu^+(A), \lambda_\mu^+(B)\}$

$1-\lambda_\mu^+(AB) \ge 1-\max\{\lambda_\mu^+(A), \lambda_\mu^+(B)\}$

$\ge \min\{1-\lambda_\mu^+(A), 1-\lambda_\mu^+(B)\}$

That is, $1-\lambda_\mu^+(AB) \ge \min\{1-\lambda_\mu^+(A), 1-\lambda_\mu^+(B)\}$

$\lambda_\mu^-(AB) \ge \min\{\lambda_\mu^-(A), \lambda_\mu^-(B)\}$

$-1-\lambda_\mu^-(AB) \le -1-\min\{\lambda_\mu^-(A), \lambda_\mu^-(B)\}$

That is, $-1-\lambda_\mu^-(AB) \le \max\{-1-\lambda_\mu^-(A), -1-\lambda_\mu^-(B)\}$

Clearly, $1-\lambda_\mu^+(A^{-1}) = 1-\lambda_\mu^+(A)$,

$-1-\lambda_\mu^-(A^{-1}) = -1-\lambda_\mu^-(A)$.

Hence, $\Box\lambda_\Omega$ is a bipolar intuitionistic anti fuzzy HX subgroup on ϑ.

3.12 Theorem

Let Ω be a bipolar intuitionistic fuzzy subset on G .Let λ_Ω be a bipolar intuitionistic anti fuzzy HX subgroup on ϑ then $\Diamond\lambda_\Omega$ is a bipolar intuitionistic anti fuzzy HX subgroup on ϑ.

Proof

Let λ_Ω be a bipolar intuitionistic anti fuzzy HX subgroup on ϑ. Then

Now , let $\lambda_\gamma^+(AB) \geq \min\{\lambda_\gamma^+(A), \lambda_\gamma^+(B)\}$

$1-\lambda_\gamma^+(AB) \leq 1-\min\{\lambda_\gamma^+(A), \lambda_\gamma^+(B)\}$

$\leq \max\{1-\lambda_\gamma^+(A), 1-\lambda_\gamma^+(B)\}$

That is, $1-\lambda_\gamma^+(AB) \leq \max\{1-\lambda_\gamma^+(A), 1-\lambda_\gamma^+(B)\}$

$\lambda_\gamma^-(AB) \leq \max\{\lambda_\gamma^-(A), \lambda_\gamma^-(B)\}$

$-1-\lambda_\gamma^-(AB) \geq -1-\max\{\lambda_\gamma^-(A), \lambda_\gamma^-(B)\}$

$\geq \min\{-1-\lambda_\gamma^-(A), -1-\lambda_\gamma^-(B)\}$

That is, $-1-\lambda_\gamma^-(AB) \geq \min\{-1-\lambda_\gamma^-(A), -1-\lambda_\gamma^-(B)\}$

Clearly, $1-\lambda_\gamma^+(A^{-1}) = 1-\lambda_\gamma^+(A)$,

$-1-\lambda_\gamma^-(A^{-1}) = -1-\lambda_\gamma^-(A)$.

Hence, $\Diamond\lambda_\Omega$ is a bipolar intuitionistic anti fuzzy HX subgroup on ϑ.

CONCLUSION

In this paper we introduce the concept of bipolar intuitionistic anti fuzzy HX group and discuss the basic results on bipolar intuitionistic anti fuzzy HX subgroup. Further investigation may be in bipolar intuitionistic anti-fuzzy normal HX group on HX group which will give a new horizon in the further study.

ACKNOWLEDGEMENT

The authors would like to express their sincere thanks to all our friends for their help to make this paper a successful one.

REFERENCES

1. Atanassov. K.T, Intuitionistic fuzzy sets, Fuzzy Sets and Systems,20,1986,pp. 87-96.
2. Biswas .R, Fuzzy subgroups and anti-fuzzy subgroups, Fuzzy Sets and Systems, 35, 1990, pp.121-124.
3. Li Hongxing, HX group, BUSEFAL, 33, 1987, pp.31 – 37.
4. Luo Chengzhong, Mi Honghai , Li Hongxing, Fuzzy HX group, BUSEFAL, 41-14, 1989, pp.97 – 106.
5. Muthuraj. R, Sridharan. M, Bipolar anti fuzzy HX group and its lower level Sub HX groups, Journal of Physical Sciences, 16, 2012, pp. 157 - 169.
6. Muthuraj. R, Sridharan. M, Operations on Bipolar fuzzy HX subgroups, International Journal of Engineering and Associates, Volume 3, Issue 5, 2014 , pp.12-18.
7. Muthuraj. R, Manikandan. K.H, Intuitionisic Q-fuzzy HX group and its <α,β>, level sub HX group, CIIT Fuzzy System, Volume 3(9), 2011.
8. Sankar. K, Ezhilmaran. D, Bipolar intuitionistic fuzzy graphs with application, International Journal of Research and Scientific innovation, III(XII), 2016, pp. 44 - 52.
9. Rosenfeld A, Fuzzy Groups, J. Math. Anal. Appl. 35, 1971,pp. 512-517.
10. Zadeh L.A, Fuzzy Sets, Inform and Control, 8,1965, pp. 338-365.
11. Zhang W.R, Bipolar fuzzy sets, Proc. of FUZZ-IEEE, 1998, pp. 835-840.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Trees Exposed to Air Pollutants in Traffic and Industrial Sites at Dindigul Town"

V. Prathipa[1]*, A. Sahaya Raja[2] and A. Uma Maheswari [3]

[1]Department of Chemistry, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India
[2]PG and Research Department of Chemistry, G.T.N. Arts College (Autonomous), Dindigul, Tamil Nadu, India
[3]Department of Chemistry, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India

Received: 16 Nov 2020 Revised: 18 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
V. Prathipa
Department of Chemistry, PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India
Email : spsahayamdgl@gmail.com

ABSTRACT

The study examined the variation of Air Pollution Tolerance Indices (APTI) of trees around the selected sites of study area. Site -1 is a residential area which was considered as control site, site- 2 is located in a heavily polluted traffic junction and site-3 is in an industrially polluted region. Bio monitoring of air pollution had been carried out in these sites. Biochemical parameters taken into consideration were total chlorophyll, relative moisture content, pH and ascorbic acid of matured leaf samples. The present study suggests that plants have the potential to serve as excellent monitors of air pollution. The study summarized the results on bio monitoring of local plant species along various sites. *Moringa olifera* showed higher tolerance for automobile pollution throughout the study period. Tree species *Delonix regia* showed higher tolerance for industrial pollution during this analysis. Thus, it can be an effective indicator for air pollution.

Key words: Pollution, *Moringa olifera,* Biochemical, traffic, industrial.

INTRODUCTION

Plants are the only living organisms which have to suffer a lot from automobile exhaust pollution because they remain static at their habitat. The vegetation naturally cleanses the atmosphere by absorbing gases and some particulate matter through leaves. Plants have a very large surface area and their leaves function as an efficient pollutant trapping device. Some plants have been classified according to their degree of sensitivity and tolerance towards various air pollutants (Turkand Wirth, 1975,) and water pollutants (Thambavani .D.S and Prathipa. V et al., 2010,2011,2012 and 2013, Saralathambavani. D and Prathipa,.V et al., 2010, 2011,2012 and 2013 V.Prathipa et al., 2015,

R. Rajendran et al.,2015). Sensitive plants species are suggested to act as bio-indicators. Levels of air pollution tolerance vary from species to species depending on the capacity of plants to withstand the effect of pollutants without showing any external damages (Tiwari, and Bansal, 1994).Leaf is the plant part, which is most sensitive to pollution. The pollution indicator value of the leaf has been exploited by a large number of workers. One of the most common impacts of air pollution is the gradual disappearance of chlorophyll. The decrease in chlorophyll content was depending upon the increasing pollution load in traffic areas. The level of toxicity may be responsible for lowering the levels of total chlorophyll (Prakash Govind etal, 2002). The pH may increase the efficiency of the conversion from hexose sugar to ascorbic acid while low leaf pH extract showed good correlation with sensitivity to air pollution and also reduces photosynthesis in plants. The leaf extract pH in plants increased due to basic pollutants present at the polluted site. The relative water content (RWC) is a useful indicator of the state of the water balance of the plant. The large quantity of water in plant body helps in maintaining its physiological balance under stress conditions. The plants with maximum and minimum APTI values serve as tolerant and sensitive species. APTI of sampling species at different sites were examined during the study period.

METHODOLOGY

Study location

Dindigul is the interior region of Tamil Nadu. It lies on the banks of Kudavanar River. The total landscape of Dindigul is 6058 Sqkm. The urban population is 3, 76,445. Inspite of its geographical location there are about 110 tanneries both registered and non-registered in and around Dindigul. Dindigul is noted for its locks. Also iron safe of good quality and durability are made here. A lock-manufacturing unit under co-operative sector is functioning here. It is one of the largest trading centres in Tamil Nadu for chewing tobacco and Rojasupari which are produced in this town. They are being sent to various places in and around Tamil Nadu. Dindigul is flourishing with handloom industry at Chinnalapatti, which islocated at 11 Km away from Dindigul on the Madurai – Dindigul road. Polluted site was located at the bus stand in Dindigul town which is the busiest area in Dindigul and traffic jam condition prevalent here throughout the day, possess a high number of two wheelers, three wheelers, and four wheelers. Another polluted site was sampled near the tannery area where many numbers of tanneries located at Thomaiyarpuram bypass, Dindigul, while the control site was located at Lakshmanapuram, Dindigul. The control site was situated about 12km away from polluted site where pollution level is almost very low. The fresh leaf samples were collected from both sites, polluted and control, grown on the edge of the road almost with similar topography or conditions and all these leaves were free from pathogens or any diseases.

Plant selection

1. *Delonix regia,* 2. *Moringa olifera,* 3. *Azardiracta indica,* 4. *Pongamia glabra,* 5. *Tarmarindus indica,* 6.*Mangifer indica,* 7.*Tectona grandis,* 8.*Ficus religiosa,* 9.*Ficus benghalensis,* 10.*Eucalyptus globules,* 11.*Carcia papaya,* 12.*Ficus racemosa,* 13.*Embilica officionails,* 14.*Bambusoideae,*15.*Citrus limon.*

Sample collection

Trees were randomly selected from the immediate vicinity of the station and were labelled for experiment. Two replicates of fully matured leaf samples of the selected plant species were collected monthly, mixed to get a homogeneous sample. Then plant leaves were kept in polythene bags preserved in refrigerator for further analysis.

Analysis of selected biochemical parameters

RWC is determined by using the method described by Barrs and Weatherly (1962).Each sample was placed in a pre-weighed airtight vital. Leaf sample should be placed in a vial slightly longer than the sample, with its basal part to the bottom samples should reach the lab as soon as possible. In the lab, vials were weighed to obtain leaf sample weight(W),after which the sample was immediately hydrated to full turgidity for 3-4 h under normal room light and temperature. After hydration, the samples were taken out of water and were well dried of any surface moisture

quickly a light with filter/tissue paper and immediately weighed to obtain fully turgid weight (TW).Samples were then oven dried at 80^{o}C for 24h and weighed(after being cooled down in a desiccators) to determine dry weight (DW).

Calculation

RWC(%) = (W-DW)/(TW-DW)*100
Where, W-Sample fresh weight
TW- Sample turgid weight
DW- Sample dry weight

For the measurement of leaf extract pH, 2g of the sample was homogenized with 20ml of deionised water and pH of the suspension was measured with a digital pH meter with a glass combined electrode. Estimation of total chlorophyll content (TCH) was done according to the method described by Arnon (1949).3 g fresh leaves were blended and then extracted with 10ml of 80 percent acetone and left for 15min. The liquid portion was decanted into another test-tube and centrifuged at 2,500 rpm for 3 min. Thesupernatant was then collected and the absorbance was then taken at 645nm and 663 nm using aspectro photometer. Ascorbic acid was determined following the method of Agarwal (1985).10g of the leaf samples were transferred into a glass pestle mortar and macerated well with 4 percent oxalic acid. The contents were transferred toa 100ml volumetric flask by filtering through a muslin cloth and repeated the extractions with percent oxalic acid. This is titrated against 0.02 percent of aselective reagent 2,6 dichlorophenol indophenol dye solution taken in the burette, a permanent pale pink color is obtained.

Determination of APTI

The air pollution tolerance indices of fifteen common plants were determined following the method ofsingh and Roa (1983). The formula of is given as:
APTI = A(T+P) +R/10
Where, A-Ascorbic acid content(mg/g),T-Total chlorophyll (mg/g),P-pH of leaf extract and R-Relative water content of leaf(%). The results were statistically analyzed and interpreted using SPSS software version 17.To isolate which groups differed from the others with respect to the months, plants and study stations.

Experimental findings and discussion

The values obtained for the analysis of selected biochemical parameters of plants were analysed statistically. All the bio-chemical parameters exhibited significant variation from species to species and station to station as shown 0.07 percent ($p<0.04$).

Air pollution tolerance index

APTI of 15 plant species at residential, heavy traffic and industrial sites of the study area. All biochemical parameters that were analysed for APTI plays significant role in determine resistivity and susceptibility of plant species. In the month of December 2012 APTI value ranged from 2.9 to 12.05. At site -1 the lowest value observed in *F.bengamina* (2.97) and the highest value was observed in *A.indica* (9.95).Almost all the species showed variation in their tolerance towards air pollution between control and polluted sites. Exceptionally, *Embilica officinalis* showed no significant variation (4.9 at S1, 4.8 at S2,4.75at S3).Species likeT. indica(4.81 to 7.2), *P. Glabra* (6.8 to 8.4), *Ficus benghalensis*(5.9 to 7.4), *Ficus religiosa* (6.3 to 9.3),*Tectona grandis*(5.52 to 7.50) and *Ficus racemosa* (2.9 to 8.6) showed tolerance towards polluted sites. *Ficus racemosa* showed maximum variation. Plants such as *Mangifer indica* (13.0) and *Delonix regia* showed higher tolerance at S2 and S3 sites respectively. During January ,2013 the calculated values ranged between 2.4 to 10.9. Plants like *Delonix regia, Moringa olifera , Azardiracta indica , Pongamia glabra* and *Tarmarindus indica* exhibited steady increase of APTI value from site 1 to site 3.The values found to be decreased from control to polluted sites in trees like *Mangifer indica* (9.14 to 7.1) and *Carciapapaya* (9.8 to 6.04). Decrease may be due to reduction in the tolerance for pollutants during that cold month. Notable increase was observed in *Ficus benghalensis* (4.25 to

9.86), *Tectona grandis*(5.1 to 7.5),*Carcia papaya* (5.12 to 8.2) and *Ficus racemosa* (3.8 to 9.01) at S3 and these trees showed higher tolerance for industrial pollution and *Moringa olifera* (9.9) showed higher tolerance for automobile pollution. It was observed in the month of February 2013 that almost all the species observed with higher tolerance. In contrary ,Species like *Tarmarindus indica* (6.2 to 1.5), *Moringa olifera* (10.0 to 2.5),*Mangifer indica* (9.0 to 8.02) and *Eucalyptus globules* (7.42 to 5.71) became sensitive. The maximum and minimum values observed were 10.7 and 2.7, respectively . It was observed that *Mangifer indica* showed higher tolerance at S2 (10.91) and *Azardiracta indica* showed higher tolerance for S3(8.02). It was evident in the month of march;2013 higher tolerance was observed in *Ficus racemosa* (17.84) at S3. The analyzed values ranged between 3.15 to 17.85. Species like c *indica* (6.01 to 9.3), *Citrus limon* (4.25 to 6.55), *Bambusoideae* (4 to 8.3) and *Ficus benghalensis*(6.95 to 9.10) showed increased tolerance to air pollution. Trees such as *Delonix regia* (13.9),*Tarmarindus indica* (11.7) and *Pongamia glabra* (12.8) showed significant increase in their tolerance towards industrial air pollutants. In view of data obtained for the month of April, 2013 most of the selected species showed increased APTI at all the stations but it varied with the pollution load. The calculated values ranged between 3.35 to 14.86. The highest value of tolerance was shown by *Moringa olifera* at the area polluted by least value of tolerance was shown by *Ficus benghalensis* at S1. Tree species *Moringa olifera* (8.60 to 6.99) exhibited decreasing values of tolerance in traffic and industrial sites. Plants such as Delonix *regia* (13.25),*Pongamia glabra* (11.96) and *Mangifer indica* (9.03) were observed with significant higher tolerance at S3. On the basis of APTI data for the month of month of May 2013 the tolerance value ranged between 1.45 to 19.13. High tolerance was shown by *Delonix regia* at industrial site. Least value was exhibited by *Tectona grandis* at residential area. Plants such as *Azardiracta indica* (17.49),*Mangifer indica* (13.5), *Pongamia glabra* (8.80) and *Tectona grandis* (9.74) in S2 showed higher values than other selected sites. *Delonix regia*(19.2), *Embilica officionails* (15.10), *Ficus benghalensis* (10.06) and *Ficus racemosa* (10.12) exhibited significant tolerance at S3.

CONCLUSION

The present study suggests that plants have the potential to serve as excellent monitors of air pollution. The study summarized the results on bio monitoring of local plant species along various sites. *Moringa olifera* showed higher tolerance for automobile pollution. From the biochemical parameter analysis, the tolerance may be due to the increase of chlorophyll content. Several researchers have exhibited increase in chlorophyll content under air pollution. Tree species *Delonix regia* showed higher for industrial pollution during this study period. It is inferred from the biochemical analysis that increase of ascorbic acid content may be responsible for tolerance of that species in industrial site. Pollution load dependent increase of ascorbic acid content of plant species may be due to increased rate of production of reactive oxygen species (ROS) during photo-oxidation of SO_2 to SO_3 where sulphites are generated from SO_2 absorbed. Tree species became sensitive for air pollutants. So, it can be an effective indicator for air pollution.

REFERENCES

1. Agarwal,M. (1985).Plants factors as indicators of SO_2 and O_3 Pollutants. Symp on Bio-monitoring stateenvironment.New Delhi, Proceedings, PP.225-231.
2. Arnon, D.I.(1949).Copper enzymes in isolated chloroplasts.Plant physiol.,24:1-15.
3. Barrs,H.D and Weatherly ,P.T.(1962). Are-examination of the relative turgidity technique for estimating water deficits in leaves. Australian J. Biol, Sci., 15:413-428
4. Prakash Govind, Gupta vinay, Poonia shefali, Sangita, Sharma Sujata (2002). "Effectof sulphur dioxide exposure on the chlorophyll contents in Raphanus sativus L andBrassica rapa L" (Dept Bot, CCs Univ, Meerut-250 005) Plant Arch, 2(2), 165-170.
5. Tiwari S, Bansal S (1994). "Air Pollution tolerance indices of some planted trees inurban areas of Bhopal." (Kirodimal Govt PG Coll, Raigarh, MP-496 001) ActaEcologica, 16(1), 1-8.
6. Turk, R and Wirth V (1975).pH dependence of SO_2 damage to lichens. Oecologia.,19: 285-295.

7. Thambavani.D. S., Prathipa.V (2010)A correlation of particulate matter with gaseous pollutants in ambient air of Dindigul town, Asian Journal of Environmental Science,Vol.5 No.2 pp.89-93 ref.16
8. Thambavani.D. S., Prathipa.V (2011),Assessment of leather tannery effluent using multiple indicators,Asian Journal of Experimental Chemistry ,Vol.6 No.2 pp.47-57
9. Thambavani.D. S., Prathipa.V (2011), Physico-chemical characteristics of leather tannery effluent-current scenario in Dindigul town (Tamil Nadu), India, Asian Journal of Environmental Science, Vol.6 No.2 pp.119-124
10. Sarala Thambavani .D and Prathipa. V. (2012) Bio monitoring of air pollution around urban and industrial sites Journal of research in Biology,1: 007-014
11. V.Prathipa, A.Sahaya Raja (2015), Air Quality Assessment and Air Quality Index of Dindigul Town (Tamil Nadu), India– A Case Study, International Journal of PharmTech Research,Vol.8, No.6, pp 45-55.
12. Thambavani, D. S., Prathipa.V (2012), Air quality index for Dindigul town,Asian Journal of Environmental Science,Vol.7 No.1 pp.13-20
13. Saralathambavani.D., Prathipa,.V (2012),Strategies of heavy metal uptake by plants growing under urban environment,Asian Journal of Soil Science,Vol.7 No.2 pp.304-311
14. Saralathambavani.D., Prathipa,.V (2012) A study of heavy metal contamination in the urban soil of Dindigul town, Tamil Nadu,Asian Journal of Soil Science,Vol.7 No.2 pp.150-158
15. Thambavani, D. S., Prathipa.V (2012) Quantitative assessment of soil metal pollution with principal component analysis, geo accumulation index and enrichment index, Asian Journal of Environmental Science, Vol.7 No.2 pp.125-134
16. Thambavani, D. S., Prathipa.V (2012),Biological monitoring of road side plants exposed to air pollution, Asian Journal of Environmental Science, Vol.7 No.1 pp.38-46
17. Thambavani, D. S., Prathipa.V (2012), Assessment of metal enrichments in industrial soil of Dindigul Town, Tamil Nadu,Asian Journal of Soil Science, Vol.7 No.1 pp.98-103
18. Thambavani, D. S., Prathipa.V (2013), Assessing heavy metal contamination in urban soils - an index analysis approach,Journal of Applicable Chemistry ,Vol.2 No.4 pp.887-897
19. V. Prathipa, A. Sahaya Raja, D. Sarala Thambavani (2015), A Study of traffic related air pollution at Dindigul district, Tamil Nadu, India, Journal of Environmental Science and Pollution Research,1(1), 6–7.
20. R. Rajendran, A. Pandia Rajan, A. Sahaya Raja, V. Prathipa, M.S. Dheenadayalan (2015), Assessment of physico–chemical parameters of River Cauvery in and around Nerur, Journal of Environmental Science and Pollution Research. 1(1), 17–19.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Rainfall Correlation of Length Weight Relationship and Fecundity of Spiny Rock Crab (*Thalamita crenata)*

Gideon A. Ebarsabal[1]* and Edilmar P. Masuhay[2]

[1]Surigao State College of Technology, Surigao City, Philippines.
[2]Surigao State College of Technology-Mainit Campus, Surigao del Norte, Philippines.

Received: 02 Oct 2020 Revised: 07 Dec 2020 Accepted: 12 Jan 2021

***Address for Correspondence**
Gideon A. Ebarsabal
Surigao State College of Technology,
Surigao City, Philippines.

ABSTRACT

This research was performed to determine the correlation of rainfall between the length-weight relationship and fecundity of spiny rock crab (*Thalamita crenata*) caught in the coastal islands barangays of Surigao City. 576 crabs consisting of 288 males of mean length of 3.9 cm, mean weight of 41.25 g, mean b value of 2.7 and 288 females of 3.8 cm and 36.29 g and 2.4 b values, respectively were tested. 109 crabs measuring 2.3-4.9 cm showed mean fecundity of 13,700. Rainfall was significantly correlated to male, female crabs, and fecundity $P > .0001$ with r^2 of 0.536, 0.083, and 0.14, respectively. Male b values were heavier during rainy days and leaner during the dry season. Females showed almost the same b values in a year.

Keywords: carapace, correlation, growth pattern, isometric, allometric.

INTRODUCTION

The presence of extensive mangrove areas along coastal islands of Surigao City supports aquatic organisms. This area serves as a buffer zone during the rainy season. It is greatly affected by the rainfall occurrence and can change the whole ecosystem's chemical structure. *Thalamita crenata* is a relatively small-sized crab species collected by artisanal fishermen only for local consumption as a source of protein. They live their entire lives in the mangrove area and very sensitive to changes in temperature brought about by rainfall. Studies of the Length-weight relationship provide a practical assessment of stocks aquatic species (Gulland, 1983; Enin, 1994; Stergou and Moutopuios, 2001; Pauly, 1993 cited by Patil & Patil, 2012). This may compare between populations of the same species or between species in gonad development and well-being of the animal stock. Further, length-width/weight relationship is more suitable for assessing not only fish but also crustacean (Sukumaran & Neelakantan, 1970; Tabash, 2001). Relationships between carapace length and weight of the crabs can be used to calculate the standing stock biomass, condition indices, analysis of ontogenetic changes, and several other aspects of crustacean population dynamics (Atar & Seçer, 2003).

Fecundity is the number of ripening eggs in the female before the next spawning season. Crabs vary from species to species and also vary within the same species due to different factors such as age, size, nourishment, ecological conditions of the water body, etc. Variation may primarily a reflection of variation in the size of the crab at maturity (Arshad, et. al. 2015). It bears some broad relationship to the environment according to the eggs. The longer warm season may affect demographic variables with rapid growth, development, and reproduction (Hines, et. al. 2010). Crustaceans, including brachyuran crabs, exhibit geographic variation in reproductive traits (Lonsdale and Levinton, 1985; Reaka, 1986; Hines, 1989; Dugan, et al. 1991; Lardies and Castilla, 2001; Brante, et. al. 2003). Freshwater crab Barytelphusa gurini length-weight relationship was conditioned by the seasonal monsoon that occurred in a year (Patil, et. al. 2012). Accordingly, Sigana (2002) stated that the reproductive performance of blue crab which changes the ecological parameters specifically rainfall that increases or decreases the salinity level. Further, Myla, et al. (2013) expounded that the correlation between the temperature and salinity of Mud Crab Scylla serrata (Forskal) was highly significant and may lead to stock management forecasting knowledge affecting weather condition. This paper presents the correlation of rainfall to length-weight relationship and fecundity of crabs.

METHODS

The study area was in the coastal islands in Surigao City 9043'44.18"N 125038'07.65"E. *T. crenata* were handpicked by fisher folks during night time. The specimen was randomly selected at the plastic baskets. A total of 576 pieces of 288 male and female *T. crenata*, was utilized in determining the length and weight in size structure, combined male and female berried and not berried, male, female not berried, berried, male and female body condition, rainfall in the wet and dry season. The wet season falls in December, January, February, August, September, October, and November while dry season in March, April, and May June, and July. They were measured in length (cm) using caliper and weight (g) using a digital weighing scale (0.01).

Sampling Procedure

This study was conducted in Tagana-an, Surigao del Norte from October 2016 to August 2017. Fifty (50) samples were bought from the fishermen and randomly selected with twenty-five (25) males and twenty-five (25) females.
After the collection, 50 crabs were placed in a pail and brought to the SSCT – Mainit Campus science laboratory for data sampling. The carapace length (cm) of the crabs was measured using a plastic ruler. Likewise, weight was identified using a digital weighing scale (.01 g).

Data Analysis

Length Weight Relationship

Body condition was analyzed using W=aLb classified as isometric growth b=3, negative allometric b<3 and positive allometry b>3. Begenal and Tesch (1978); and Patil (2012) reported when, (b=3) isometric growth takes place (animal grows without changing body shape), but when b value is below or above 3 allometry growth takes place (animal changes its shape as it grows larger).

Fecundity Determination

The eggs were removed and dislodged using a scalpel from the broad abdomen. Eggs were counted form the 3 subsamples on the dissecting set using the gravimetric method.
Fecundity was calculated using the formula (Hunter, et. al. 1989):

$$\text{Fecundity} = \frac{\text{Weight of the gonad (g)}}{\text{Mean weight of sub samples (g)}} \text{ X } 200$$

Monthly rainfall was derived from the data in Claver, Surigao del Norte station. Pearson correlation coefficient was used to determine the strength of the correlation (Cohen, 1988; Cohen, 1988 cited by Hemphill, 2003): the scatterplots were checked the significant outliers using the Shapiro-Wilk test of normality of two variables.

RESULTS

Salinity levels are influenced by several factors including rainfall, evaporation, an inflow of river water, and wind. The distribution and abundance of marine fish are heavily dependent on the salinity, temperature, and oxygen content of the different water layers. The amount of salt in the water (salinity) has many impacts on the fish and crabs (invertebrates). Some species have very specific optimal ranges for salinity while others easily transition. Crabs can live in a wide range of salinity from nearly freshwater to ocean saltwater. Monthly mean rainfall affects the temperature and salinity of the ecosystem. This interferes with the physiological functions of the animals inhabiting the mangrove environment. Rainfall changes the temperature level of the physical and chemical conditions of the surrounding. It varies the salinity of the water structure. Mangrove areas as buffer zone are directly affected by the disturbance causing the animals to adjust their homeostasis. *T. crenata* succumb to the adverse condition caused by the ecological changes brought about by rainfall (Sigana, 2002). High and low-level rainfall especially in the advent of climate change may affect the biological factor of crabs. Rainfall of Surigao del Norte normally followed five months dry season from March to July and the rainy season from August to February. The dry season falls when rainfall reached 250 mm below (https://www.worldweatheronline.com/claver-weather-averages/surigao-del.../ph.aspx).

However, from October 2016 to September 2017 most rainfall incidence in January was at 624.1 mm and moderate in November at 433.33 mm respectively. It revealed almost the same level at 221.22 to 64.5 mm in other months. This means that only two months was the wet season and the rest was the dry season. Fecundity is the number of oocytes or eggs produced in the spawning period. Rainfall affects directly on the production of gonad which determines the number of eggs (Sodamola, et.al. n.d in Sudanonautes Africanus, Milne–Edwards, 1869). Sampling revealed higher oocytes number from February to May 2017 ranges from 14,117 to 28,501 eggs. While on October 2016 to January 2017 it has few eggs ranging from 5,266 to 10,057. July to August 2017 showed moderate to higher numbers at 28,501 pieces. Zero eggs happened during the months of June and September. The results of the correlation of rainfall to fecundity and b value of males and females showed significantly correlated $P<.001$. However, the r2 ranged from .08 and .5 for female and male b value and .1 in fecundity. Rainfall and fecundity revealed a statistically significant negative relationship. The b value of male and female crabs showed a statistically significant positive relationship.

DISCUSSION

Increasing rainfall decrease the number of eggs produced by crabs. Heavier male crabs were caught from December 2016 to March 2017 coincide with high rainfall from November 2016 to January 2017. While leaner male crabs were gathered on April 2017 December 2017 coincide during low rainfall from March 2017 to December 2017. Female crabs showed almost the same lower b values over the whole year. The result of the study revealed a negative correlation as rainfall increased reversely decreased the number of egg production by female crabs. The reproductive capacity of blue crabs reduces at the onset of the rainy season and increases as the seasonal monsoon improved (Songrak, et.al. 2014). Male crabs were heavier during excessive rainfall resulted from the lesser activity at low temperatures and provide more energy reserved to produce tissues for growth (Roy, Singha, Ali, & Rhaman, 2012). Male crabs were thinner at lesser rainfall resulting from the good temperature that suits to the biological reproductively of female Blue Swimming Crab Portunus pelagicus (Sumpton,et.al. 2015); (Olusoji, et. al. 2010).

REFERENCES

1. Arshad, Q., Siddiqui, S., Ramachandran, S., Goga, U., Bonsu, A., Patel, M., ... & Bronstein, A. M. (2015). Right hemisphere dominance directly predicts both baseline V1 cortical excitability and the degree of top-down modulation exerted over low-level brain structures. *Neuroscience*, *311*, 484-489;
2. ATAR, H. H., & SEÇER, S. (2003). Width/length-weight relationships of the blue crab (*Callinectes sapidus* Rathbun 1896) population living in Beymelek Lagoon Lake. *Turkish Journal of Veterinary and Animal Sciences*, *27*(2), 443-447;
3. Brante, A., Fernández, M., Eckerle, L., Mark, F., Pörtner, H. O., & Arntz, W. (2003). Reproductive investment in the crab Cancer setosus along a latitudinal cline: egg production, embryo losses and embryo ventilation. *Marine Ecology Progress Series*, *251*, 221-232;
4. Cohen, J. (1988). Set correlation and contingency tables. *Applied psychological measurement*, *12*(4), 425-434;
5. Dugan, J. E., Wenner, A. M., & Hubbard, D. M. (1991). Geographic variation in the reproductive biology of the sand crab Emerita analoga (Stimpson) on the California coast. *Journal of Experimental Marine Biology and Ecology*, *150*(1), 63-81;
6. Enin, U. I. (1994). Length-weight parameters and condition factor of two West African prawns. *Revue d'hydrobiologie tropicale*, *27*(2), 121-127;
7. Gulland, J. A. (1983). *Fish stock assessmenta manual of basic methods* (No. 639.2021 G8);
8. Hemphill, J. F. (2003). Interpreting the magnitudes of correlation coefficients;
9. Hines, A. H. (1989). Geographic variation in size at maturity in brachyuran crabs. *Bulletin of Marine Science*, *45*(2), 356-368;
10. Hines, J. E., Nichols, J. D., Royle, J. A., MacKenzie, D. I., Gopalaswamy, A. M., Kumar, N. S., & Karanth, K. U. (2010). Tigers on trails: occupancy modeling for cluster sampling. *Ecological Applications*, *20*(5), 1456-1466;
11. Lardies, M., & Castilla, J. (2001). Latitudinal variation in the reproductive biology of the commensal crab Pinnaxodeschilensis (Decapoda: Pinnotheridae) along the Chilean coast. *Marine Biology*, *139*(6), 1125-1133;
12. Lonsdale, D. J., & Levinton, J. S. (1985). Latitudinal differentiation in copepod growth: an adaptation to temperature. *Ecology*, *66*(5), 1397-1407;
13. MYLA, S. C., TIRUMANI, V. B., & PATURI, R. C. G. (2017). Body weight, carapace length and width relationship and condition factor of the mud crab Scylla serrata (Forskal) in mangrove ecosystem. *Notulae Scientia Biologicae*, *9*(3), 338-343;
14. Olusoji, O. B., Anifowose, O. J., & Sodamola, M. Y. (2010). Length-weight relationships, condition factor and fecundity of the West Africa freshwater crab, Sudanonautes africanus (Milne-Edwards 1883), in Western Nigeria. *West African Journal of Applied Ecology*, *16*(1).
15. Patil, K. M., & Patil, M. U. (2012). Length-weight relationship and condition factor of freshwater crab Barytelphusa gurini,(Decapoda, Brachyura). *Journal of experimental Sciences*;
16. Reaka, M. L. (1986). Biogeographic patterns of body size in stomatopod Crustacea: ecological and evolutionary consequences. *Biogeography of the Crustacea: crustacean issues*, *4*, 209-235;
17. Roy, B. J., Singha, N. K., Ali, S. H., & Rhaman, M. G. (2012). Exploitation of marine swimming crab in Cox s Bazar, Bangladesh. *Bangladesh Journal of Zoology*, *40*(2), 241-245;
18. Sigana, D. O. (2002). Breeding Cycle of Thalamita crenata (Latreille, 1829) at Gazi Creek (Maftaha Bay), Kenya;
19. Sodamola, M. O., Bello-Olusoji, O. A., & Adejola, Y. A. Gonadal Developments of West African Freshwater Crab (Sudanonautes Africanus, Milne–Edwards, 1869) on the Banks of Asejire Reservoir, Nigeria;
20. Songrak, A., Bodhisuwan, W., Yoocharem, N., Udomwong, W., & Darbanandana, T. (2014). Reproductive biology of the blue swimming crab, Portunus pelagicus (Linnaeus, 1758) in the coastal waters of Trang province, Southern Thailand. *Journal of Fisheries and Environment*, *38*(2), 27-40;
21. Sukumaran, K. K., & Neelakantan, B. (1999). Spawning biology of two marine portunid crabs, Portunus (Portunus) sanguinolentus (Herbst) and Portunus (Portunus) pelagicus (Linnaeus) from the Karnataka Coast;

22. Sumpton, W. D., Campbell, M. J., O'Neill, M. F., McLennan, M., Campbell, A. B., Leigh, G. M., ... & Lloyd-Jones, L. (2015). Assessment of the blue swimmer crab (Portunus armatus) fishery in Queensland;
23. Tabash, F. A. (2001). Assessment and ecological characterization of the blue crab (jaiba, Callinectes arcuatus) in the Gulf of Nicoya, Costa Rica.

Fig. 1. Coastal island barangays of Surigao City (@Surigao City, Google Map)

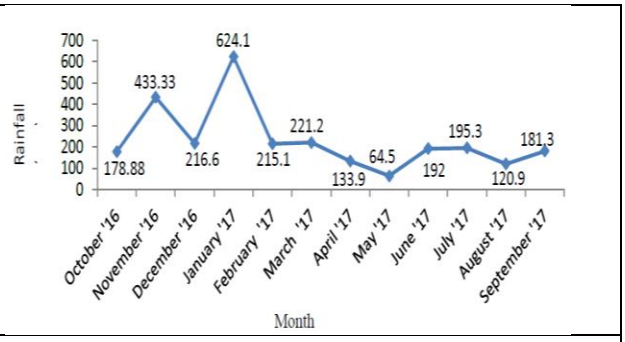


Fig. 2. Monthly mean rainfall from October 2016 to September 2017

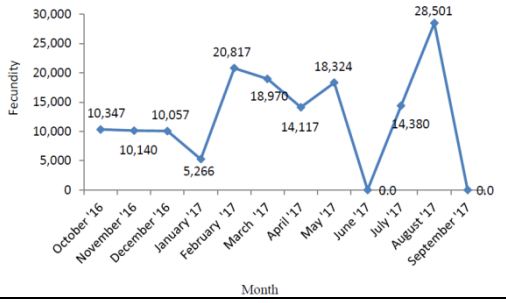


Fig. 3. Monthly mean fecundity of swimming crab from October 2016 to September 2017

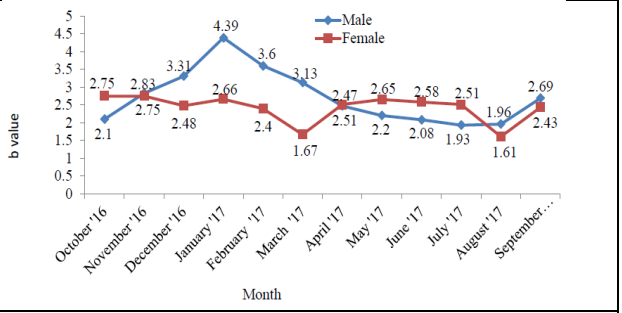


Fig. 4. Monthly b value of swimming crab from October 2016 to September

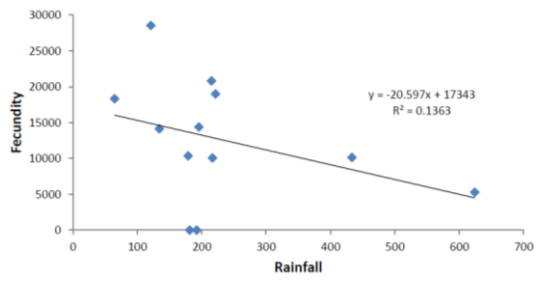


Fig. 5. Linear regression of rainfall and fecundity of crabs

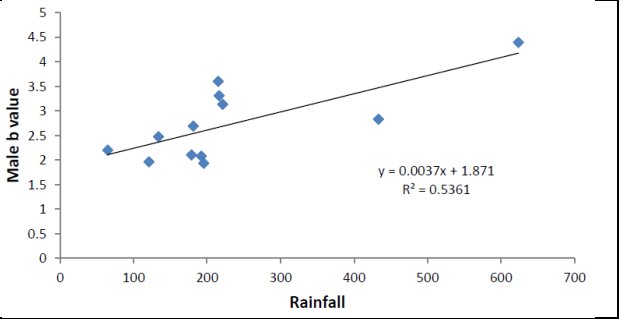


Fig. 6. Linear regression of rainfall and male b value of crabs

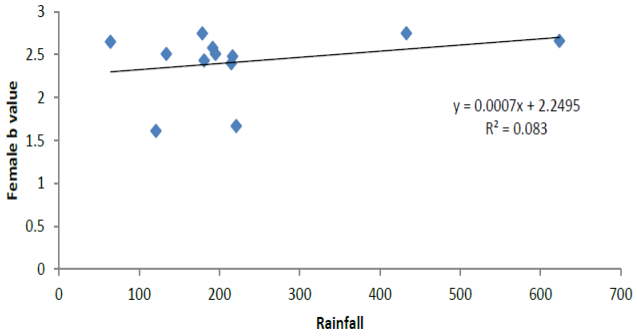


Fig. 7. Linear regression of rainfall and female b value of crabs

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Intuitionistic Fuzzy Normal HX Ring

G. Sabarinathan[1]*, K.H. Manikandan[1], M. Sridharan[1], M.S. Muthuraman[1] and R. Muthuraj[2]

[1]Department of Mathematics, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.
[2]PG and Research, Department of Mathematics, H.H.The Rajah's College, Pudukkottai, Tamil Nadu, India.

Received: 12 May 2020 Revised: 17 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
G. Sabarinathan
Department of Mathematics,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: sabarinathan.g@gmail.com

ABSTRACT

This research work defines an intuitionistic fuzzy normal HX ring of a HX ring and some of their important properties. Next we discuss the important operators of an intuitionistic fuzzy subset of an intuitionistic fuzzy normal HX ring and some of their properties.

Keywords: intuitionistic fuzzy set, fuzzy ring, fuzzy HX ring, intuitionistic fuzzy normal HX ring,

INTRODUCTION

In 1965, Zadeh [10] introduced the concept of fuzzy set. In 1967, Rosenfeld [9] defined fuzzy subgroup and discussed some of its properties. In 1982 Wang-jin Liu introduced the concept of fuzzy ring and fuzzy ideal. In 1988, Professor Li Hong Xing [4] proposed the concept of HX ring and derived some of its properties, then Professor Zhong [2] gave the structures of HX ring on a class of ring. R. Muthuraj et.al [6]., introduced the concept of fuzzy HX ring. In this paper we define a new algebraic structure of an intuitionistic fuzzy normal HX ring of a HX ring and investigate some related properties. We define the necessity and possibility operators of an intuitionistic fuzzy subset of an intuitionistic fuzzy normal HX ring and discuss some of its properties. Also we discuss the image and pre-image of an intuitionistic fuzzy set in an intuitionistic fuzzy normal HX ring and discuss some of its properties.

PRELIMINARIES

In this section, we gave the fundamental definitions that will be used in the paper. Throughout this paper, R = (R,+,·) is a Ring, e is the additive identity element of R and xy, we mean x.y

2.1 Definition

Let R be a ring. In $2^R-\{\phi\}$, a non-empty set $\vartheta \subset 2^R-\{\phi\}$ with two binary operation ' + ' and '.' is said to be a HX ring on R if ϑ is a ring with respect to the algebraic operation defined by

i. $A + B = \{a + b / a \in A \text{ and } b \in B\}$, which its null element is denoted by Q , and the negative element of A is denoted by – A.

ii. $AB = \{ab / a \in A \text{ and } b \in B\}$,

iii. $A (B + C) = AB + AC$ and $(B + C) A = BA + CA$. for all $A,B,C \in \vartheta$.

2.2 Definition

Let R be a ring. Let μ be a fuzzy ring defined on R. Let $\vartheta \subset 2^R-\{\phi\}$ be a HX ring. A fuzzy subset λ^μ of ϑ is called a fuzzy HX ring on ϑ or a fuzzy ring induced by μ if the following conditions are satisfied. For all $A,B \in \vartheta$,

i. $\lambda^\mu (A - B) \geq \min \{ \lambda^\mu (A), \lambda^\mu (B) \}$, ii. $\lambda^\mu (AB) \geq \min \{ \lambda^\mu (A), \lambda^\mu (B) \}$

where $\lambda^\mu (A) = \max\{ \mu(x) / \text{ for all } x \in A \subseteq R \}$.

2.3 Definition

Let R be a ring. Let μ be a fuzzy ring defined on R. Let $\vartheta \subset 2^R-\{\phi\}$ be a HX ring. A fuzzy subset λ^μ of ϑ is called a fuzzy normal HX subring on ϑ if, for all $A,B \in \vartheta$, $\lambda^\mu(AB) = \lambda^\mu(BA)$,where $\lambda^\mu (A) = \max\{ \mu(x) / \text{ for all } x \in A \subseteq R \}$.

PROPERTIES OF AN INTUITIONISTIC FUZZY NORMAL HX SUBRING

3.1 Definition

Let R be a ring. Let μ be a fuzzy ring on R and a nonempty set $\vartheta \subset 2^R-\{\phi\}$ is a HX ring. An intuitionistic fuzzy subset $\lambda^H = \langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle$ of a HX ring ϑ is said to be an intuitionistic fuzzy HX (IFHXSR) subring of ϑ if the following conditions are satisfied. For all $A , B \in \vartheta$,

i. $\lambda^\mu(A-B) \geq \min\{ \lambda^\mu(A) , \lambda^\mu(B) \}$, ii. $\lambda^\mu(AB) \geq \min\{ \lambda^\mu(A) , \lambda^\mu(B) \}$,

iii. $\lambda^\gamma(A-B) \leq \max\{ \lambda^\gamma(A) , \lambda^\gamma(B) \}$, iv. $\lambda^\gamma(AB) \leq \max \{ \lambda^\gamma(A) , \lambda^\gamma(B) \}$

Where $\lambda^\mu(A) = \max\{ \mu(x) / x \in A \subseteq R \}$, $\lambda^\gamma(A) = \min\{\gamma(x) / x \in A \subseteq R\}$.

3.2 Definition

Let R be a ring. Consider $H = \{ \langle x, \mu(x), \gamma(x) \rangle / x \in R \}$ be an intuitionistic fuzzy set defined on R, where $\mu : R \to [0,1]$, $\gamma : R \to [0,1]$ such that $0 \leq \mu(x) + \gamma(x) \leq 1$. Let $\vartheta \subset 2^R-\{\phi\}$ be a HX ring. An intuitionistic fuzzy HX subring (IFHXSR) $\lambda^H = \{\langle A, \lambda^\mu(A), \lambda^\gamma(A) \rangle / A \in \vartheta, 0 \leq \lambda^\mu(A) + \lambda^\gamma(A) \leq 1\}$ of a HX ring ϑ is said to be an intuitionistic fuzzy normal HX subring (IFNHXSR) if for all $A,B \in \vartheta$,

(i) $\lambda^\mu(AB) = \lambda^\mu(BA)$ (ii) $\lambda^\gamma(AB) = \lambda^\gamma(BA)$

where, $\lambda^\mu(A) = \max \{\mu(x) / \text{ for all } x \in A \subseteq R\}$, $\lambda^\gamma(A) = \min \{\gamma(x) / \text{ for all } x \in A \subseteq R\}$

3.3 Theorem

If λ^H and λ^K are any two intuitionistic fuzzy normal HX subrings of a HX ring ϑ, then $\lambda^H \cap \lambda^K$ is also an intuitionistic fuzzy normal HX subring of a HX ring ϑ.

Proof

Let $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta \}$ and $\lambda^K = \{\langle A , \Theta^\eta(A) , \Theta^\delta(A) \rangle / A \in \vartheta \}$ be any two intuitionistic fuzzy normal HX subrings of a HX ring ϑ,.

To Prove $\lambda^H \cap \lambda^K$ is also an intuitionistic fuzzy normal HX subring of a HX ring ϑ.

By Theorem 3.3[6], $\lambda^H \cap \lambda^K$ is an intuitionistic fuzzy HX subring of ϑ. We enough to show the normality condition. For any $A,B \in \vartheta$,we have

(i) $(\lambda^\mu \cap \Theta^\eta)(AB) = \min \{\lambda^\mu(AB), \Theta^\eta(AB)\}$

$= \min \{\lambda^\mu(BA), \Theta^\eta(BA)\}$

$= (\lambda^{\mu} \cap \Theta^{\eta})(BA)$

Hence, $(\lambda^{\mu} \cap \Theta^{\eta})(AB) = (\lambda^{\mu} \cap \Theta^{\eta})(BA)$

(ii) $(\lambda^{\gamma} \cap \Theta^{\delta})(AB) = \max \{\lambda^{\gamma}(AB), \Theta^{\delta}(AB)\}$

$= \max \{\lambda^{\gamma}(BA), \Theta^{\delta}(BA)\}$

$= (\lambda^{\gamma} \cap \Theta^{\delta})(BA)$

Hence, $(\lambda^{\gamma} \cap \Theta^{\delta})(AB) = (\lambda^{\gamma} \cap \Theta^{\delta})(BA)$

Therefore, the intersection of any two IFNHX subrings is also an IFNHX subring of ϑ.

3.4 Definition

Let $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A) \rangle / \text{for all } A \in \vartheta\}$ be an intuitionistic fuzzy subset of a HX ring ϑ. We define the following "necessity" and "possibility" operators :

$\Box \lambda^H = \{\langle A, \lambda^{\mu}(A), 1 - \lambda^{\mu}(A) \rangle / A \in \vartheta\}$, $\Diamond \lambda^H = \{\langle A, 1 - \lambda^{\gamma}(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$

3.5 Theorem

If λ^H is an intuitionistic fuzzy normal HX subring of a HX ring ϑ then $\Box \lambda^H$ is an intuitionistic fuzzy normal HX subring of a HX ring ϑ.

Proof

By Theorem 3.6[6], $\Box \lambda^H = \{\langle A, \lambda^{\mu}(A), (\lambda^{\mu})^c(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy HX subring of ϑ.

To prove that $\Box \lambda^H$ is an intuitionistic fuzzy normal HX subring of ϑ.

Let $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

We have i. $\lambda^{\mu}(AB) = \lambda^{\mu}(BA)$ ii. $\lambda^{\gamma}(AB) = \lambda^{\gamma}(BA)$

Now, $(\lambda^{\mu})^c(AB) = 1 - (\lambda^{\mu})(AB)$

$= 1 - (\lambda^{\mu})(BA)$

$= (\lambda^{\mu})^c(BA)$

Hence , $\lambda^{\mu}(AB) = \lambda^{\mu}(BA)$ and $(\lambda^{\mu})^c(AB) = (\lambda^{\mu})^c(BA)$

Therefore, $\Box \lambda^H = \{\langle A, \lambda^{\mu}(A), (\lambda^{\mu})^c(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy normal HX subring of ϑ.

3.6 Theorem

If λ^H is an intuitionistic fuzzy normal HX subring of a HX ring ϑ then $\Diamond \lambda^H$ is an intuitionistic fuzzy normal HX subring of a HX ring ϑ.

Proof

By Theorem 3.7[6], $\Diamond \lambda^H = \{\langle A, (\lambda^{\gamma})^c(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy HX subring of ϑ.

To prove that $\Diamond \lambda^H$ is an intuitionistic fuzzy normal HX subring of ϑ.

Let $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

We have i. $\lambda^{\mu}(AB) = \lambda^{\mu}(BA)$ ii. $\lambda^{\gamma}(AB) = \lambda^{\gamma}(BA)$

Now $(\lambda^{\gamma})^c(AB) = 1 - (\lambda^{\gamma})(AB)$

$= 1 - (\lambda^{\gamma})(BA)$

$= (\lambda^{\gamma})^c(BA)$

Hence , $(\lambda^{\gamma})^c(AB) = (\lambda^{\gamma})^c(BA)$ and $\lambda^{\gamma}(AB) = \lambda^{\gamma}(BA)$

Therefore, $\Diamond \lambda^H = \{\langle A, (\lambda^{\gamma})^c(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$ is a intuitionistic fuzzy normal HX subring of ϑ.

3.7 Theorem

An IFS $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy normal HX subring of a HX ring ϑ if and only if the fuzzy subsets $\lambda^{\mu}(A)$, $(\lambda^{\gamma})^c(A)$ are fuzzy normal HX subrings of a HX ring ϑ.

Proof

Let $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

We have i. $\lambda^\mu(AB) = \lambda^\mu(BA)$ ii. $\lambda^\gamma(AB) = \lambda^\gamma(BA)$.

Clearly, $\lambda^\mu(A)$ is a fuzzy normal HX subring of ϑ by (i)

Now we have to show $(\lambda^\gamma)^c$ is a fuzzy normal HX subring of ϑ.

$$(\lambda^\gamma)^c(AB) = 1 - (\lambda^\gamma)(AB)$$
$$= 1 - (\lambda^\gamma)(BA)$$
$$= (\lambda^\gamma)^c(BA)$$

Thus, $(\lambda^\gamma)^c$ is a fuzzy normal HX subring of ϑ.

Conversely, $\lambda^\mu(A)$ and $(\lambda^\gamma)^c(A)$ are fuzzy normal HX subrings of a HX ring ϑ

To prove that $\lambda^H = \{\langle A ,\lambda^\mu(A),\lambda^\gamma(A)\rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

Now, we know that

$$(\lambda^\gamma)^c(AB) = (\lambda^\gamma)^c(BA)$$
$$1 - (\lambda^\gamma)(AB) = 1 - (\lambda^\gamma)(BA)$$
$$(\lambda^\gamma)(AB) = (\lambda^\gamma)(BA)$$

Already, we have $\lambda^\mu(AB) = \lambda^\mu(BA)$.

Hence, $\lambda^H = \{\langle A, \lambda^\mu(A),\lambda^\gamma(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

3.8 Theorem

An IFS $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy normal HX subring of a HX ring ϑ if and only if the fuzzy subsets $(\lambda^\mu)^c$ and (λ^γ) are fuzzy normal HX subrings of a HX ring ϑ.

Proof

Let $\lambda^H = \{\langle A ,\lambda^\mu(A), \lambda^\gamma(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ. We have (i) $\lambda^\mu(AB) = \lambda^\mu(BA)$ (ii) $\lambda^\gamma(AB) = \lambda^\gamma(BA)$.

From (ii) it is clear that (λ^γ) is a fuzzy normal HX subring of ϑ.

Now, $(\lambda^\mu)^c(AB) = 1 - (\lambda^\mu)(AB)$

$$= 1 - (\lambda^\mu)(BA)$$
$$= (\lambda^\mu)^c(BA)$$

Hence, $(\lambda^\mu)^c$ and (λ^γ) are fuzzy normal HX subrings of a HX ring ϑ.

Conversely, Let $(\lambda^\mu)^c$ and (λ^γ) are fuzzy normal HX subrings of a HX ring ϑ

To prove that $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta\}$ be a intuitionistic fuzzy normal HX subring of ϑ.

Now, $(\lambda^\mu)^c(AB) = (\lambda^\mu)^c(BA)$

$$1 - (\lambda^\mu)(AB) = 1 - (\lambda^\mu)(BA)$$
$$\lambda^\mu(AB) = \lambda^\mu(BA)$$

Thus $\lambda^\mu(AB) = \lambda^\mu(BA)$ and $\lambda^\gamma(AB) = \lambda^\gamma(BA)$

Hence, $\lambda^H = \{\langle A,\lambda^\mu(A), \lambda^\gamma(A)\rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

3.9 Theorem

An IFS $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta\}$ is an intuitionistic fuzzy normal HX subring of a HX ring ϑ if and only if the fuzzy subsets $(\lambda^\mu)^c$ and $(\lambda^\gamma)^c$ are fuzzy normal HX subrings of a HX ring ϑ.

Proof

Let $\lambda^H = \{\langle A , \lambda^\mu(A) , \lambda^\gamma(A) \rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ. We have (i) $\lambda^\mu(AB) = \lambda^\mu(BA)$ (ii) $\lambda^\gamma(AB) = \lambda^\nu(BA)$.

Now, $(\lambda^\mu)^c(AB) = 1 - (\lambda^\mu)(AB)$

$$= 1 - (\lambda^\mu)(BA)$$

$= (\lambda^{\mu})^c (BA)$

and $(\lambda^{\gamma})^c (AB) = 1 - (\lambda^{\gamma})(AB)$

$= 1 - (\lambda^{\gamma})(BA)$

$= (\lambda^{\gamma})^c (BA)$

Hence, $(\lambda^{\mu})^c$ and $(\lambda^{\gamma})^c$ are fuzzy normal HX subrings of a HX ring ϑ.

Conversely, Let $(\lambda^{\mu})^c$ and $(\lambda^{\gamma})^c$ are fuzzy normal HX subrings of a HX ring ϑ

To prove that $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A)\rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

Now, $(\lambda^{\mu})^c (AB) = (\lambda^{\mu})^c (BA)$

$1 - (\lambda^{\mu})(AB) = 1 - (\lambda^{\mu})(BA)$

$\lambda^{\mu}(AB) = \lambda^{\mu}(BA)$

Therefore, $\lambda^{\mu}(AB) = \lambda^{\mu}(BA)$

And also, $(\lambda^{\gamma})^c (AB) = (\lambda^{\gamma})^c (BA)$

$1 - (\lambda^{\gamma})(AB) = 1 - (\lambda^{\gamma})(BA)$

$\lambda^{\gamma}(AB) = \lambda^{\gamma} (BA)$.

Hence, $\lambda^H = \{\langle A, \lambda^{\mu}(A), \lambda^{\gamma}(A)\rangle / A \in \vartheta\}$ be an intuitionistic fuzzy normal HX subring of ϑ.

3.10 Definition [6]

Let ϑ_1 and ϑ_2 be any two HX rings. Then the function $f : \vartheta_1 \to \vartheta_2$ is said to be a homomorphism if it satisfies the following axioms:

i) $f(A+B) = f(A) + f(B)$ and ii) $f(AB) = f(A)\, f(B)$, for all $A,B \in \vartheta_1$.

3.11 Definition [6]

Let ϑ_1 and ϑ_2 be any two HX rings. Then the function $f : \vartheta_1 \to \vartheta_2$ is said to be an anti homomorphism if it satisfies the following axioms:

i) $f(A+B) = f(B) + f(A)$ and ii) $f(AB) = f(B)\, f(A)$, for all $A,B \in \vartheta_1$.

3.12 Definition

Let R_1 and R_2 be any two rings. Let $\vartheta_1 \subset 2^{R1} - \{\phi\}$ and $\vartheta_2 \subset 2^{R2} - \{\phi\}$ be any two HX rings. Let $A = \{\langle x, \mu_A(x), \gamma_A(x)\rangle / x \in R_1\}$ and $B = \{\langle y, \alpha_B(y), \beta_B(y)\rangle / y \in R_2\}$ be any two intuitionistic fuzzy sets on R_1 and R_2 respectively. Let $C = \{\langle U, \lambda_C^{\mu}(U), \theta_C^{\gamma}(U)\rangle / U \in \vartheta_1\}$ and $D = \{\langle V, \eta_D^{\alpha}(V), \psi_D^{\beta}(V)\rangle / V \in \vartheta_2\}$ any two intuitionistic fuzzy sets in ϑ_1 and ϑ_2 resp. Let f be a function from ϑ_1 into ϑ_2 then the image of C on ϑ_1 under f is defined as

$$\eta_D^{\alpha}(V) = \begin{cases} \max\left\{\lambda_C^{\mu}(U) : U \in f^{-1}(V)\right\}, f^{-1}(V) \neq \varphi \\ 0 \qquad\qquad , otherwise \end{cases}, \quad \psi_D^{\beta}(V) = \begin{cases} \min\left\{\theta_C^{\gamma}(U) : U \in f^{-1}(V)\right\}, f^{-1}(V) \neq \varphi \\ 1 \qquad\qquad , otherwise \end{cases}$$

Where $\eta_D^{\alpha} = f(\lambda_C^{\mu})$ also Pre-image of D on ϑ_2 under f is defined as

$\left(f^{-1}(\eta_D^{\alpha})\right)(U) = \eta_D^{\alpha}(f(U))$, $\left(f^{-1}(\psi_D^{\beta})\right)(U) = \psi_D^{\beta}(f(U))$.

3.13 Theorem

Let R_1 and R_2 be any two rings. Let $A = \{\langle x, \mu_A(x), \gamma_A(x)\rangle / x \in R_1\}$ and $B = \{\langle y, \alpha_B(y), \beta_B(y)\rangle / y \in R_2\}$ be any two intuitionistic fuzzy sets on R_1 and R_2 respectively. Let $C = \{\langle U, \lambda_C^{\mu}(U), \theta_C^{\gamma}(U)\rangle / U \in \vartheta_1\}$ and $D = \{\langle V, \eta_D^{\alpha}(V), \psi_D^{\beta}(V)\rangle / V \in \vartheta_2\}$ be any two intuitionistic fuzzy sets in ϑ_1 and ϑ_2 respectively. Let f be an onto homomorphism from ϑ_1 to ϑ_2. If C be an intuitionistic fuzzy normal HX subring of ϑ_1 then f(C) is also an intuitionistic fuzzy normal HX subring of ϑ_2.

Proof

Let C be an intuitionistic fuzzy normal HX subring of ϑ_1 then

$(i) \quad \lambda_C^{\mu}(UT) = \lambda_C^{\mu}(TU)$, $(ii) \quad \theta_C^{\gamma}(UT) = \theta_C^{\gamma}(TU)$

By Theorem 3.13 [6], f(C) is an intuitionistic fuzzy HX subring of ϑ_2.

To Prove that f(C) is an intuitionistic fuzzy normal HX subring of ϑ_2.

Let V = f(U) , W = f(T) $\in \vartheta_2$, where U,T$\in \vartheta_1$. Now

$$[f(\lambda_C^{\mu})]\,[f(U)\ f(T)] = [f(\lambda_C^{\mu})]\,[f(UT)]$$
$$= \lambda_C^{\mu}(UT)$$
$$= \lambda_C^{\mu}(TU)$$
$$= [f(\lambda_C^{\mu})]\,[f(TU)]$$
$$[f(\lambda_C^{\mu})]\,[f(U)\ f(T)] = [f(\lambda_C^{\mu})]\,[f(T)f(U)]$$

And, $[f(\theta_c^{\gamma})][f(U)f(T)] = [f(\theta_c^{\gamma})][f(UT)]$

$$= \theta_c^{\gamma}(UT)$$
$$= \theta_c^{\gamma}(TU)$$
$$= [f(\theta_c^{\gamma})][f(TU)]$$
$$[f(\theta_c^{\gamma})][f(U)f(T)] = [f(\theta_c^{\gamma})][f(T)f(U)]$$

Hence, D = f(C) is an intuitionistic fuzzy normal HX subring of ϑ_2.

3.14 Theorem

Let R_1 and R_2 be any two rings. Let A = { ⟨ x, $\mu_A(x)$, $\gamma_A(x)$ ⟩ / x$\in R_1$ } and B = { ⟨ y, $\alpha_B(y)$, $\beta_B(y)$ ⟩ / y$\in R_2$ } be any two intuitionistic fuzzy sets on R_1 and R_2 respectively. Let C = { ⟨U, $\lambda_C^{\mu}(U)$, $\theta_C^{\gamma}(U)$ ⟩ / U$\in \vartheta_1$} and D = {⟨ V, $\eta_D^{\alpha}(V)$, $\psi_D^{\beta}(V)$ ⟩ / V$\in \vartheta_2$ } be any two intuitionistic fuzzy sets in ϑ_1 and ϑ_2 resp. Let f be an onto homomorphism from ϑ_1 to ϑ_2. If D be the intuitionistic fuzzy normal HX subring of ϑ_2 then f^{-1}(D) is an intuitionistic fuzzy normal HX subring of ϑ_1.

Proof

Let D be an intuitionistic fuzzy normal HX subring of ϑ_2 then

$(i) \quad \eta_D^{\alpha}[VW] = \eta_D^{\alpha}[WV]$ $(ii) \quad \psi_D^{\beta}[VW] = \psi_D^{\beta}[WV]$

By Theorem 3.14 [6], f^{-1}(D) is an intuitionistic fuzzy HX subring of ϑ_1.

To Prove that f^{-1}(D) is an intuitionistic fuzzy normal HX subring of ϑ_1.

Let U,T$\in \vartheta_1$ and f(U) = V , f(T) =W$\in \vartheta_2$.

$$[f^{-1}(\eta_D^{\alpha})]\,(UT) = \eta_D^{\alpha}\,[f(UT)]$$
$$= \eta_D^{\alpha}\,[f(U)f(T)]$$
$$= \eta_D^{\alpha}\,[VW]$$
$$= \eta_D^{\alpha}\,[WV]$$
$$= \eta_D^{\alpha}\,[f(T)f(U)]$$
$$= \eta_D^{\alpha}\,[f(TU)]$$
$$= [f^{-1}(\eta_D^{\alpha})]\,(TU)$$

Also, $[f^{-1}(\psi_D^{\beta})](UT) = \psi_D^{\beta}[f(UT)]$

$= \psi_D^{\beta}[f(U)f(T)]$

$= \psi_D^{\beta}[VW]$

$= \psi_D^{\beta}[WV]$

$[f^{-1}(\psi_D^{\beta})](UT) = [f^{-1}(\psi_D^{\beta})](TU)$

Therefore, $f^{-1}(D)$ is an intuitionistic fuzzy normal HX subring of ϑ_1.

3.15 Theorem

Let R_1 and R_2 be any two rings. Let $A = \{\langle x, \mu_A(x), \gamma_A(x)\rangle / x \in R_1\}$ and $B = \{\langle y, \alpha_B(y), \beta_B(y)\rangle / y \in R_2\}$ be any two intuitionistic fuzzy sets on R_1 and R_2 respectively. Let $C = \{\langle U, \lambda_C^{\mu}(U), \theta_C^{\gamma}(U)\rangle / U \in \vartheta_1\}$ and $D = \{\langle V, \eta_D^{\alpha}(V), \psi_D^{\beta}(V)\rangle / V \in \vartheta_2\}$ be any two intuitionistic fuzzy sets in ϑ_1 and ϑ_2 respectively. Let f be an onto anti-homomorphism from ϑ_1 to ϑ_2. If C is an intuitionistic fuzzy normal HX subring of ϑ_1 then f(C) is an intuitionistic fuzzy normal HX subring of ϑ_2.

Proof

Let C be an intuitionistic fuzzy normal HX subring of ϑ_1 then

$(i)\quad \lambda_C^{\mu}(UT) = \lambda_C^{\mu}(TU) \qquad (ii)\quad \theta_C^{\gamma}(UT) = \theta_C^{\gamma}(TU)$

By Theorem 3.15 [6], f(C) is an intuitionistic fuzzy HX subring of ϑ_2.

To Prove that f(C) is an intuitionistic fuzzy normal HX subring of ϑ_2.

Let $V = f(U)$, $W = f(T) \in \vartheta_2$, where $U,T \in \vartheta_1$. Now,

$[f(\lambda_C^{\mu})][f(U)\ f(T)] = [f(\lambda_C^{\mu})][f(TU)]$

$= \lambda_C^{\mu}(TU)$

$= \lambda_C^{\mu}(UT)$

$= [f(\lambda_C^{\mu})][f(UT)]$

$[f(\lambda_C^{\mu})][f(U)\ f(T)] = [f(\lambda_C^{\mu})][f(T)f(U)]$

Also, $[f(\theta_c^{\gamma})][f(U)f(T)] = [f(\theta_c^{\gamma})][f(TU)]$

$= \theta_c^{\gamma}(TU)$

$= \theta_c^{\gamma}(UT)$

$= [f(\theta_c^{\gamma})][f(UT)]$

$[f(\theta_c^{\gamma})][f(U)f(T)] = [f(\theta_c^{\gamma})][f(T)f(U)]$

Thus D = f(C) is an intuitionistic fuzzy normal HX subring of ϑ_2.

3.16 Theorem

Let R_1 and R_2 be any two rings. Let $A = \{\langle x, \mu_A(x), \gamma_A(x)\rangle / x \in R_1\}$ and $B = \{\langle y, \alpha_B(y), \beta_B(y)\rangle / y \in R_2\}$ be any two intuitionistic fuzzy sets on R_1 and R_2 respectively. Let $C = \{\langle U, \lambda_C^{\mu}(U), \theta_C^{\gamma}(U)\rangle / U \in \vartheta_1\}$ and $D = \{\langle V, \eta_D^{\alpha}(V), \psi_D^{\beta}(V)\rangle / V \in \vartheta_2\}$ be any two intuitionistic fuzzy sets in ϑ_1 and ϑ_2 resp. Let

f be an onto anti- homomorphism from ϑ_1 to ϑ_2. If D is an intuitionistic fuzzy normal HX subring of ϑ_2 then $f^{-1}(D)$ is an intuitionistic fuzzy normal HX subring of ϑ_1.

Proof

Let D be an intuitionistic fuzzy normal HX subring of ϑ_2 then

(i) $\eta_D^{\alpha}[VW] = \eta_D^{\alpha}[WV]$ (ii) $\psi_D^{\beta}[VW] = \psi_D^{\beta}[WV]$

By Theorem 3.16[6], $f^{-1}(D)$ is an intuitionistic fuzzy HX subring of ϑ_1.

To Prove that $f^{-1}(D)$ is an intuitionistic fuzzy normal HX subring of ϑ_1.

Let $U,T \in \vartheta_1$ and $f(U) = V$, $f(T) = W \in \vartheta_2$. Now

$$[f^{-1}(\eta_D^{\alpha})](UT) = \eta_D^{\alpha}[f(UT)]$$
$$= \eta_D^{\alpha}[f(U)f(T)]$$
$$= \eta_D^{\alpha}[VW]$$
$$= \eta_D^{\alpha}[WV]$$
$$= \eta_D^{\alpha}[f(T)f(U)]$$
$$= \eta_D^{\alpha}[f(TU)]$$
$$[f^{-1}(\eta_D^{\alpha})](UT) = [f^{-1}(\eta_D^{\alpha})](TU)$$

Also, $[f^{-1}(\psi_D^{\beta})](UT) = \psi_D^{\beta}[f(UT)]$
$$= \psi_D^{\beta}[f(U)f(T)]$$
$$= \psi_D^{\beta}[VW]$$
$$= \psi_D^{\beta}[WV]$$
$$[f^{-1}(\psi_D^{\beta})](UT) = [f^{-1}(\psi_D^{\beta})](TU)$$

Thus, $f^{-1}(D)$ is an intuitionistic fuzzy normal HX subring of ϑ_1.

ACKNOWLEDGEMENT

The authors would like to express their sincere thanks to all our friends for their help to make this paper a successful one.

REFERENCES

1. Atanassov. K.T, intuitionistic fuzzy sets, Fuzzy Sets and System 20 (1986) ,pp 87-96.
2. Bing-xueYao and Yubin-Zhong, The construction of power ring, Fuzzy information and Engineering (ICFIE),ASC 40,pp.181-187,2007.
3. Dheena. P and Mohanraaj. G, On intuitionistic fuzzy k ideals of semi rings, International Journal of computational cognition, Volume 9, No.2, 45-50, June 2011.
4. Li Hong Xing, HX ring, BUSEFAL, 34(1) 3-8, January 1988.
5. Muthuraj.R, Manikandan K.H., Sithar Selvam. P.M., Intuitionistic Q-fuzzy normal HX group, International Journal of Mathematical Archive , Vol.2,no10.,pp 1828-1832.
6. Muthuraj. R, Muthuraman. M.S., Intuitionistic fuzzy HX ring ,IOSR Journal of Mathematics (IOSR-JM) Volume 10, Issue 4,Ver II , 3-12 (Jul-Aug 2014).

7. Muthuraj. R and Muthuraman. M.S, Intuitionistic anti fuzzy semiprimary HX ideal in HX ring, International journal of applied engineering research, 2015, vol.10, no.39, pp. 30050 30056.
8. Muthuraj. R and Muthuraman. M.S, Intuitionistic fuzzy HX k – ideal of a semi HX ring, SS International journal of pure and applied mathematics, 2015, pp. 31-41.
9. Rosenfeld. A., Fuzzy groups, J. Math. Anal.,35(1971),512-517.
10. Zadeh. L.A., Fuzzy sets, Information and control,8,338-353.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Review Paper on Implementation of Neural Network for FPGA System Design

Varghese M P[1] and T Muthumanickam[2]*

[1]Research Scholar, Department of Electronics and Communication Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.
[2]Professor and Head of Department of Electronics and Communication Engineering, Vinayaka Mission's Kirupananda Variyar Engineering College, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

Received: 28 Nov 2020 Revised: 04 Dec 2020 Accepted: 28 Dec 2020

***Address for Correspondence**
T Muthumanickam
Professor and Head of Department of Electronics and Communication Engineering,
Vinayaka Mission's Kirupananda Variyar Engineering College,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.

ABSTRACT

In this paper, an investigation is carried out on how neural networks can be implemented for different stages of the FPGA placement flow. A machine-learning framework can be used to model the underlying relationship between characteristics of circuits and obtain an optimized implementation on an FPGA. The efficiency of the framework can be demonstrated by applying framework for reducing the cost of performing placement and routing. The framework recommends the best placement flow for different circuits. The framework also predicts various quality metrics without incurring the cost of performing placement and routing. In recent years, it is very much demanding to deploy Deep Neural Networks on devices such as mobile wearable devices, mobiles and drones, to process visual data acquired by the cameras present in the systems. Also, training DNNs locally benefit model customization and data privacy security. Many systems are powered by batteries or will be having limited energy sources, FPGA is commonly used as the primary processing engine to satisfy both demands in energy-efficiency and performance. Training a DNN with FPGAs has not been well exploited by the community to its full extend.

Keywords: FPGA, Energy, Prediction, Neural Networks, ANN

INTRODUCTION

The main problem encountered in a VLSI circuit design is Energy consumption, for which CMOS is the prominent technology. Focus on low power is not only because of the recent demands for portable applications. Battery-powered gadgets have created a demand for high energy-efficient circuit. Mobile phones represent a very huge industry, creates a scope for innovation along with profitability. The scope for gadgets that are portable is going to depend on the improvement of low-cost devices with long battery life and complex functionality. A major concern has always been power consumption. This review paper focuses on the requirement of an energy-efficient model for overcoming energy consumption in a FPGA system, focusing on measuring power-dissipation in different components and, ultimately, evaluating various ways of reducing total power consumption in a FPGA unit using Neural network. The major advantage of using Artificial Neural Network is that it is a part of the Artificial Intelligence Deep Learning domain, which makes it easy to model complex systems. A comprehensive knowledge about the underlying module technology is not a requisite. As FPGAs continue to scale in accordance with Moore's law, so is the size of the applications targeted for them. The result is increase in runtime for optimization stages in the Computer Aided Design flow, larger amounts of data generated and passed from one stage to next, pressure at every stage to create high-quality solutions required for later stages. This trend continues to explore other approaches that can be used for problems throughout the CAD flow.

No guarantee of routing success is a major problem faced while designing Field Programmable Gate Array (FPGA) and it can take hours to place. Therefore, it is important for the placement tool to know whether a design is routable at an early stage. Estimating routability at an early stage, can be beneficial for the placer to avoid pursuing dead-end paths that do not lead to a feasible routing solution and early feedback enable the placer to improve its optimization strategy. For example, if a placement resolved to be easy to route may result in the placer avoiding further optimization and thus runtime is reduced. On the other hand, a placement which is hard to route causes the placer to increases the effort to optimize. However, developing a routability prediction model fast enough to be repeatedly applied during placement, and accurate enough to provide useful feedback to the placer, cannot be avoided. In FPGA design flow, prediction of routability is associated closely with the problem of interconnect congestion prediction and requires estimating the amount of routing resources needed proceeding to routing. Estimation of congestion has assumed different forms. Among those, most commonly used are global routers. However, the global routers have more runtimes and, therefore, can only implore not that much frequently during placement, thus limiting their effectiveness overall. Moreover, global routers fail to account for the effects of local nets on routability, which is emerged during detailed routing. Stochastic models can also be proposed. These models have little to no knowledge of connection geometry typically. Instead, they use probability distributions for connection lengths empirically derived from analysis of circuit.

LITERATURE SURVEY

In paper by Yudong Tao [1], Challenges in Energy-Efficient Deep Neural Network Training with FPGA is analysed. In this paper, the advantages and disadvantages of implementing Deep Neural Networks on FPGAs is compared with GPUs and CPUs are summarized. FPGA is a substitute platform for training DNNs at the edge with the constraints of energy efficiency. The solutions to train DNNs on the portable devices using FPGAs however have not been well exploited. The challenges of applying DNN training on FPGAs mainly focuses on the complexity of resource utilization and the requirements of both hardware and software design knowledge to obtain required solution. So, the paper proposes to leverage the automated hardware design based on HLS to accelerate the development cycle and to achieve a better system performance. To figure out the attainment of the FPGA-based DNN training systems and trigger the research in this domain, a complete performance metric is proposed with the examination of on-chip resource usage, efficiency in training, efficiency in energy and model accuracy. Three critical computer vision tasks have been identified, namely object detection, video classification and image classification and

design an evaluation workflow to measure the design quality of the solutions for these three tasks. This paper summarizes the present status of utilizing FPGA for DNN computation and analyses the main challenges in implementing DNN training on FPGAs. Other than that, an evaluation workflow and a performance metric are proposed to compare the FPGA-based systems for DNN training in terms of model performance for specific computer vision tasks, energy efficiency, training efficiency, and usage of on-chip resources. As it can be described as an emerging technique in the computer vision field, the deep neural network achieved exceptional performance in different applications. The convolutional neural network has proven to be an efficient approach to perceive high-level concepts and abstract from unstructured visual data, such as videos and images is an example. Moreover, other DNN architectures, such as the graph convolutional neural network and generative adversarial network have been recently suggested and applied to a broader range of analytical computer vision applications, including cloud point segmentation, image denoising, image generation etc. Numerous hardware platforms for Deep Neural Network computations are compared and introduced to illustrate the advantages and disadvantages of different platforms. Comparison of FPGA, GPU, CPU and for DNN Computations are carried out. Specific techniques that can be used to accelerate DNN computation with FPGAs are summarized and discussed in the paper. The energy efficiency of FPGAs for DNN training and inference is also examined. The efficiency of DNN becomes high the model size grows higher to achieve more representation capability. So, both inference and model training of DNNs require the computation accelerators to maintain sufficient computation power. Compared to CPUs that have a few high-performance floating-point computing cores and correct the latency of executing instructions, GPUs assign the tasks amid a large number of floating-point computing cores and allow extensive parallelism to maximize the throughput. FPGAs on the contrast, have the potential to address these issues. FPGAs allows the integrated circuit reconfiguration and provide the flexibility to implement wide ranges of operations and instructions. Current FPGAs contain many on-chip resources and components, including block RAMs, flip-flops, communication cores, look-up tables, arithmetic-logic units, etc.

Primary challenges of using DNN training on FPGAs are given below

a. When DNNs are trained, measuring the gradients of network parameters depends on both the inputs of the current layer and the gradients of the following layer. The heterogeneity and length of the data dependency paths of various layers make it challenging to design a FPGA system that could adequately pipeline the processes and avert external memory accesses.

b. The knowledge required for the FPGA design and DNN design are different. While designing an advanced CNN training framework requires in-depth knowledge in computer vision, deep learning, and optimization, deploying DNNs on FPGAs requires knowledge about logic circuit design and HDL language. In paper by Cheng Luo [5], Efficient deep neural network training by FPGA-based batch level parallelism is analysed. Deep neural networks (DNNs) training needs a particular amount of time and resources to accomplish admissible results, which extremely limits its distribution in resource-limited platforms. This paper proposes a Dark FPGA, a novel customizable framework to accelerate efficiently the entire DNN training on a single FPGA platform. First, batch-level parallelism is analysed to implement efficient FPGA-based DNN training. Second, a novel hardware architecture is being optimised by a batch-oriented data design and tiling techniques to efficiently exploit parallelism is arranged. In addition, an analytical model is advanced in order to achieve the best design parameters for the Dark FPGA accelerator with respect to a specific network specification and FPGA resource constraints. The results appear that the accelerator is able to achieve about 10 times faster than CPU training and about one third of the energy consumption than GPU training using 8-bit integers for training VGG-like networks.

a. The training process, compared to inference process, delivers additional computations and different operations that can be performed in backward propagation. As a result, this leads to differences in requirements for computational resources and hardware architecture.

b. Existing FPGA accelerators for inference usually exploit layer-level and image-level parallelism for effective computing. On contradiction, FPGA accelerators for training have to proceed with batches of training examples in parallel. As a result, effective exploitation of the batch-level parallelism has to contribute significant acceleration.

c. Throughput is the main performance metric of concern for training, while inference is latency responsive. This cause batch-level parallelism to be ignored at inference accelerators. In order to solve these problems, this paper proposes a novel FPGA architecture for DNN training by the introduction of a batch-oriented data pattern which is referred to as channel-height width- batch pattern. The CHWB pattern allots training samples of distant batches at adjacent memory addresses, which allows parallel data transfer and processing to be attained within one cycle. The proposed architecture can support the entire training process inside a single FPGA and accelerate it with batch-level parallelism. An exhaustive exploration of the design space with distant levels of parallelism and their corresponding architectures with respect to resource consumption and performance is also presented in the paper.

The work proposed Dark FPGA, a novel FPGA framework for effective training of deep neural networks, with a low-precision DNN training algorithm. The Dark FPGA accelerator explores batch-level parallelism, which provides efficient training acceleration for both backward propagation as well as forward propagation on a homogeneous FPGA system. Optimization strategies like tiling strategies and batch-focused data sequence CHWB are employed to improve overall performance. Moreover, an optimization tool is developed for finding the optimal design parameters for specific network description. The paper by G. Grewal [4], seeks to address the disconnect between different stages of the FPGA CAD flow that often influences the quality of results of the implemented designs. A machine-learning framework that consists of a suite of regression and classification techniques that is used to model the relationship between the characteristics of circuits and the best CAD algorithm to use for determining an optimized implementation on an FPGA. The efficiency of the framework is achieved by applying this framework to the placement stage that would recommend the best placement flow for different circuits. In addition, the framework is used to predict different quality metrics without incurring the cost of performing routing and placement.

The most important optimization problems faced in the Field Programmable Gate Array (FPGA) design process today are NP-complete. As FPGAs continue to scale in accordance with Moore's law, so does the size of the applications target for them. The result would be data generated in larger amounts of and passed to different stages, creating a surge in runtime for key optimization stages in the CAD flow, and pressure at every stage to generate high-quality solutions appropriate for later stages in the flow. Machine Learning (ML) can assist a designer in making the decision and CAD tools in solving the problem. When a new circuit has to be placed, an ML model has been trained and used in order to recommend the best placement flow, where best can refer to runtime or Quality-of-Result. Furthermore, Machine Learning models can be developed to precisely and efficiently approximate important parameters such as the circuit's switch utilization or Rent exponent. The information thus obtained can be used to guide a CAD algorithm to perform effective and intelligent optimization given parameters based on these costs predicted, rather than the traditional blind search performed by many CAD algorithms. Present algorithms do not distinguish between benchmarks when performing the search for a near optimalsolution.ML can act as a recommender and guide to achieve specific optimization strategies either during a given stage or before a given stage in the CAD flow. To be specific, it proposes an ML framework that uses training data to understand the underlying relationship between circuits and the CAD algorithms used to map them onto a particular FPGA device. The framework does not resolve the problem at an arbitrary stage in the flow. Furthermore, it seeks to assist the tool or designer in problem solving. Machine Learning algorithms consist of a set of powerful methods that is used for training the data to develop optimized learning models. In order to create an accurate learning model, the training data must be sufficiently vast and rich to capture the underlying behaviour of the problem.

The possible abilities of the framework are established by applying it to placement stage of the FPGA flow. The main benefits of this work include the following:

a. The paper proposes a novel ML-based framework for suggesting the most adequate placement procedure to use for a new circuit. Seven distant academic placement flows are studied, each based on the award-winning placer, as well as the Viva do placement tool. Four different placement objectives are examined: dynamic power, critical path delay, routed wirelength and minimizing estimated wirelength.

b. The paper has demonstrated the affability of the proposed framework by using it to accurately and efficiently predict various quality metrics without performing routing and placement, like dynamic power, routed wirelength, estimated wirelength, postrouting column, circuit's Rent exponent, critical path delay, row utilization. It is important to assert that the previous applications, models and data sets are by no means exhaustive, but they provide to show the possible capabilities of the framework.

In the paper by Alhyari [7], the ability to accurately and efficiently estimate the routability of a circuit based on its placement is one of the most challenging and difficult tasks in the Field Programmable Gate Array (FPGA) flow. In the paper, they present a deep-learning framework that is based on a Convolutional Neural Network model that is able to predicts the routability of a placement. They also tries to integrate the deep-learning model into placement too land demonstrate how the model is used to(a) increase the placer's ability to offer routable placements for hard-to route circuits using feedback focussing on routability estimates generated by the proposed model and (b) avoid costly, but futile, place-and-route iterations. The model has been evaluated and trained using over 26K placement images which is derived from 372 benchmarks and shows that the proposed framework accomplishes a routability prediction accuracy of 97%, while demonstrating runtimes of only a few milliseconds. In the current situation, the largest Field Programmable Gate Array design can easily take many hours to place and there is no guarantee of routing success. So, it is very crucial for the placement tool to know at an early stage on whether the design is routable or not. Also, an early prediction of routability can help the placer to avoid pursuing dead-end paths which do not guide to a feasible routing solution and early stage feedback can also enable the placer to provide an optimization strategy. As an example, a placement that is considered to be easy to route can result in the placer avoiding further optimization and thus it leads to reduction in runtime. While on the other hand, a placement that is determined to be hard to route can cause the placer to improve the optimization effort. After all, developing a routability prediction model that is fast enough to be continuously applied during placement and accurate enough to provide useful feedback to the placer, is important.

PROPOSED METHOD

The system proposed contains two stages: an online testing/deployment stage and an offline training stage. The offline stage engages building either a regression model or a classification model. Regression models are used to assess continuous values (e.g., a circuit's Rent exponent, critical-path delay, routed wirelength, etc.).Classification models are used to endorse the best flow for a new circuit, where best can refer to any objective that a designer may wish the flow to optimize.

The important steps that are included in proposal are

i) Achieving a set of relevant benchmark circuits for training the system.
ii) Find a suitable set of flows among the existing flows.
iii) Developing the training data from the benchmark circuits and determining the best flow for every benchmark for the different objectives that a designer may wish to achieve. These values are used as class labels for the records that make up the training data.
iv) Automatically exacting features from the benchmarks that will allow the regression or classification models to learn the relationship that endure between the desired output and structure of the circuits.

v) Choosing features that can be used to ease the problem of dimensionality, if the original number of features is either too huge to handle or many of the dimensions are highly correlated, and hence redundant.

vi) The final features and computed class labels for each circuit form the training data. These are used as inputs to various machine-learning techniques to train the classification and regression models.

Given a new circuit to place, the online stage will perform the following steps

i) Determine the feature values required for the new circuit.

ii) The trained model can be used to either estimate an objective value or recommend the best placement flow to use based on the objectives to be optimized.

iii) Execute the Machine Learning framework on the circuit.

iv) The circuit should be added to the offline stage's database of known circuits, enabling the framework's performance to further improve as it gains experience.

CONCLUSION

The prediction of FPGA routing and placement have become a necessity. So, a model can be created using Neural network that predicts whether routability and placement of FPGA, once the neural network is trained. Solutions for implementing neural networks using FPGAs is described. The main purpose of this work was to compare several neuron variants, mainly in the domain of speed and complexity, and to suggest a solution for connecting neurons into a neural network. Model will be robust and efficient in forecast of congestion routable problems encountered in FPGA placement. The model will be very advantageous in designing the FPGA circuit. Model can be compared to other placement prediction models to demonstrate its usefulness and excellence.

REFERENCES

1. Y. Tao, R. Ma, M. Shyu and S. Chen, "Challenges in Energy-Efficient Deep Neural Network Training with FPGA," 2020 IEEE/CVF Conference on Computer Vision and Pattern Recognition Workshops (CVPRW), Seattle, WA, USA, 2020, pp. 1602-1611.
2. Z. Abuowaimer, D. Maarouf, T. Martin, J. Foxcroft, G. Grewal,S. Areibi, and A. Vannelli. GPLace3.0: Routability Driven Analytic Placer for UltraScale FPGA Architectures. ACM Transaction on Design Automation of Electronic Systems, 23(5):66:1–66:3, August 2018.
3. Sergei Alyamkin, Matthew Ardi, Alexander C Berg, Achille Brighton, Bo Chen, Yiran Chen, Hsin-Pai Cheng, Zichen Fan, Chen Feng, Bo Fu, et al. Low-power computer vision: Status, challenges, and opportunities. IEEE Journal on Emerging and Selected Topics in Circuits and Systems, 9(2):411–421, 2019.
4. G. Grewal, S. Areibi, M. Westrik, Z. Abuowaimer and B. Zhao, "Automatic Flow Selection and Quality-of-Result Estimation for FPGA Placement," 2017 IEEE International Parallel and Distributed Processing Symposium Workshops (IPDPSW), Lake Buena Vista, FL, 2017, pp. 115-123.
5. C. Luo, M. Sit, H. Fan, S. Liu, W. Luk and C. Guo, "Towards Efficient Deep Neural Network Training by FPGA-Based Batch-Level Parallelism," 2019 IEEE 27th Annual International Symposium on Field-Programmable Custom Computing Machines (FCCM), San Diego, CA, USA, 2019.
6. Lukas Bossard, Matthieu Guillaumin, and Luc Van Gool. Food-101 - mining discriminative components with random forests. In David J. Fleet, Tom´as Pajdla, Bernt Schiele, and TinneTuytelaars, editors, European Conference on Computer Vision, volume 8694 of Lecture Notes in Computer Science, pages 446–461, 2014.M. Wegmuller, J. P. von der Weid, P. Oberson, and N. Gisin, "High resolution fiber distributed measurements with coherent OFDR," in *Proc. ECOC'00*, 2000, paper 11.3.4, p. 109.

7. Alhyari, A. Shamli, Z. Abuwaimer, S. Areibi and G. Grewal, "A Deep Learning Framework to Predict Routability for FPGA Circuit Placement," 2019 29th International Conference on Field Programmable Logic and Applications (FPL), Barcelona, Spain, 2019, pp. 334-341.
8. E. Wang, J. J. Davis, R. Zhao, H.-C. Ng, X. Niu, W. Luk, P. Y. K. Cheung, and G. A. Constantinides, "Deep Neural Network Approximationf or Custom Hardware: Where We've Been, WhereWe're Going," ACM Computing Surveys, vol. 52, no. 2, 2019.
9. M. Ghasemzadeh, M. Samragh, and F. Koushanfar, "ReBNet: Residual Binarized Neural Network," in IEEE International Symposium on Field-programmable Custom Computing Machines, 2018.
10. S. Yang, A. Gayasen, C. Mulpuri, S. Reddy, and R. Aggarwal. Routability-driven FPGA placement contest. In Proceedings of the International Symposium on Physical Design, pages 139–143. ACM, 2016.
11. M. Kurek, P. Deisenroth, W. Luk, and T. Todman, "Knowledge Transfer in Automatic Optimization Reconfigurable Designs," in IEEE International Symposium on Field Programmable Custom Computing Machines, Washington DC, USA, May 2016, pp. 84–87.

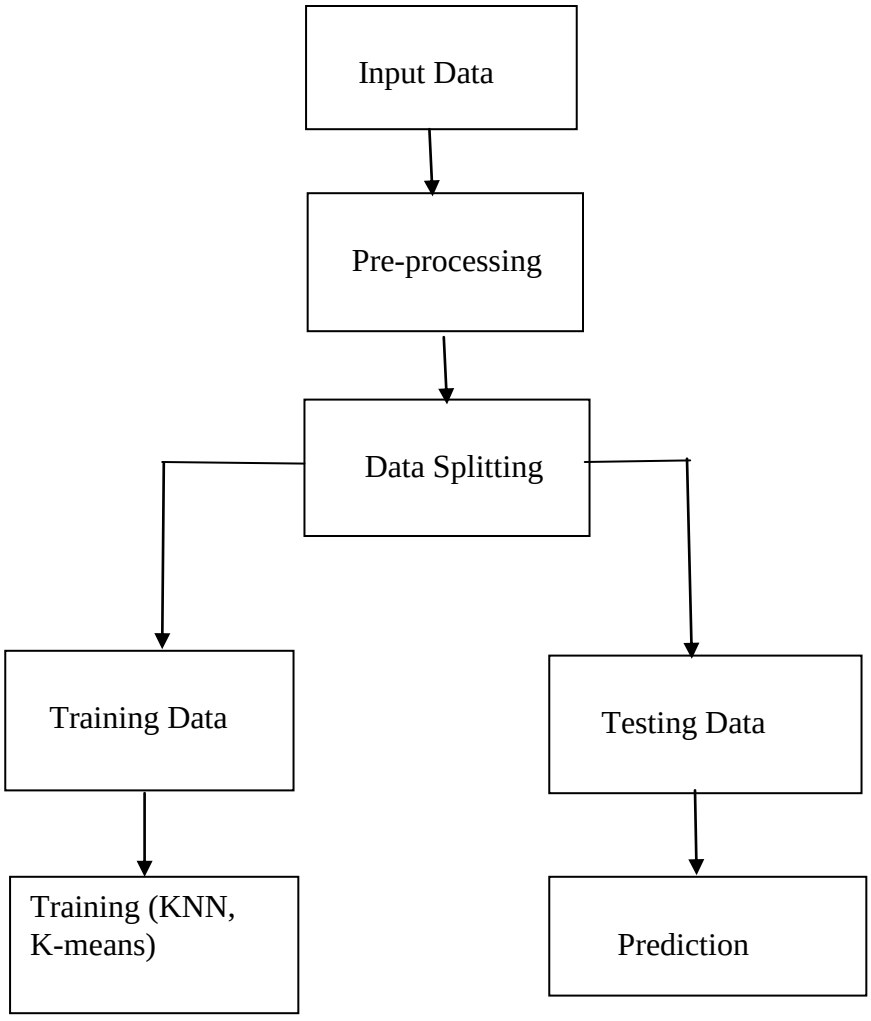


Figure.1. ML EDA Framework: Testing & Updating

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Physico – Chemical Analysis of Selected Municipal Drinking Water Samples of Dindigul District"

Ignatius Navis Karthika[1]*, M.A.Pushparaj[2] and A. Sahaya Raja[3]

[1]Department of Chemistry, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India
[2]Department of Civil Engineering, R.V.S College of Engineering, Dindigul, Tamil Nadu, India
[3]PG and Research Department of Chemistry, G.T.N. Arts College (Autonomous), Dindigul, Tamil Nadu, India

Received: 14 Nov 2020 Revised: 16 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
Ignatius Navis Karthika
Department of Chemistry,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email : spsahayamdgl@gmail.com

ABSTRACT

The present study was carried out by collecting two municipal water samples during 2016 and 2020.The results were compared with standards prescribed by WHO and ISI 10500.Total 10 parameters were analysed. On comparing the water samples collected during 2016 and 2020 in the present investigation observed all physico –chemical parameters during the year 2020 were within the water quality standards and it is fit for drinking purpose.

Keywords : Physico-chemical parameters, monsoon, dilution, municipal water samples.

INTRODUCTION

Human civilization originated, developed and thrived in places within easy reach of freshwater sources. Amongst global resources, water is emerging as perhaps the most critical butmis used natural resource .Water is the elixir of life without which no biotacould survive in the biosphere. The pH decreased with increasing levels of sewage bio solids and the available plant nutrients increased markedly. Water, it is one of the prime necessities of life. We can hardly live for a few days without water. There are many resources of water on the earth .Due to its unique properties water is of multiple uses such as drinking, irrigation, industrial and energy production for living organism (Delphine Rose. 2008, Thambavani .D.S and Prathipa. V et al., 2010,2011,2012 and 2013, Saralathambavani. D and Prathipa,.V et al., 2010, 2011,2012 and 2013 V.Prathipa et al., 2015, R. Rajendran et al.,2015). Although water is very abundant on the earth . Water being a good and universal solvent dissolves almost all substance which it comes in contact. Generally natural water is not pure. It contains impurities of various kinds of both dissolved gases,

suspended impurities etc. These are natural impurities derived from the atmosphere. Fresh and natural water is essential for survival of man. The present study compares the water quality of municipal water for two different years namely 2016 and 2020 BIS (1983), whether it is acceptable for drinking or not all are deeply observed and compared with ISI 10500 and WHO specification.

Experimental findings and analysis

Dindigul is also known as Holland of Tamilnadu. It is famous for tannery units, safe locks, veggies and rock fort. Also iron safe of good quality and durability are made here. A lock-manufacturing unit under co-operative sector is functioning here. It is one of the largest trading centers in Tamil Nadu for chewing tobacco and Rojasupari which are produced in this town. They are being sent to various places in and around Tamil Nadu. Dindigul is flourishing with handloom industry at Chinnalapatti, which is located at 11 Km away from Dindigul on the Madurai – Dindigul road. Water samples are collected directly through tube well water as well as municipal water for their daily need. Water samples are collected in two litre polythene bottles from two stations. Analysis was carried out for various water quality parameters such as pH, EC, Total hardness, TDS, Turbidity, Total alkalinity, Calcium, Magnesium, Chlorides and Sulphates using prescribed methods APHA (1998). The reagents used for the analysis were AR grade and distilled water was used for preparation of solutions. Water samples collected and analysed during the year 2016 at the same sampling stations was taken for comparison APHA (1985). The overall results of the Physicochemical parameters for water samples are shown in table-1, graph 1 and 2.

pH

pH value indicates acid – alkalinity range of water .It is affected by environment factors such as temperature. pH observed at S1 is 7.4 and S2 is 7.2.The values were within the maximum permissible limits, prescribed by WHO and Indian standards for drinking water.

Electrical conductivity (EC)

Electrical conductivity is the water capacity to convey electric current .It signifies the amount of total dissolved solids. The values observed in this study at S1 and S2 are 1232 and 1240m Mhos/cm respectively. The values show that medium amount of dissolved inorganic salts.

Total dissolved solids

Water containing 500 mg/L of TDS is not considered for drinking water supplies but in unavailable case 1500mg/L is also allowed .TDS values observed at S1 is 360 mg/L and S2 is 340 mg/L which and fit as in IS 10500 and WHO specification.

Turbidity

In the present investigation we found 3.5 NTU turbidity in S1 water samples while 3.6 NTU in S2 water sample. The both values are within the permissible limits of standard specifications.

Total alkalinity and Total hardness

Chemical pure water is neutral having equal amount of hydrogen and hydroxyl ion. The values observed are 150 mg/L at S1 and 124 mg/L at S2 which are within the permissible limit as per Indian standards but above the limits as per Indian standards but above the limits prescribed WHO. Water samples at S1-172 and S2- 168 are below the permissible limit

Calcium and Magnesium

Calcium concentration found 35 and 29 mg/L at station S1 and S2 respectively which is below permissible limit. Magnesium content in the investigated water sample was 119mg/L and 120 mg/L which are close to permissible limit of Indian standard specification for drinking water.

Chlorides and sulphates

The values of chlorides observed are 130mg/L atS1 and 125 mg/L at S2 which are much lowers are compared to permissible limits as prescribed by SIS and WHO. Sulphates occurs naturally in water because of leaching of some minerals .We noted 132 mg/L in S1 and 128 mg/L in S2 water sample.

CONCLUSION

Dilutions was the solution to pollution when populations were small in olden days. Now in recent years increase in population lead to increase in pollution. Despite the nationwide lockdown since March 25 in India there is a drastic change in the meteorology of our nation particularly in our study zone. Our study zone has experienced good rainfall after a period of six years. This has led to the decrease in pollution in ground water and municipal water. Hence dilution has played a vital role in bringing the water quality within the permissible level.

REFERENCES

1. APHA (1998).Standard methods or examination of water and waste water treatment 20thEd.N.W.Washington D.C
2. APHA (1985). Standard methods for examination of water and waste Washington ,D.C.10th ed.
3. BIS (1983).Indian standard specifications for drinking water IS:10500.Bureau of Indian standards ,New Delhi.
4. WHO (1998).International standards for drinking water quality vol.I, "Recommanadtions" world health organization ,Geneva ,PP130.
5. Delphine Rose (2008).Characteristics of ground water quality of selected areas Dindigul District,Tamilnadu ,J.Aqua .Biol.23(1):40-43.
6. Thambavani.D. S., Prathipa.V (2010)A correlation of particulate matter with gaseous pollutants in ambient air of Dindigul town, Asian Journal of Environmental Science,Vol.5 No.2 pp.89-93 ref.16
7. Thambavani.D. S., Prathipa.V (2011),Assessment of leather tannery effluent using multiple indicators,Asian Journal of Experimental Chemistry ,Vol.6 No.2 pp.47-57
8. Thambavani.D. S., Prathipa.V (2011), Physico-chemical characteristics of leather tannery effluent-current scenario in Dindigul town (Tamil Nadu), India, Asian Journal of Environmental Science, Vol.6 No.2 pp.119-124
9. Sarala Thambavani .D and Prathipa. V. (2012) Bio monitoring of air pollution around urban and industrial sites Journal of research in Biology,1: 007-014
10. V.Prathipa, A.Sahaya Raja (2015), Air Quality Assessment and Air Quality Index of Dindigul Town (Tamil Nadu), India– A Case Study, International Journal of Pharm Tech Research,Vol.8, No.6, pp 45-55.
11. Thambavani, D. S., Prathipa.V (2012), Air quality index for Dindigul town,Asian Journal of Environmental Science,Vol.7 No.1 pp.13-20
12. Saralathambavani.D., Prathipa,.V (2012),Strategies of heavy metal uptake by plants growing under urban environment, Asian Journal of Soil Science,Vol.7 No.2 pp.304-311
13. Saralathambavani.D., Prathipa,.V (2012) A study of heavy metal contamination in the urban soil of Dindigul town, Tamil Nadu, Asian Journal of Soil Science,Vol.7 No.2 pp.150-158
14. Thambavani, D. S., Prathipa.V (2012) Quantitative assessment of soil metal pollution with principal component analysis, geo accumulation index and enrichment index, Asian Journal of Environmental Science, Vol.7 No.2 pp.125-134
15. Thambavani, D. S., Prathipa.V (2012),Biological monitoring of road side plants exposed to air pollution, Asian Journal of Environmental Science, Vol.7 No.1 pp.38-46
16. Thambavani, D. S., Prathipa.V (2012), Assessment of metal enrichments in industrial soil of Dindigul Town, Tamil Nadu, Asian Journal of Soil Science, Vol.7 No.1 pp.98-103

17. Thambavani, D. S., Prathipa.V (2013), Assessing heavy metal contamination in urban soils - an index analysis approach, Journal of Applicable Chemistry ,Vol.2 No.4 pp.887-897
18. V. Prathipa, A. Sahaya Raja, D. Sarala Thambavani (2015), A Study of traffic related air pollution at Dindigul district, Tamil Nadu, India, Journal of Environmental Science and Pollution Research,1(1), 6–7.
19. R. Rajendran, A. Pandia Rajan, A. Sahaya Raja, V. Prathipa, M.S. Dheenadayalan (2015), Assessment of physico–chemical parameters of River Cauvery in and around Nerur, Journal of Environmental Science and Pollution Research. 1(1), 17–19.

Table -1 Average results of physico –chemical parameters

S.No	Parameters	Sampling station				WHO	IS10500
		S1 R.M.colony	S1 R.M.colony	S2 Seelapadi	S2 Seelapadi		
YEAR		2016	2020	2016	2020		
1	pH	6.64	7.4	6.92	7.2	7-8.5	6.5-8.5
2	EC	1898	1232	1911	1240	1400	-
3	TDS	997	360	990	340	1000	500
4	Turbidity	3.8	3.5	3.2	3.6	5.0	10.0
5	Total akalinity	148	150	124	124	120	200
6	Total hardness	320	172	324	168	500	300
7	Calcium	80	35	98	29	100	75
8	Magnesium	130	119	143	120	150	30
9	Chlorides	198	130	204	125	250	250
10	Sulphates	167	132	159	128	250	200

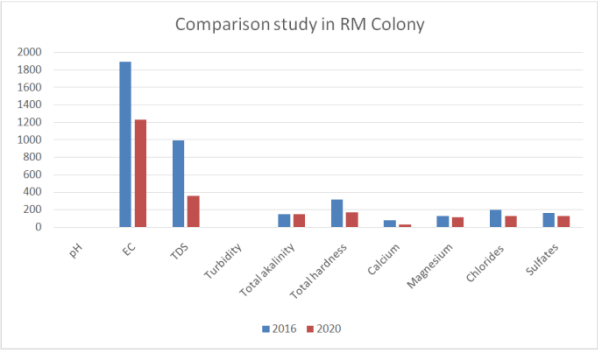


Fig.1. Comparison study in RM Colony

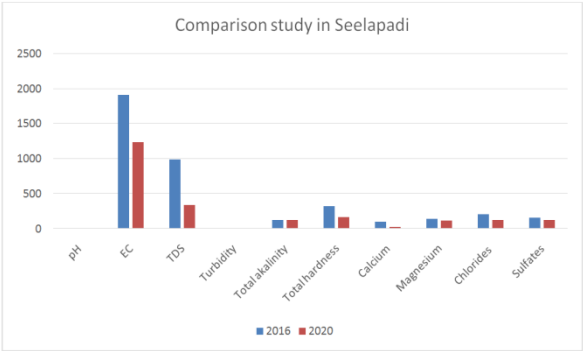


Fig.2. Comparison study in Seelapadi

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Analysis of Vital Physico-Chemical Parameters and Ranking of Ground Water Samples in and Around Dindigul District

V. Prathipa[1], A. Sahaya Raja[2]* and A. Uma Maheswari [3]

[1]Department of Chemistry, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India
[2]PG and Research Department of Chemistry, G.T.N. Arts College (Autonomous), Dindigul, Tamil Nadu, India.
[3]Department of Chemistry, Vels Institute of Science Technology and Advanced Studies, Pallavaram, Chennai, Tamil Nadu, India

Received: 14 Nov 2020 Revised: 17 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
A. Sahaya Raja
PG and Research Department of Chemistry,
G.T.N. Arts College (Autonomous),
Dindigul, Tamil Nadu, India
Email: spsahayamdgl@gmail.com

ABSTRACT

The present work was undertaken to analyse the various water quality parameters such as pH, Electrical conductivity, Total dissolved solids (TDS), Total alkalinity (TA) and Total hardness (TH). Ground water samples were collected from different residential areas of Dindigul District .The results were collected with the values specified by World Health Organization (WHO) for drinking water quality. It was found that the ground water was polluted at few residential areas, while others showed physical parameters within the range and the water was found good and fit for drinking and irrigation purpose.

Keywords: Irrigation, Residential areas, Ground water, Drinking water, Water quality parameters,

INTRODUCTION

Water pollution is due to slight alternation in physical, chemical and biological characteristics of water, which may harm humans and aquatic biota. It is more difficult to pollute ground water than surface water because the soil can either stop pollutant reaching ground water or help to reduce its concentration. In and around urban areas, domestic and industrial effluents, septic tanks solid waste refuse dumps and their leachates are the potential sources of ground water pollution and also accidental spillages may lead to ground water contamination. It is evident that many parts of the industrial areas in India are colonized and are in very close vicinity of the industries are using ground water for drinking ,cleaning ,bathing, domestic purpose .Historically ,ground water supplies were thought to be free of pathogenic microbes due to the natural filtering ability of the subsurface environment and the

distance a microbe would have to travel in order to reach the ground water source. Contaminants that find their way into ground water may originate due to lack of treatment, improper management of waste water disposal.(Thambavani .D.S and Prathipa. V et al., 2010, 2011, 2012 and 2013, Saralathambavani. D and Prathipa,.V et al., 2010, 2011,2012 and 2013 V. Prathipa et al., 2015, R. Rajendran et al.,2015, I.N. Karthika et al 2015,2018). Some water borne pathogenic diseases that may coincide with faecal coliform contamination include ear infections, dysentery, typhoid fever, viral and bacterial gastroenteritis and Hepatitis 'A' .The presence of faecal coliform tends to affect humans more than it does aquatic creatures, though not exclusively .To estimate the levels of ground water contamination, ground water quality analysis have been discussed and concluded.

EXPERIMENTAL FINDINGS AND DISCUSSIONS

Study location: The present study covers selected urban area of Dindigul District

Selection of sampling points: After a quick survey of the city, three types of locations were chosen for collecting ground water samples. Each type of location has five sampling stations which mostly include hand pumps and dug wells. The sampling stations were classified on occupation wise using stratified random sampling techniques as HIG (I- IV), MIG (I-V), LIG (I-V) and IA (I-V).

Sample collection: Samples from various ground water sources were collected for the evaluation of physical parameters.

Analytical procedure: The collected samples were analysed in the laboratory as per standards methods.

pH: It was determined with the help of a pH meter using a glass electrode and reference electrode. The pH meter was calibrated by buffer solutions (Borax buffer). After calibrating, the pH meter with buffer solutions the electrode assembly was removed and washed with distilled water .Now it was dipped in to the water sample and the pH of the sample was read from the meter.

Electrical conductivity: It is the measure of the ability of an aqueous solution to carry the electric current .It was determined by conductivity measure method.

$K = 1{,}000{,}000x/Rm\ [1+0.019(t-25)]$

Where K= Conductivity in μ mhos/cmat 25^{o}C
X= Cell constant in cm^{-1}
Rm = Measured resistance of sample in ohm
t = Temperature of measurement

Total Dissolved Solids (TDS): The TDS in water samples were estimated as the residue left after the evaporation of filtered sample.
Total Dissolved Solids (mg/l) =(B-A)*1000/v
Where, A = Initial mass of evaporating dish (g)
B = Final mass of evaporating dish (g)
V= Volume of water sample taken in ml.

Total Alkalinity (TA): It is the quantitative ability of water to react with a strong acid at a designated pH. It was determined by neutralization titration with a strong acid H_2SO_4 using methyl orange and phenolphthalein indicators.

Total Alkalinity (TA) = 1/50*(A+B)/100*50*1000 ppm
Where, A= Volume of N/50H_2SO_4 used to phenolphthalein endpoint (ml)
B = Additional Volume of N/50H_2SO_4 used to phenolphthalein endpoint (ml)

Total Hardness (TH)
The total hardness of water refers to the sum of concentrations of alkaline earth metal cations present in it. The total hardness was determined by complexometry using EDTA as titrant.

RESULTS AND DISCUSSIONS

pH
The results presented in table revealed that the pH range in the four residential areas under investigations was between 7.0 and 8.5. It is from the station of view of portability and plant growth as well. Infact lightly alkaline water is better for plant growth compared to acidic water (pH<7.0).It goes that the pH of ground water over the year in all the five residential areas is well within the desirable range.

Electrical Conductivity (EC)
High EC values make water unsuitable for irrigation, boilers etc., The mean EC values of ground water samples collected round the year are given in Table -2.
From table-2 it is clear that the mean value of industrial area was the highest .It may be attributed to large amount of industrial waste seeping into ground to contaminate water heavy metal ions and anions.

Total Dissolved Solids (TDS)
In the present investigations, the mean values of TDS were found in different stations in table -3.
TDS of more than500 mg/l make the water undesirable for drinking purpose. Present investigations have revealed that maximum was from the sample collected from lower income group and Industrial area where TDS was as high as 1342 and 1390 mg/l respectively.

Total Alkalinity
The result presented in table -4 revealed that the lowest mean value of total alkalinity was found for the HIG areas and the highest for the LIG. It was definitely on the higher side.

Total hardness
Present studies have revealed in table -5 that total hardness of ground water in HIG and MIG areas were well within the permissible limits. It was slightly greater in LIG and Industrial area was high alarm. Water with excess hardness is known to cause heart disease and kidney problem.

REFERENCES

1. APHA –AWWA-WPCF (1998).Standard methods for examination of water and waste water.19th ed. Washington District of Columbia: America public health association America water works Association, Water pollution control federation.
2. BIS (2003).Bureau of Indian standards New Delhi IS:10500:A 1991 edition -2.2 (2003-2009)

3. Manivasakam, N.(1987), Industrial effluents origin ,Characteristics , effects ,analysis and treatment . Sakthi publications, Coimbatore.
4. Sarala thambavani D and Prathipa. V (2011), Physico –Chemical characteristics of leather tannery effluent – current Scenario in Dindigul town .Asian journal of Environmental Science vol(6).119-124
5. Trivedy, R.K and Goel ,P.K .(1986), Chemical and Biological methods for water pollution studies .Enviro media ,Karad ,India .
6. Thambavani. D. S., Prathipa. V (2010),A correlation of particulate matter with gaseous pollutants in ambient air of Dindigul town, Asian Journal of Environmental Science,Vol.5 No.2 pp.89-93 ref.16
7. Thambavani. D. S., Prathipa. V (2011),Assessment of leather tannery effluent using multiple indicators, Asian Journal of Experimental Chemistry ,Vol.6 No.2 pp.47-57
8. Thambavani. D. S., Prathipa. V (2011), Physico-chemical characteristics of leather tannery effluent-current scenario in Dindigul town (Tamil Nadu), India, Asian Journal of Environmental Science, Vol.6 No.2 pp.119-124
9. Sarala Thambavani .D and Prathipa. V. (2012) Bio monitoring of air pollution around urban and industrial sites Journal of research in Biology,1: 007-014
10. V. Prathipa, A. Sahaya Raja (2015), Air Quality Assessment and Air Quality Index of Dindigul Town (Tamil Nadu), India– A Case Study, International Journal of PharmTech Research,Vol.8, No.6, pp 45-55.
11. Thambavani, D. S., Prathipa.V (2012), Air quality index for Dindigul town, Asian Journal of Environmental Science,Vol.7 No.1 pp.13-20
12. Saralathambavani. D., Prathipa,.V (2012),Strategies of heavy metal uptake by plants growing under urban environment, Asian Journal of Soil Science,Vol.7 No.2 pp.304-311
13. Saralathambavani. D., Prathipa.V (2012) A study of heavy metal contamination in the urban soil of Dindigul town, Tamil Nadu, Asian Journal of Soil Science,Vol.7 No.2 pp.150-158
14. Thambavani, D. S., Prathipa. V (2012) Quantitative assessment of soil metal pollution with principal component analysis, geo accumulation index and enrichment index, Asian Journal of Environmental Science, Vol.7 No.2 pp.125-134
15. Thambavani, D. S., Prathipa.V (2012),Biological monitoring of road side plants exposed to air pollution, Asian Journal of Environmental Science, Vol.7 No.1 pp.38-46
16. Thambavani, D. S., Prathipa.V (2012), Assessment of metal enrichments in industrial soil of Dindigul Town, Tamil Nadu, Asian Journal of Soil Science, Vol.7 No.1 pp.98-103
17. Thambavani, D. S., Prathipa.V (2013), Assessing heavy metal contamination in urban soils - an index analysis approach,Journal of Applicable Chemistry ,Vol.2 No.4 pp.887-897
18. V. Prathipa, A. Sahaya Raja, D. Sarala Thambavani (2015), A Study of traffic related air pollution at Dindigul district, Tamil Nadu, India, Journal of Environmental Science and Pollution Research,1(1), 6–7.
19. R. Rajendran, A. Pandia Rajan, A. Sahaya Raja, V. Prathipa, M.S. Dheenadayalan (2015), Assessment of physico–chemical parameters of River Cauvery in and around Nerur, Journal of Environmental Science and Pollution Research. 1(1), 17–19.
20. I.N. Karthika, M.S. Dheenadayalan (2015), Study of ground water quality at selected locations in Dindigul district, India, J. Adv. Chem. Sci. 1 (2) (2015) 67–69.
21. Karthika.IN, Thara. K, Dheenadayalan.MS (2018),Physico-Chemical Study of the Ground Water Quality at Selected Locations in Periyakulam, Theni district, Tamilnadu, India, Materials Today-Proceedings, Volume: 5, Issue: 1, Pages: 422-428.

Table -1 The range of pH values of different four types of residential areas of Dindigul

S.no	Residential areas	pH range		Annual mean
		Lowest	Highest	
1.	Higher income group (HIG)	7.72	8.05	7.19
2.	Minimum income group (MIG)	7.70	8.06	8.01
3.	Lower income group (LIG)	7.0	7.9	7.15
4.	Industrial area (IA)	7.30	8.72	7.9

Table -2 The EC values of different residential areas of Dindigul				
S.No	Residential areas	EC(µ mhos/cm)		Annual mean
		Lowest	Highest	
1.	Higher income group (HIG)	1002	1665	1395
2.	Minimum income group (MIG)	810	2275	1650.5
3.	Lower income group (LIG)	1195	2495	1724.6
4.	Industrial area (IA)	1363	2665	1820

The maximum permissible limit of EC is 400µ mhos/cm

Table -3 The TDS values of different residential areas of Dindigul				
S.No	Residential areas	TDS (mg/l)		Annual mean
		Lowest	Highest	
1.	Higher income group (HIG)	590	845	725
2.	Minimum income group (MIG)	675	1130	805.5
3.	Lower income group (LIG)	869	1342	1018.2
4.	Industrial area (IA)	890	1390	1051

Maximum permissible limit =500mg/l

Table -4 The alkalinity values of different residential areas of Dindigul				
S.No	Residential areas	Alkalinity (mg/l)		Annual mean
		Lowest	Highest	
1.	Higher income group (HIG)	120	150	152.25
2.	Minimum income group (MIG)	395.8	720.10	605.45
3.	Lower income group (LIG)	406.3	760.8	645.8
4.	Industrial area (IA)	432.6	756.0	552.5

Maximum permissible limit =500mg/l

Table - 5 The Total hardness values of different residential areas of Dindigul				
S.No	Residential areas	Total hardness (mg/l)		Annual mean
		Lowest	Highest	
1.	Higher income group (HIG)	200	285	142.1
2.	Minimum income group (MIG)	202	290	218.4
3.	Lower income group (LIG)	470	1420	471.7
4.	Industrial area (IA)	620	2440	590.5

Maximum permissible limit =300mg/l

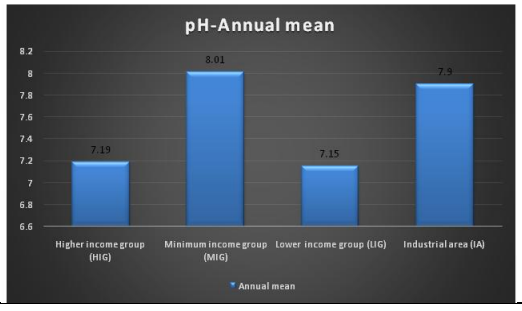


Fig.1.pH

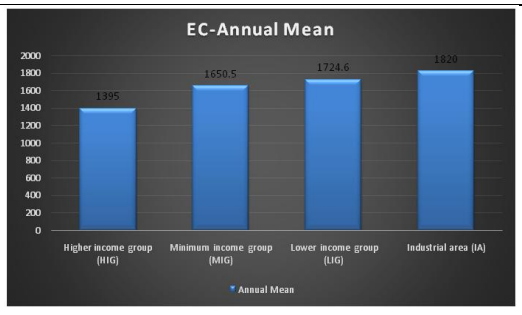


Fig.2.Electrical Conductivity

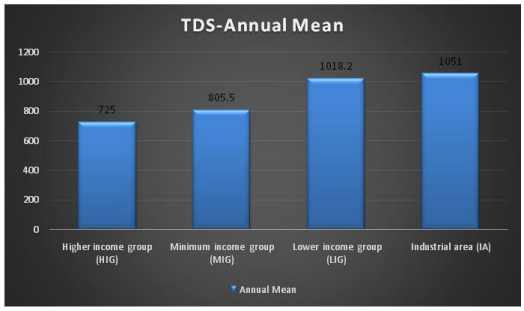


Fig.3.Total Dissolved Solids (TDS)

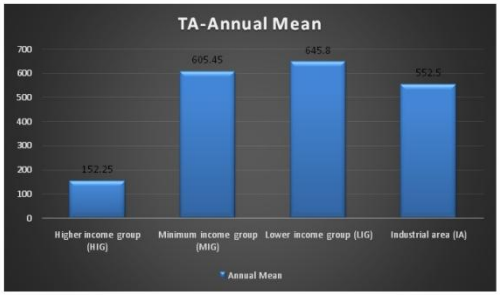


Fig.4.Total Alkalinity

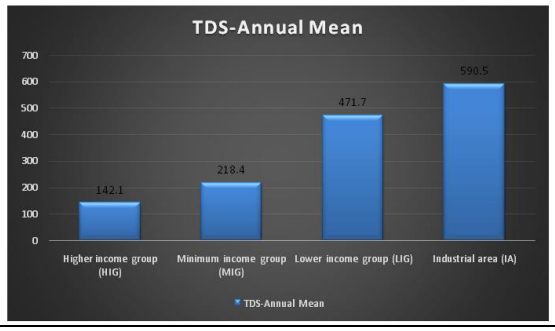


Fig.5.Total hardness

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Intuitionistic Anti L - Fuzzy HX Ring

K.H.Manikandan[1]*, M.Sridharan[1], M.S.Muthuraman[1], G.Sabarinathan[1] and R.Muthuraj[2]

[1]Department of Mathematics, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.
[2]PG and Research, Department of Mathematics, H.H.The Rajah's College, Pudukkottai, Tamil Nadu, India.

Received: 15 Nov 2020 Revised: 17 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
K.H. Manikandan
Department of Mathematics,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: manimaths7783@gmail.com

ABSTRACT

In this paper, we define the notion of intuitionistic anti L- fuzzy HX subring of a HX ring and some of their related properties are investigated. We define the necessity and possibility operators of an intuitionistic anti L-fuzzy subset of an intuitionistic anti L- fuzzy HX subring and discuss some of its properties.

Keywords: intuitionistic fuzzy set, fuzzy HX ring, intuitionistic L- fuzzy HX subring, intuitionistic anti L- fuzzy HX subring, anti product in intuitionistic anti L- fuzzy HX subring.

INTRODUCTION

In 1965, Zadeh [12] introduced the concept of fuzzy subset μ of a set X as a function from X into the closed unit interval [0, 1] and studied their properties. Fuzzy set theory is a useful tool to describe situations in which the data or imprecise or vague and it is applied to logic, set theory, group theory, ring theory, real analysis, measure theory etc. In 1967, Rosenfeld [11] defined the idea of fuzzy subgroups and gave some of its properties. Li Hong Xing [4] introduced the concept of HX group. With the successful upgrade of algebraic structure of group many researchers considered the algebraic structure of some other algebraic systems in which ring was considered as first. In 1988, Professor Li Hong Xing [5] proposed the concept of HX ring and derived some of its properties, then Professor Zhong [2,3] gave the structures of HX ring on a class of ring. R. Muthuraj et.al [10]., introduced the concept of fuzzy HX ring. In this paper we define a new algebraic structure of an intuitionistic anti L-fuzzy HX subring of a HX ring and investigate some related properties. We define the necessity and possibility operators of an intuitionistic fuzzy subset of an intuitionistic anti L-fuzzy HX subring and discuss some of its properties.

PRELIMINARY

In this section, we site the fundamental definitions that will be used in the sequel. Throughout this paper, R = (R ,+, ·) is a Ring, e is the additive identity element of R and xy, we mean x.y

2.1 Definition [5]

Let R be a ring. In $2^R - \{\phi\}$, a non-empty set $\vartheta \subset 2^R - \{\phi\}$ with two binary operation ' + ' and '.' is said to be a HX ring on R if ϑ is a ring with respect to the algebraic operation defined by

iv. A + B = {a + b / a ∈ A and b ∈ B} , which its null element is denoted by Q , and the negative element of A is denoted by – A.

v. AB = {ab / a ∈ A and b ∈ B},

vi. A (B + C) = AB + AC and (B + C) A = BA + CA.

INTUITIONISTIC ANTI L-FUZZY HX SUBRING OF A HX RING

In this section we define the concept of an intuitionistic anti fuzzy HX subring of a HX ring and discuss some related results.

3.1 Definition

Let R be a ring. Let H = { ⟨x, μ(x), η(x)⟩ / x∈R } be an intuitionistic L-fuzzy set defined on a ring R, where μ : R→[0,1], η : R→[0,1] such that $0 \le \mu(x) + \eta(x) \le 1$. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. An intuitionistic L-fuzzy subset $\lambda_H = \{ \langle A, \lambda_\mu(A), \lambda_\eta(A)\rangle / A \in \Re$ and $0 \le \lambda_\mu(A) + \lambda_\eta(A) \le 1\}$ of $\Re$ is called an intuitionistic L-fuzzy HX subring of $\Re$ or an intuitionistic L-fuzzy subring induced by H if the following conditions are satisfied. For all A,B ∈$\Re$,

i. $\lambda_\mu(A - B) \ge \lambda_\mu(A) \wedge \lambda_\mu(B)$ ii. $\lambda_\mu(AB) \ge \lambda_\mu(A) \wedge \lambda_\mu(B)$

iii. $\lambda_\eta(A - B) \le \lambda_\eta(A) \vee \lambda_\eta(B)$ iv. $\lambda_\eta(AB) \le \lambda_\eta(A) \vee \lambda_\eta(B)$.

where $\lambda_\mu(A) = \min\{ \mu(x) /$ for all $x \in A \subseteq R \}$ and $\lambda_\eta(A) = \max\{\eta(x) /$ for all $x \in A \subseteq R \}$.

3.2 Definition

Let R be a ring. Let H = { ⟨x, μ(x), η(x)⟩ / x∈R } be an intuitionistic L-fuzzy set defined on a ring R, where μ : R→[0,1], η : R→[0,1] such that $0 \le \mu(x) + \eta(x) \le 1$. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. An intuitionistic L-fuzzy subset $\lambda_H = \{ \langle A, \lambda_\mu(A), \lambda_\eta(A)\rangle / A \in \Re$ and $0 \le \lambda_\mu(A) + \lambda_\eta(A) \le 1\}$ of $\Re$ is called an intuitionistic anti L-fuzzy HX subring of $\Re$ or an intuitionistic anti L-fuzzy subring induced by H if the following conditions are satisfied. For all A,B ∈$\Re$,

i. $\lambda_\mu(A - B) \le \lambda_\mu(A) \vee \lambda_\mu(B)$ ii. $\lambda_\mu(AB) \le \lambda_\mu(A) \vee \lambda_\mu(B)$

iii. $\lambda_\eta(A - B) \ge \lambda_\eta(A) \wedge \lambda_\eta(B)$ iv. $\lambda_\eta(AB) \ge \lambda_\eta(A) \wedge \lambda_\eta(B)$.

where $\lambda_\mu(A) = \max\{ \mu(x) /$ for all $x \in A \subseteq R \}$ and $\lambda_\eta(A) = \min\{\eta(x) /$ for all $x \in A \subseteq R \}$.

3.2 Remark

For an intuitionistic anti L-fuzzy HX subring λ_μ of a HX ring $\Re$, the following result is obvious. For all A, B ∈$\Re$,

i. $\lambda_\mu(A) \ge \lambda_\mu(0)$ and $\lambda_\mu(A) = \lambda_\mu(-A)$, ii. $\lambda_\mu(A - B) = 0$ implies that $\lambda_\mu(A) = \lambda_\mu(B)$.

iii. $\lambda_\eta(A) \le \lambda_\eta(0)$ and $\lambda_\eta(A) = \lambda_\eta(-A)$, iv. $\lambda_\eta(A - B) = 0$ implies that $\lambda_\eta(A) = \lambda_\eta(B)$.

3.3 Theorem

Let G and H be any two intuitionistic L-fuzzy sets on R. Let γ_G and λ_H be any two intuitionistic anti L-fuzzy HX subrings of a HX ring $\Re$ then their intersection, $\gamma_G \cap \lambda_H$ is also an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

Proof

Let $G = \{ \langle x, \alpha(x), \beta(x)\rangle / x \in R \}$ and $H = \{ \langle x, \mu(x), \eta(x)\rangle / x \in R \}$ be any two intuitionistic L-fuzzy sets defined on a ring R.

Then, $\gamma_G = \{ \langle A, \gamma_\alpha(A), \gamma_\beta(A)\rangle / A \in \Re \}$ and $\lambda_H = \{ \langle A, \lambda_\mu(A), \lambda_\eta(A)\rangle / A \in \Re \}$ be any two intuitionistic anti L-fuzzy HX subrings of a HX ring $\Re$.

$\gamma_G \cap \lambda_H = \{ \langle A, (\gamma_\alpha \cap \lambda_\mu)(A), (\gamma_\beta \cup \lambda_\eta)(A)\rangle / A \in \Re \}$

Let $A,B \in \Re$.

i. $(\gamma_\alpha \cap \lambda_\mu)(A-B) = \gamma_\alpha(A-B) \wedge \lambda_\mu(A-B)$

$\leq \{\gamma_\alpha(A) \vee \gamma_\alpha(B)\} \wedge \{\lambda_\mu(A) \vee \lambda_\mu(B)\}$

$= \{\gamma_\alpha(A) \wedge (\lambda_\mu(A) \vee \lambda_\mu(B))\} \vee \{\gamma_\alpha(B) \wedge (\lambda_\mu(A) \vee \lambda_\mu(B))\}$

$= [\gamma_\alpha(A) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(A) \wedge \lambda_\mu(B)] \vee [\gamma_\alpha(B) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(B) \wedge \lambda_\mu(B)]$

$\leq [\gamma_\alpha(A) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(B) \wedge \lambda_\mu(B)]$

$= \{(\gamma_\alpha \cap \lambda_\mu)(A) \vee (\gamma_\alpha \cap \lambda_\mu)(B)\}$

$(\gamma_\alpha \cap \lambda_\mu)(A-B) \leq \{(\gamma_\alpha \cap \lambda_\mu)(A) \vee (\gamma_\alpha \cap \lambda_\mu)(B)\}$

ii. $(\gamma_\alpha \cap \lambda_\mu)(AB) = \gamma_\alpha(AB) \wedge \lambda_\mu(AB)$

$\leq \{\gamma_\alpha(A) \vee \gamma_\alpha(B)\} \wedge \{\lambda_\mu(A) \vee \lambda_\mu(B)\}$

$= \{\gamma_\alpha(A) \wedge (\lambda_\mu(A) \vee \lambda_\mu(B))\} \vee \{\gamma_\alpha(B) \wedge (\lambda_\mu(A) \vee \lambda_\mu(B))\}$

$= [\gamma_\alpha(A) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(A) \wedge \lambda_\mu(B)] \vee [\gamma_\alpha(B) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(B) \wedge \lambda_\mu(B)]$

$\leq [\gamma_\alpha(A) \wedge (\lambda_\mu(A)] \vee [\gamma_\alpha(B) \wedge \lambda_\mu(B)]$

$= \{(\gamma_\alpha \cap \lambda_\mu)(A) \vee (\gamma_\alpha \cap \lambda_\mu)(B)\}$

$(\gamma_\alpha \cap \lambda_\mu)(AB) \leq \{(\gamma_\alpha \cap \lambda_\mu)(A) \vee (\gamma_\alpha \cap \lambda_\mu)(B)\}$

iii. $(\gamma_\beta \cup \lambda_\eta)(A-B) = \gamma_\beta(A-B) \vee \lambda_\eta(A-B)\}$

$\geq \{\gamma_\beta(A) \wedge \gamma_\beta(B)\} \vee \{\lambda_\eta(A) \wedge \lambda_\eta(B)\}$

$= \{\gamma_\beta(A) \vee (\lambda_\eta(A) \wedge \lambda_\eta(B)\} \wedge \{\gamma_\beta(B) \vee (\lambda_\eta(A) \wedge \lambda_\eta(B)\}$

$= [\gamma_\beta(A) \vee (\lambda_\eta(A)] \wedge [\gamma_\beta(A) \vee \lambda_\eta(B)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(A)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(B)]$

$\geq [\gamma_\beta(A) \vee (\lambda_\eta(A)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(B)]$

$= (\gamma_\beta \cup \lambda_\eta)(A) \wedge (\gamma_\beta \cup \lambda_\eta)(B)$

$(\gamma_\beta \cup \lambda_\mu)(A-B) \geq (\gamma_\beta \cup \gamma_\eta)(A) \wedge (\gamma_\beta \cup \lambda_\eta)(B)$

iv. $(\gamma_\beta \cup \lambda_\eta)(AB) = \gamma_\beta(AB) \vee \lambda_\eta(AB)$

$\geq \{\gamma_\beta(A) \wedge \gamma_\beta(B)\} \vee \{\lambda_\eta(A) \wedge \lambda_\eta(B)\}$

$= \{\gamma_\beta(A) \vee (\lambda_\eta(A) \wedge \lambda_\eta(B)\} \wedge \{\gamma_\beta(B) \vee (\lambda_\eta(A) \wedge \lambda_\eta(B)\}$

$= [\gamma_\beta(A) \vee (\lambda_\eta(A)] \wedge [\gamma_\beta(A) \vee \lambda_\eta(B)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(A)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(B)]$

$\geq [\gamma_\beta(A) \vee (\lambda_\eta(A)] \wedge [\gamma_\beta(B) \vee \lambda_\eta(B)]$

$= (\gamma_\beta \cup \lambda_\eta)(A) \wedge (\gamma_\beta \cup \lambda_\eta)(B)$

$(\gamma_\beta \cup \lambda_\mu)(AB) \geq (\gamma_\beta \cup \lambda_\eta)(A) \wedge (\gamma_\beta \cup \lambda_\eta)(B)$

Hence, $\gamma_G \cap \lambda_H$ is an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

3.4 Theorem

Let G and H be any two intuitionistic L-fuzzy sets on R. Let γ_G and λ_H be any two intuitionistic anti L-fuzzy HX subrings of a HX ring $\Re$ then their union, $\gamma_G \cup \lambda_H$ is also an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

Proof

Let G = { ⟨x, α(x), β(x)⟩ / x∈R } and H = { ⟨x, μ(x), η(x)⟩ / x∈R } be any two intuitionistic L-fuzzy sets defined on a ring R.

Then, γ_G = { ⟨A, γ_α(A), γ_β(A)⟩ / A∈ℜ } and λ_H = { ⟨A, λ_μ(A), λ_η(A)⟩ / A∈ℜ } be any two intuitionistic anti L-fuzzy HX subrings of a HX ring ℜ.Then,

$\gamma_G \cup \lambda_H$ = { ⟨A, $(\gamma_\alpha \cup \lambda_\mu)$(A), $(\gamma_\beta \cap \lambda_\eta)$(A)⟩ / A∈ℜ }

Let A,B ∈ ℜ

i. $(\gamma_\alpha \cup \lambda_\mu)$ (A–B) = γ_α(A–B) ∨ λ_μ(A–B)
≤ {γ_α(A) ∨ γ_α(B)} ∨{ λ_μ(A) ∨ λ_μ (B)}
={ γ_α(A) ∨ λ_μ(A)} ∨ {γ_α(B) ∨ λ_μ (B)}
= $(\gamma_\alpha \cup \lambda_\mu)$(A) ∧ $(\gamma_\alpha \cup \lambda_\mu)$(B)
$(\gamma_\alpha \cup \lambda_\mu)$ (A–B) ≤ $(\gamma_\alpha \cup \lambda_\mu)$(A) ∧ $(\gamma_\alpha \cup \lambda_\mu)$(B).

ii. $(\gamma_\alpha \cup \gamma_\mu)$ (AB) = γ_α(AB) ∨ λ_μ(AB)
≤ {γ_α(A) ∨ γ_α(B)} ∨{ λ_μ(A) ∨ λ_μ (B)}
={ γ_α(A) ∨ λ_μ(A)} ∨ {γ_α(B) ∨ λ_μ (B)}
= $(\gamma_\alpha \cup \lambda_\mu)$(A) ∧ $(\gamma_\alpha \cup \lambda_\mu)$(B)
$(\gamma_\alpha \cup \lambda_\mu)$ (AB) ≤ $(\gamma_\alpha \cup \lambda_\mu)$(A) ∧ $(\gamma_\alpha \cup \lambda_\mu)$(B).

iii. $(\gamma_\beta \cap \lambda_\eta)$ (A–B) = γ_β(A–B) ∧ λ_η(A–B)
≥ {γ_β(A) ∧ γ_β(B)}∧ { λ_η(A) ∧ λ_η (B)}
= (γ_β(A) ∧ λ_η(A)) ∧ (γ_β(B) ∧ λ_η (B))
= $(\gamma_\beta \cap \gamma_\eta)$(A) ∧ $(\gamma_\beta \cap \lambda_\eta)$(B)
$(\gamma_\beta \cap \lambda_\mu)$ (A–B) ≥ $(\gamma_\beta \cap \gamma_\eta)$(A) ∧ $(\gamma_\beta \cap \lambda_\eta)$(B)

iv. $(\gamma_\beta \cap \lambda_\eta)$ (AB) = γ_β(AB) ∧ λ_η(AB)
≥ {γ_β(A) ∧ γ_β(B)}∧ { λ_η(A) ∧ λ_η (B)}
= (γ_β(A) ∧ λ_η(A)) ∧ (γ_β(B) ∧ λ_η (B))
= $(\gamma_\beta \cap \gamma_\eta)$(A) ∧ $(\gamma_\beta \cap \lambda_\eta)$(B)
$(\gamma_\beta \cap \lambda_\mu)$ (AB) ≥ $(\gamma_\beta \cap \gamma_\eta)$(A) ∨ $(\gamma_\beta \cap \lambda_\eta)$(B)

Hence, $\gamma_G \cup \lambda_H$ is an intuitionistic anti L-fuzzy HX subring of a HX ring ℜ.

3.5 Definition

Let G = { ⟨x, α(x), β(x)⟩ / x∈R } and H = { ⟨x, μ(x), η(x)⟩ / x∈R } be any two intuitionistic L-fuzzy sets defined on a ring R. Let $\Re_1 \subset 2^{R_1} - \{\phi\}$ and $\Re_2 \subset 2^{R_2} - \{\phi\}$ be any two HX rings. Let γ_G = { ⟨A, γ_α(A), γ_β(A)⟩ / A∈ℜ } and λ_H = {⟨A, λ_μ(A), λ_η(A)⟩ /A∈ℜ } be any two intuitionistic L-fuzzy subsets of a HX ring ℜ, then the anti product of γ_G and λ_H is defined as

$(\gamma_G \times \lambda_H)$ = {⟨(A,B), $(\gamma_\alpha \cap \lambda_\mu)$(A,B), $(\gamma_\beta \cup \lambda_\eta)$(A, B)⟩ /(A, B) ∈ $\Re_1 \times \Re_2$},

where, $(\gamma_\alpha \cap \lambda_\mu)$(A, B) = γ_α(A) ∧ λ_μ(B) , for all (A, B) ∈ $\Re_1 \times \Re_2$,

$(\gamma_\beta \cup \lambda_\eta)$(A, B) = γ_β(A) ∨ λ_η(B)}, for all (A, B) ∈ $\Re_1 \times \Re_2$.

3.6 Theorem

Let G and H be any two intuitionistic L-fuzzy sets of R_1 and R_2 respectively. Let $\Re_1 \subset 2^{R_1} - \{\phi\}$ and $\Re_2 \subset 2^{R_2} - \{\phi\}$ be any two HX rings. If γ^G and λ^H are any two intuitionistic anti L-fuzzy HX subrings of $\Re_1$ and $\Re_2$ respectively then, $\gamma^G \times \lambda^H$ is also an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re_1 \times \Re_2$.

Proof

Let G = { ⟨x, α(x), β(x)⟩ / x∈R } and H = { ⟨x, μ(x), η(x)⟩ / x∈R } be any two intuitionistic L-fuzzy sets defined on a ring R.

Then, γ_G = { ⟨A, γ_α(A), γ_β(A)⟩ / A∈ℜ } and λ_H = { ⟨A, λ_μ(A), λ_η(A)⟩ / A∈ℜ } be any two intuitionistic anti L-fuzzy HX subrings of a HX ring ℜ.Then,

$(\gamma_G \times \lambda_H)$ = {⟨(A,B), $(\gamma_\alpha \cap \lambda_\mu)$(A,B), $(\gamma_\beta \cup \lambda_\eta)$(A, B)⟩ /(A, B) ∈ $\Re_1 \times \Re_2$},

where, $(\gamma_\alpha \cap \lambda_\mu)(A, B) = \gamma_\alpha(A) \wedge \lambda_\mu(B)$, for all (A, B) ∈ $\Re_1 \times \Re_2$,

$(\gamma_\beta \cup \lambda_\eta)(A, B) = \gamma_\beta(A) \vee \lambda_\eta(B)$}, for all (A, B) ∈ $\Re_1 \times \Re_2$.

Here C = (A,B) and D = (E,F)

i. $(\gamma_\alpha \cap \lambda_\mu)(C - D) = \gamma_\alpha(C - D) \wedge \lambda_\mu(C - D)$

$\leq \{\gamma_\alpha(C) \vee \gamma_\alpha(D)\} \wedge \{\lambda_\mu(C) \vee \lambda_\mu(D)\}$

$= \{\gamma_\alpha(C) \wedge (\lambda_\mu(C) \vee \lambda_\mu(D))\} \vee \{\gamma_\alpha(D) \wedge (\lambda_\mu(C) \vee \lambda_\mu(D))$

$= (\gamma_\alpha(C) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(C) \wedge \lambda_\mu(D)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(D))$

$\leq (\gamma_\alpha(C) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(D))$

$= (\gamma_\alpha \cap \lambda_\mu)(C) \vee (\gamma_\alpha \cap \lambda_\mu)(D)$

$(\gamma_\alpha \cap \lambda_\mu)(C - D) \leq (\gamma_\alpha \cap \lambda_\mu)(C) \vee (\gamma_\alpha \cap \lambda_\mu)(D)$.

ii. $(\gamma_\alpha \cap \gamma_\mu)(CD) = \gamma_\alpha(CD) \wedge \lambda_\mu(CD)$

$\leq \{\gamma_\alpha(C) \vee \gamma_\alpha(D)\} \wedge \{\lambda_\mu(C) \vee \lambda_\mu(D)\}$

$= \{\gamma_\alpha(C) \wedge (\lambda_\mu(C) \vee \lambda_\mu(D))\} \vee \{\gamma_\alpha(D) \wedge (\lambda_\mu(C) \vee \lambda_\mu(D))$

$= (\gamma_\alpha(C) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(C) \wedge \lambda_\mu(D)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(D))$

$\leq (\gamma_\alpha(C) \wedge \lambda_\mu(C)) \vee (\gamma_\alpha(D) \wedge \lambda_\mu(D))$

$= (\gamma_\alpha \cap \lambda_\mu)(C) \vee (\gamma_\alpha \cap \lambda_\mu)(D)$

$(\gamma_\alpha \cap \lambda_\mu)(CD) \leq (\gamma_\alpha \cap \lambda_\mu)(C) \vee (\gamma_\alpha \cap \lambda_\mu)(D)$

iii. $(\gamma_\beta \cup \lambda_\eta)(C–D) = \gamma_\beta(C–D) \vee \lambda_\eta(C–D)\}$

$\geq \{\gamma_\beta(C) \wedge \gamma_\beta(D)\} \vee \{\lambda_\eta(C) \wedge \lambda_\eta(D)\}\}$

$= \{\gamma_\beta(C) \vee (\lambda_\eta(C) \wedge \lambda_\eta(D))\} \wedge \{\gamma_\beta(D) \vee (\lambda_\eta(C) \wedge \lambda_\eta(D))\}$

$= [\gamma_\beta(C) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(C) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(D) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(D) \vee \lambda_\eta(D))$

$\geq (\gamma_\beta(C) \vee \lambda_\eta(C)) \wedge (\gamma_\beta(D) \vee \lambda_\eta(D))$

$= (\gamma_\beta \cup \gamma_\eta)(C) \wedge (\gamma_\beta \cup \lambda_\eta)(D)$

$(\gamma_\beta \cup \lambda_\mu)(C–D) \geq (\gamma_\beta \cup \gamma_\eta)(C) \wedge (\gamma_\beta \cup \lambda_\eta)(D)$

iv. $(\gamma_\beta \cup \lambda_\eta)(CD) = \gamma_\beta(CD) \wedge \lambda_\eta(CD)$

$\geq \{\gamma_\beta(C) \wedge \gamma_\beta(D)\} \vee \{\lambda_\eta(C) \wedge \lambda_\eta(D)\}\}$

$= \{\gamma_\beta(C) \vee (\lambda_\eta(C) \wedge \lambda_\eta(D))\} \wedge \{\gamma_\beta(D) \vee (\lambda_\eta(C) \wedge \lambda_\eta(D))\}$

$= [\gamma_\beta(C) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(C) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(D) \vee \lambda_\eta(C)] \wedge [\gamma_\beta(D) \vee \lambda_\eta(D))$

$\geq (\gamma_\beta(C) \vee \lambda_\eta(C)) \wedge (\gamma_\beta(D) \vee \lambda_\eta(D))$

$= (\gamma_\beta \cup \gamma_\eta)(C) \wedge (\gamma_\beta \cup \lambda_\eta)(D)$

$(\gamma_\beta \cup \lambda_\mu)(CD) \geq (\gamma_\beta \cup \gamma_\eta)(C) \wedge (\gamma_\beta \cup \lambda_\eta)(D)$

Hence, $\gamma_G \times \lambda_H$ is an intuitionistic anti L-fuzzy HX subring of a HX ring ℜ.

3.7 Definition

Let H be an intuitionistic L-fuzzy set of R. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. Let λ_H be an intuitionistic L-fuzzy set of ℜ. We define the following "necessity" and possibility" operations:

□ λ_H = { ⟨ A, λ_μ(A), 1– λ_μ(A) ⟩ / A∈ℜ}

◊ λ_H = { ⟨ A, 1– λ_η(A), λ_η(A) ⟩ / A∈ℜ}.

3.8 Theorem

Let H be an intuitionistic L-fuzzy set on R. Let λ_H be an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$ then □ λ_H is an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

Proof

Let λ_H be an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$. Then,

i. $\lambda_\mu (A - B) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

ii. $\lambda_\mu (AB) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

iii. $\lambda_\eta(A - B) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$

iv. $\lambda_\eta(AB) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$.

Now, $\lambda_\mu (A - B) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

$1 - \lambda_\mu (A - B) \geq 1 - (\lambda_\mu (A) \vee \lambda_\mu (B))$

$\geq (1 - \lambda_\mu (A)) \wedge (1 - \lambda_\mu (B))$

That is, $1 - \lambda_\mu (A - B) \geq (1 - \lambda_\mu (A)) \wedge (1 - \lambda_\mu (B))$

We have, $\lambda_\mu (AB) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

$1 - \lambda_\mu (AB) \geq 1 - (\lambda_\mu (A) \vee \lambda_\mu (B))$

$\geq (1 - \lambda_\mu (A)) \wedge (1 - \lambda_\mu (B))$

That is, $1 - \lambda_\mu (AB) \geq (1 - \lambda_\mu (A)) \wedge (1 - \lambda_\mu (B))$

Hence, □ λ_H is an intuitionistic anti-fuzzy HX subring of a HX ring $\Re$.

3.9 Theorem

Let H be an intuitionistic L-fuzzy set on R. Let λ_H be an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$ then $\Diamond\lambda_H$ is an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

Proof

Let λ_H be an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$. Then,

i. $\lambda_\mu (A - B) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

ii. $\lambda_\mu (AB) \leq \lambda_\mu (A) \vee \lambda_\mu (B)$

iii. $\lambda_\eta(A - B) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$

iv. $\lambda_\eta(AB) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$.

Now, $\lambda_\eta (A - B) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$

$1 - \lambda_\eta(A - B) \leq 1 - (\lambda_\eta(A) \wedge \lambda_\eta(B))$

$\leq (1 - \lambda_\eta (A)) \vee (1 - \lambda_\eta (B))$

That is, $1 - \lambda_\eta (A - B) \leq (1 - \lambda_\eta (A)) \vee (1 - \lambda_\eta (B))$

We have, $\lambda_\eta (AB) \geq \lambda_\eta(A) \wedge \lambda_\eta(B)$

$1 - \lambda_\eta (AB) \leq 1 - (\lambda_\eta(A) \wedge \lambda_\eta(B))$

$\leq (1 - \lambda_\eta (A)) \vee (1 - \lambda_\eta (B))$

That is, $1 - \lambda_\eta (AB) \leq (1 - \lambda_\eta (A)) \vee (1 - \lambda_\eta (B))$

Hence, $\Diamond$ λ_H is an intuitionistic anti L-fuzzy HX subring of a HX ring $\Re$.

CONCLUSION

In this paper we introduce the concept of intuitionistic anti L-fuzzy HX ring and discuss the basic results on HX ring. Further investigation may be in intuitionistic anti L-fuzzy HX ideals on HX ring which will give a new horizon in the further study.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to all our friends for their help to make this paper a successful one.

REFERENCES

1. Atanassov. K.T, intuitionistic fuzzy sets, Fuzzy Sets and System 20 (1986) ,pp 87-96.
2. Bing-xueYao and Yubin-Zhong, Upgrade of algebraic structure of ring, Fuzzy information and Engineering (2009)2:219-228.
3. Bing-xue Yao and Yubin-Zhong, The construction of power ring, Fuzzy information and Engineering (ICFIE), ASC 40,pp.181-187,2007.
4. Li Hong Xing, HX group, BUSEFAL, 33(4), 31-37, October 1987.
5. Li Hong Xing, HX ring, BUSEFAL, 34(1) 3-8, January 1988.
6. Liu.W.J., Fuzzy invariant subgroups and fuzzy ideals, Fuzzy sets and systems,8,133-139.
7. Muthuraj. R, Manikandan K.H., Intuitionistic Q-fuzzy HX subgroup and $< \alpha,\beta >$ level sub HX groups, CIIT International DOI – FS 122011010.
8. Muthuraj.R, Sridharan.M, Bipolar fuzzy HX group and its level sub HX groups, International Journal of Mathematical Archive,5(1), 230-239,2014.
9. Muthuraj.R, Muthuraman.M.S., Intuitionistic fuzzy HX ring ,IOSR Journal of Mathematics (IOSR-JM) Volume 10, Issue 4,Ver II , 3-12 (Jul-Aug 2014).
10. Muthuraj.R, Ramila Gandhi.N., Induced fuzzy k-ideal and its level k-ideals on a HX ring, IOSR Journal of Mathematics (IOSR-JM) Volume 9, Issue 6 (Jan 2014) , pp 138-143.
11. Rosenfeld. A., Fuzzy groups, J.Math.Anal.,35(1971),512-517.
12. Zadeh.L.A., Fuzzy sets, Information and control,8,338-353

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Synthesis of Biologically Active Quinoline Substituted Pyrazole, Pyrazolone and It's Derivative

Sathiyaseelan Manisekar[1]*, Prabakaran Kalaivanan[1], Ramya Rajendran[2] and Parvathi Ramakrishnan[3]

[1]Department of Chemistry, Annai College of Arts and Science, Kovilacheri, Tamil Nadu, India.
[2]Department of Chemistry, kalaimahal College of Arts and Science, Sembanarkoil, Tamil Nadu, India.
[3]Department of Physics, Annai College of Engineering and Technologies, Kovilacheri, Tamil Nadu, India.

Received: 17 Nov 2020 Revised: 19 Dec 2020 Accepted: 22 Jan 2021

***Address for Correspondence**
Sathiyaseelan Manisekar
Department of Chemistry,
Annai College of Arts and Science,
Kovilacheri, Tamil Nadu, India.
Email: sathiyaseelanmanisekar@gmail.com

ABSTRACT

In the present investigation a new class of pyrazole, pyrazolone and its derivatives containing quinoline moiety have been designed and synthesized from available starting materials aniline and ethyl acetoacetate with thermodynamic favoured condensation followed by cyclization, chlorination, nucleophilic hydrazination and condensation reaction to obtain goal and characterized by FT-IR, ^{1}H-NMR and ^{13}C-NMR spectroscopy in good yield.

Keywords: Pyrazole, Pyrazolone, Quinoline, Microwave, Anti Microbial.

INTRODUCTION

Pyrazoles and pyrazolones are the important members of heterocyclic compounds. Pyrazoles are potential bioactive agents due to their wide spectrum of pharmacological activities like anti-inflammatory [1], antimicrobial [2], antitumor [3], anticonvulsant, antitubercular and hypoglycemic [4-8]. Quinoline [9-13] has its own prominence in drug discovery programs. Quinoline along with its derivatives is reported to exhibit a wide spectrum of biological properties such as antimicrobial [14], antimalarial [15], antitubercular [16], anti-inflammatory [17], anti-HIV [18] and anti-cancer activities [19]. Our laboratory has surveyed the applications and reported a variety of approaches to synthesis derivatives in excellent yield [14, 20-23]. In present work, we planned to design both pyrazole and quinoline moiety, presented compound were synthesized and it was confirmed by FT-IR, ^{1}H-NMR and ^{13}C-NMR techniques.

MATERIALS AND METHODS

Melting points are uncorrected. Infrared spectra were recorded on a Perkin-Elmer Paragon 1000 FT-IR spectrophotometer as potassium bromide discs unless otherwise indicated. 1H-NMR spectra were obtained on a Brucker (400 MHz) instrument in $CDCl_3$ solutions using tetramethylsilane as an internal standard. *J* Values are given in Hz. Column chromatography utilised Merck silica gel 60 and hexane and ethylacetate as eluants. All the basic chemicals were purchased from Merck (India).

Synthesis of 4-methyl-2-hydroxyquinoline 3

In the first step of the reaction, microwave assisted condensation between aniline and ethylacetoacetate with catalytic amount of *p*-toluenesulphonic acid, the progress was monitor after every 30s by TLC. After the irradiation the product was washed with petroleum ether and light yellowish white crystal of 4-methyl-2-hydroxyquinoline were collected.

Preparation of 4-methyl-2-hydroxyquinoline 3

Yield 1.44g(90%), dark yellowish crystal, m.p: 223°C; IR(KBr, υ_{max}, cm^{-1}) : 3350, 1562, 1248 ;^{1}H-NMR (400 MHz, $CDCl_3$) : δ8.19-7.36 (m, 4H, C_5, C_6, C_7 & C_8-H), δ6.32 (s, 1H, C_3-H), δ2.38(s,3H, C_4-CH_3-H) ; ^{13}C-NMR (100 MHz, $CDCl_3$) (Fig.3): δ179.16, 149.43, 144.96, 140.55, 135.87, 130.76, 128.92, 127.06, 123.56, 22.56.

Synthesis of 2-chloro-4-methylquinoline 4

0.01 mole (1.59g) of 4-methyl-2-hydroxyquinoline **3** was dissolved in $POCl_3$ and was allowed to reflux at 75°C for 4 hours, regular intervals TLC was checked after reaction completion purified through column chromatography using petroleum ether and ethyl acetate (98:2) as an eluant.

Preparation of 2-chloro-4-methylquinoline 4

Yield: 1.68g (95%); light yellow crystal, m.p: 124°C; IR(KBr, υ_{max}, cm^{-1}) (Fig.1): 1592, 744;^{1}H-NMR (400 MHz, $CDCl_3$) (Fig.2): δ8.09 (d, 1H, J=8.5HZ, C_5-H), δ7.82 (d, 1H, J = 8.0 Hz, C_8-H), δ7.73 (t, 1H, J = 8.5 Hz, C_7-H), δ7.56(t, 1H, J = 7.0 Hz, C_6-H), δ6.56 (d, 1H, C_3-H), δ2.3(s,3H, CH_3-H) ; ^{13}C-NMR (100 MHz, $CDCl_3$) (Fig.3): δ139.16, 135.45, 134.86, 130.85, 129.47, 128.96, 128.42, 127.26, 123.56, 23.56.

Synthesis of 2-hydrazinyl-4-methylquinoline 5

0.01 mole (2.39 g) of 2-chloro-4-methylquinoline **4** was dissolved in ethanol and excess of hydrazine hydrate was added with a trace amount of sodium carbonate. The initial colour of the mixture was yellow; it was allowed to reflux at 75°C for 5 hours. The red coloration and TLC observation indicates the reaction progress, then it was poured into the crushed ice; it was extracted with ethyl acetate, concentrated and purified through column chromatography using petroleum ether and ethyl acetate (95:5) as an eluant.

Preparation of 2-hydrazinyl-4-methylquinoline 5

Yield: 1.56g(90%); greasy mass- unidentified melting point; IR(KBr, υ_{max}, cm^{-1}): 3082, 3058, 3032, 2883, 1603; ^{1}H-NMR (500MHz, $CDCl_3$) : δ 8.06 (s, 1H, C_5-H), δ 7.68-7.89 (m, 4H, C_3, C_6, C_7& C_8 -H), δ 4.0(s, 1H, NH-H), δ2.78(σ, 3H, CH_3-H), δ 2.10(s, 2H, NH_2-H); ^{13}C-NMR (125 MHz, $CDCl_3$) : δ137.98, 137.65, 134.83, 133.86, 133.25, 130.45, 129.69, 128.87, 128.14, 19.44.

Synthesis of 2-(3,5-dimethyl-1h-pyrazol-1-yl)-4-methylquinoline and its derivative 6a&b

2-Hydrazinyl-4-methylquinoline 5 (0.01mole, 1.73g) was weighed and mixed with pentane-2,4-dione or 1-phenylbutane-1,3-dione (0.01 mole) in methanol and a catalytic amount of sodium hydroxide was added and allowed to reflux at 75°C for 9 hours. The condensed material was then poured into ice-cold water; the precipitate was extracted with ethyl acetate, concentrated and purified through column chromatography.

Preparation of 2-(3,5-dimethyl-1h-pyrazol-1-yl)-4-methylquinoline 6a

Yield 1.90 g (80%); brown solid m.p: 98°C; IR(KBr, υ_{max}, cm^{-1}) : 3057, 3025, 2918, 1655; ^{1}H-NMR (500MHz, $CDCl_3$) : δ 8.10 (d, 1H, C_5-H), δ 7.43-8.08 (m, 4H, C_3, C_6, C_7& C_8 -H), δ 6.12(σ, 1H, pyra C_4–H), δ 2.80(s, 3H, pyra C_5-CH_3-H), δ 2.60(s, 3H, C_4-CH_3-H), δ2.32(s, 3H, pyra C_3-CH_3-H); ^{13}C- NMR (125 MHz, $CDCl_3$) : δ137.91, 134.71, 132.27, 129.96, 129.86, 129.82, 127.20, 125.67, 122.39, 117.00, 116.75, 116.51, 30.99, 22.44, 16.44.

Preparation of 4-methyl-2-(5-methyl-3-phenyl-1h-pyrazol-1-yl)quinoline 6b

Yield 2.33 g (78%); brown solid m.p: 128°C; IR(KBr, υ_{max}, cm^{-1}) : 3066, 3014, 2907, 1642; ^{1}H-NMR (500MHz, $CDCl_3$) : δ 8.10 (d, 1H, C_5-H), δ 8.0 (d, 2H, ph $C_{2\&6}$-H), δ 7.90 (d, 1H, C_8-H), δ 7.80 (s, 1H, C_3-H), δ 7.73-7.41 (m, 5H, C_6, C_7, ph C_3, C_4& C_5 -H), δ 6.20(σ, 1H, pyra C_4–H), δ 2.90(s, 3H, pyra C_5-CH_3-H), δ 2.60(s, 3H, C_4-CH_3-H); ^{13}C- NMR (125 MHz, $CDCl_3$) : δ157.91, 144.73, 132.47, 129.78, 128.86, 128.12, 127.40, 125.80, 121.78, 117.18, 115.85, 106.51, 31.55, 23.54.

Synthesis of 3-methyl-1-(4-methylquinolin-2-yl)-1h-pyrazol-5(4h)-one and its derivative 7a&b

2-Hydrazinyl-4-methylquinoline **5** (0.01mole, 1.73g) was weighed and mixed with ethyl acetoacetate or ethyl 3-oxo-3-phenylpropanoate (0.01 mole) in methanol and a catalytic amount of sodium hydroxide was added and allowed to reflux at 75°C for 9 hours. The condensed material was then poured into ice-cold water; the precipitate was extracted with ethyl acetate, concentrated and purified through column chromatography.

Preparation of 3-methyl-1-(4-methylquinolin-2-yl)-1h-pyrazol-5(4h)-one 7a

Yield 1.92 g (80%); dark brown solid m.p: 87°C; IR(KBr, υ_{max}, cm^{-1}): 3062, 3058, 2849, 1668; ^{1}H NMR (500MHz, $CDCl_3$) : δ 7.25-8.25 (m, 5H, C_3, C_5, C_6, C_7& C_8 –H), δ.2.78 (σ, 3H, pyraC_4-CH_3-H), δ.2.21 (δ, 2H, pyraC_4-H),δ.1.82 (σ, 3H, X_4-CH_3-H); ^{13}C- NMR (125 MHz, $CDCl_3$) : δ182.54, 137.08, 136.65, 135.87, 133.96, 133.20, 129.45, 128.69, 127.87, 127.19,25.12, 22.34, 18.44.

Preparation of 1-(4-methylquinolin-2-yl)-3-phenyl-1h-pyrazol-5(4h)-one 7b

Yield 2.32 g (77%); dark brown solid m.p: 109°C; IR(KBr, υ_{max}, cm^{-1}): 3058, 3044, 2836, 1654; ^{1}H NMR (500MHz, $CDCl_3$) : δ 8.30-7.20(m, 9H, C_5, C_6, C_7, C_8, phC_2, C_3, C_4, C_5& C_6 –H), δ6.6 (s, 1H, C_2-H), δ.2.70 (σ, 3H, C_4-CH_3-H), δ.2.21 (σ, 2H, pyraC_4-H); ^{13}C- NMR (125 MHz, $CDCl_3$) : δ187.66, 146.32, 137.08, 136.65, 135.87, 133.96, 133.20, 129.45, 128.69, 127.87, 126.67, 126.10, 125.76, 107.21, 28.82, 22.61.

RESULT AND DISCUSSION

We were start with available starting materials like aniline and ethyl acetoacetate under microwave condition with catalytic amount of *p*-toluenesulphonic acid. The product of 4-methyl-2-hydroxyquinoline 3 was confirmed by alcoholic-OH stretching appears at 3350 and C-O stretching appears at 1248cm^{-1}. The 4-methyl-2-hydroxyquinoline 3 successfully chlorinated with $POCl_3$, the light yellow color product of 2–chloro-4-methylquinoline 4 was confirmed by appearance of C-Cl stretching at 744cm^{-1} and disappearance of hydroxyl group broad singlet at δ 10.40 which is indicated as nucleophilic replacement of hydroxyl group by the chlorine group. 2-hydrazinyl-4-methylquinoline 5 achieved by nucleophilic replacement of chloride by the hydrazinyl group with hydrazine, the IR spectrum showed the disappearance of a C-Cl stretching at 744cm^{-1} which was present in 4. Furthermore, the ^{1}H-NMR spectrum indicated a two-proton singlet at δ 4.0 and a one-proton singlet at δ 2.10, it was confirm formation of compound 5.

Finally, When 5 was refluxed with pentane-2,4-dione or 1-phenylbutane-1,3-dione, base catalyzed a new final compound, 2-(3,5-dimethyl-1H-pyrazol-1-yl)-4-methylquinoline 6a&b resulted. The^{1}H-NMR the disappearance of a characteristic two-proton singlet at δ 4.0 and a one-proton singlet at δ 2.10 whilst the appearance of pyra-C_3 & C_5 2 x CH_3-H singlet at δ 2.30 & 2.94, and a characteristic two methyl group at pyrazole ring, the signal at δ 22.44 & 16.44 in ^{13}C-NMR spectrum supported the structure of compound 6a. And then appearance of pyra C_5-CH_3-H singlet at δ2.90,pyra C_3- phenyl signals at δ 8.0 (d, 2H, ph C_2 & $_6$-H), and δ 7.73-7.41 (m, 3H, ph C_3, C_4 & C_5 -H) and ^{13}C-NMR spectrum shows characteristic phenyl carbon signal at δ 132.47, 129.78, 128.86, 128.12, supported the structure of

compound 6b. Above final step also carried with ethyl acetoacetate or ethyl 3-oxo-3-phenylpropanoate; 3-methyl-1-(4-methylquinolin-2-yl)-1H-pyrazol-5(4H)-one 7 was resulted. The IR spectrum shows C-H stretching frequency at 2849 cm^{-1} and C=O stretching at 1668 cm^{-1} are the appreciable area of ketone; in ^{1}H-NMR and the disappearance of a characteristic two-proton singlet at δ 4.0 and a one-proton singlet at δ 2.10. in ^{13}C-NMR and characteristic ketone carbonyl carbon signal at δ 182 in are supporting the structure 7a. And then The IR spectraum shows C-H stretching frequency at 2836 cm^{-1} and C=O stretching at 1654 cm^{-1} are the appreciable area of ketone; in ^{1}H-NMR and the disappearance of a characteristic two-proton singlet at δ 4.0 and a one-proton singlet at δ 2.10. in ^{13}C-NMR and characteristic ketone carbonyl carbon signal at δ 187 in are supporting the structure 7b.

Anti Microbial Activity

Ciprofloxacin was used as references to evaluate the potency of the synthesized compounds against gram positive bacteria Bacillus subtilis, *Staphylococcus aureus*, and gram negative bacteria Escherichia coli, Proteus vulgaris and Fluconazole reference used in four fungus *Aspergillus niger, Aspergillus flavus, Candida albicans* and *Fusarium oxysporium* by using disc diffusion method at 10 μg/disc. The cultural media used for bacteria was nutrient agar medium and for fungi it was potato-dextrose-agar medium. Amongst all of them compound 7b has been found to be relatively active against gram positive & gram negative bacteria and all four funguses in comparison to other compounds.

CONCLUSION

A new compound was synthesized from four step procedure and the compound 7b has been found to be relatively active against gram positive & gram negative bacteria and all four funguses in comparison to other compounds.

ACKNOWLEDGEMENT

The authors are thankful to VIT, Vellore, and SRM University, Chennai for NMR spectral analysis and Annai College of Arts and Science for lab facility.

REFERENCES

1. Tewari AK and Mishra A. Synthesis and anti-inflammatory activities of N_4,N_5-disubstituted-3-methyl-1H-pyrazolo[3,4- c]pyridazines. Bioorg Med Chem. 2001; 9; 715–718.
2. Nauduri D and Reddy GB. Antibacterials and antimycotics: part 1: synthesis and activity of 2-pyrazoline derivatives. Chem Pharm Bull. 1998; 46; 1254–1260.
3. Farag AM, Ali KA, Mayhoub AS, Abdalla TM, Amr AE, Abdel- Hafez N and Abdalla MM. Design, synthesis and structure activity relationship study of novel pyrazole-based heterocycles as potential antitumor agents. Eur J Med Chem. 2010; 45; 5887–5898.
4. Anna C, Cristina L, Antonino R, Roberto R, Paolo V, Romano G, Sara B and Carla V. New "Green" Approaches to the Synthesis of Pyrazole Derivatives. Molecules. 2007; 12(7); 1482-1495.
5. Ranjana A, Vinod K, Rajiv K and Shiv PS. Approaches towards the synthesis of 5-aminopyrazoles. Beilstein J Org Chem. 2011; 7; 179-197.
6. Hany FA and Mohamed HE. Recent developments in aminopyrazole chemistry. ARKIVOC. 2009; (i); 198-250.
7. Harish KA and Sandeep J. The synthetic development of pyrazole nucleus: From reflux to microwave. Der Pharmacia Lettre. 2013; 5(1); 340-354.
8. Singaravel M, Sarkkarai A and Kambikudi RM. Synthesis, Characterization and Biological activity of Some Novel Sulphur Bridged Pyrazoles,.IJPSR. 2010; 1(9); 391-398.
9. Joule JA, Mills K and Smith GF. Heterocyclic Chemistry 3rd Edition, Reprinted by Stanley Thornes (Publishers) Ltd, UK. 1998.
10. Thomas L, Gilchrist. Heterocyclic Chemistry. 3rd Edition. Pearson (Publisher); 2005.

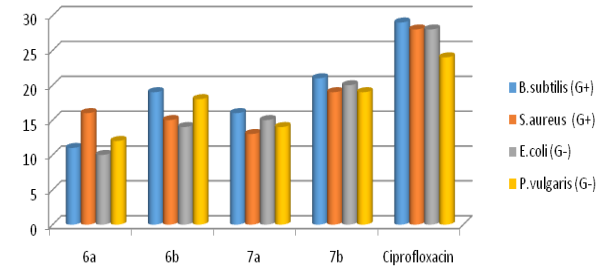
11. Sumesh E, Airody VA, Imran HC, Nishith KP and Thomas KD. New quinoline derivatives: Synthesis and investigation of antibacterial and antituberculosis properties. Eur J Med Chem. 2010; 45(8); 3374-3383.
12. Kumar S, Bawa S and Gupta H. Biological Activities of Quinoline Derivatives. Mini Rev Med Chem. 2009; 9(14); 1648-1654.
13. Akranth M, Om Prakash T, Rikta S, Rahmat A, Sandeep S, Mymoona A, Shaquiquzzaman and Mohammad, M. A. Quinoline: A versatile heterocyclic. Saudi Pharm J. 2013; 21(1); 1-12.
14. Sathiyaseelan M, Prabakaran K, Ramya R, Parvathi R, Pitchai P. synthesis, characterization and antimicrobial studies of N,N'-(butane-1,4-diylidene)bis(quinolin-2-amine) and its derivatives. IJRAR. 2018; 5(4); 530-532.
15. Nasveld P and Kitchener S. Treatment of acute vivax malaria with tafenoquine. Trans R Soc Trop Med Hyg. 2005; 99(1); 2-5.
16. Ram SU, Jaya KV, Ramakant AK, Santosh VL, Shailesh SD, Hemantkumar SD, Popat DS, Manash PS. and Jyoti C. Novel quinoline and naphthalene derivatives as potent antimycobacterial agents. Eur J Med Chem. 2010; 45(5); 1854-1867.
17. El-Feky S, Abd El-Samii ZK, Osman NA, Lashine J, Kamel MA, Thabet HK. Synthesis, molecular docking and anti-inflammatory screening of novel quinoline incorporated pyrazole derivatives using the Pfitzinger reaction II. Bioorg Chem. 2015; 58; 104-116.
18. Ahmed N, Brahmbhatt, KG, Sabde S, Mitra D, Singh IP, Bhutani KK. Synthesis and anti-HIV activity of alkylated quinoline 2,4-diols. Bioorg Med Chem. 2010; 18(8); 2872-2879.
19. Singh K, Verma V, Yadav Sreekanth V, Kumar D, Bajaj A, Kumar V. Design, regioselective synthesis and cytotoxic evaluation of 2-aminoimidazole–quinoline hybrids against cancer and primary endothelial cells. Eur J Med Chem. 2014; 87; 150-158.
20. Pitchai P, Sathiyaseelan M, Nepolraj A, Gengan RM. An elegant synthesis of an indoloquinoline alkaloid cryptackiene via vilsmeier-haack approach. Indian Journal of Chemistry. 2015; 54B; 1290-1292.
21. Babu K, Pitchai P, Sathiyaseelan M, Nepolraj A. Novel pyrazoline derivatives: Synthesis, characterization and antimicrobial studies. Der Pharma Chemica. 2015; 7(6); 95-98.
22. Sathiyaseelan M, Nepolraj A, Pitchai P, Gengan RM. Vilsmeier-Haack Methodology as a Key Step to Novel Heterocycles: Synthesis of 3-phenyl-1-(3-phenylquinolin-2-yl)-1H-pyrazole-4-carbaldehyde. Indian Journal of Advances in Chemical Science. 2016; 4(2); 172-179.
23. Nepolraj A, Sathiyaseelan M, Pitchai P, Gengan RM. An alternative approach to synthesis of 3-(2,4-dichloroquinolin-3-yl)-1-phenyl-1H-pyrazole-4-carbaldehyde using the Vilsmeier Haack reaction. International Journal of Chemical Studies. 2016; 4(2); 83-85.

Table 1: Results of Antibacterial Activity

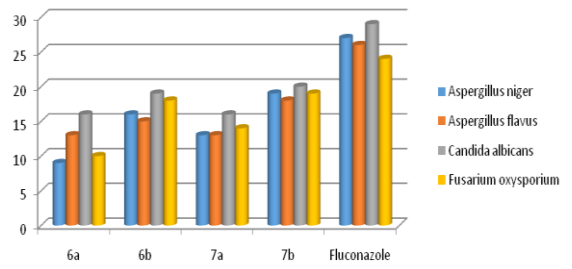
Compounds	µg/ml	*B.subtilis* (G+)	*S.aureus* (G+)	*E.coli* (G-)	*P.vulgaris* (G-)
6a	10	11	16	10	12
6b	10	19	15	14	18
7a	10	16	13	15	14
7b	10	21	18	20	19
Ciprofloxacin	10	29	28	28	24

Table 2: Results of Antifungal Activity

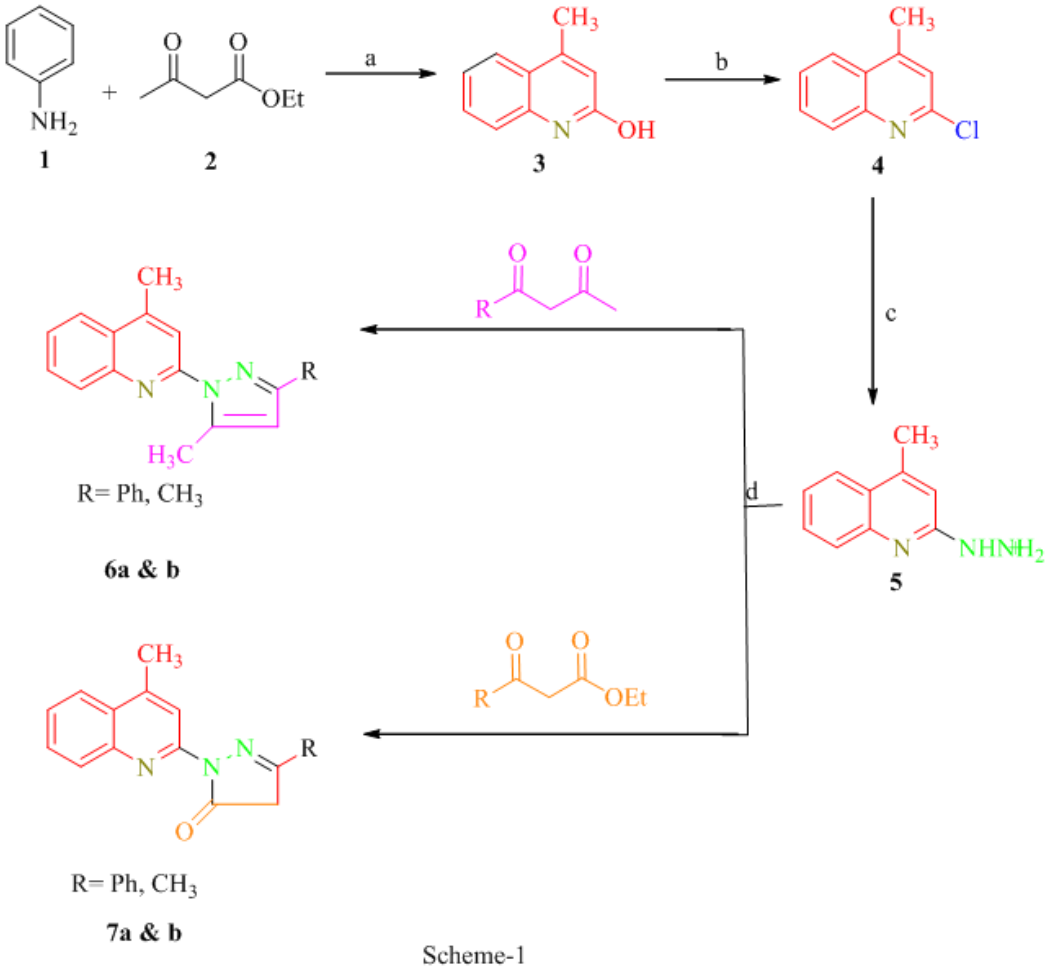
Compounds	µg/ml	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*	*Fusarium oxysporium*
6a	10	9	13	16	10
6b	10	16	15	19	18
7a	10	13	13	16	14
7b	10	19	18	20	19
Fluconazole	10	27	26	29	24



Graph 1 : graph showing comparative antibacterial activity of synthesized compounds



Graph 2 : graph showing comparative antifungal activity of synthesized compounds



Scheme-1

a) p-TSA, MWI, 240W, 3 min: b) $POCl_3$, 75°C, 4h; c) hydrazine hydrate, Na_2CO_3, EtOH, 75°C, 5h: d) MeOH, NaOH, 75°C, 9h.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Intuitionistic Anti Fuzzy HX Sub Module

M. S. Muthuraman[1]*, G. Sabarinathan[1], K. H. Manikandan[1], M. Sridharan[1] and R.Muthuraj[2]

[1]Department of Mathematics, PSNA College of Engineering and Technology,Dindigul, Tamil Nadu, India.
[2]PG and Research Department of Mathematics, H.H.The Rajah's College, Pudukkottai, Tamil Nadu, India.

Received: 13 Nov 2020 Revised: 15 Dec 2020 Accepted: 19 Jan 2021

***Address for Correspondence**
M. S. Muthuraman
Department of Mathematics,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: ramanpsna70@gmail.com

ABSTRACT

In this paper we defines the notion of an intuitionistic anti fuzzy HX sub module of a HX module. Hence we define the necessity and possibility operators of an intuitionistic fuzzy subset of an intuitionistic anti fuzzy HX submodule and discuss some of its properties.

Keywords: Fuzzy set, HX ring, intuitionistic fuzzy HX sub module, intuitionistic anti fuzzy HX sub module.

INTRODUCTION

In 1965, Zadeh [11] introduced the concept of fuzzy subset. In 1967, Rosenfeld [10] defined the idea of fuzzy subgroup and gave some of its properties. Li Hong Xing [4] introduced the concept of HX group. In 1982 Wang-jin Liu introduced the concept of fuzzy ring and fuzzy ideal. In 1988, Professor Li Hong Xing [5] proposed the concept of HX ring and derived some of its properties, then Professor Zhong [2,3] gave the structures of HX ring on a class of ring. R. Muthuraj et.al., introduced the concept of anti fuzzy HX ring. In this paper we define a new algebraic structure of an intuitionistic anti fuzzy HX sub module of a HX module and investigate some related properties. We define the operators of an intuitionistic fuzzy subset of an intuitionistic anti fuzzy HX sub module and discuss some of its properties.

PRELIMINARIES

In this section, we site the fundamental definitions that will be used in the sequel. Throughout this paper, R = (R ,+, ·) is a Ring, e is the additive identity element of R and xy, we mean x.y

2.1 Definition

Let R be a ring. In $2^R - \{\phi\}$, a non-empty set $\Re \subset 2^R - \{\phi\}$ with two binary operation ' + ' and '.' is said to be a HX ring on R if $\Re$ is a ring with respect to the algebraic operation defined by

i. A + B = {a + b / a ∈A and b ∈ B} , which its null element is denoted by Q , and the negative element of A is denoted by −A.

ii. AB = {ab / a ∈A and b ∈ B},

iii. A (B + C) = AB + AC and (B + C) A = BA + CA.

2.2 Definition

Let R be a ring. Let μ be a fuzzy ring defined on R. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. A fuzzy subset λ^μ of $\Re$is called a fuzzy HX ring on$\Re$or a fuzzy ring induced by μ if the following conditions are satisfied. For all A,B∈$\Re$,

i. λ^μ (A −B) ≥ min { λ^μ (A), λ^μ (B) } , ii. λ^μ (AB) ≥ min { λ^μ (A), λ^μ (B) }

Where λ^μ (A) = max{ μ(x) / for all x∈A ⊆ R }.

2.3 Definition

Let R be a ring. Let μ be an anti fuzzy ring defined on R. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. A fuzzy subset λ^μ of $\Re$is called an anti fuzzy HX ring on $\Re$ or an anti fuzzy ring induced by μ if the following conditions are satisfied. For all A,B∈$\Re$,

i. λ^μ (A −B) ≤ max { λ^μ (A), λ^μ (B) } , ii. λ^μ (AB) ≤ max { λ^μ (A), λ^μ (B) }

Where λ^μ (A) = min{ μ(x) / for all x∈A ⊆ R }.

2.4 Definition

Let R be a ring. Let μ be a fuzzy ring on R and a nonempty set $\Re \subset 2^R - \{\phi\}$ is a HX ring. An intuitionistic fuzzy subset $\lambda^G = \langle A , \lambda^\mu(A) , \lambda^\eta(A) \rangle$ of a HX ring $\Re$ is said to be an intuitionistic fuzzy HX (IFHXSR) subring of $\Re$ if the following conditions are satisfied. For all A , B ∈$\Re$,

i. λ^μ(A−B) ≥ min{ λ^μ(A) , λ^μ(B) }, ii. λ^μ(AB) ≥ min{ λ^μ(A) , λ^μ(B) },

iii. λ^η(A−B) ≤ max{ λ^η(A) , λ^η(B) }, iv. λ^η(AB) ≤ max { λ^η(A) , λ^η(B) }

Where λ^μ(A) = max{ μ(x) / x∈A⊆R} , λ^η(A) = min{η(x) / x∈A⊆R}.

2.5 Definition

Let R be a ring. Let μ be an anti fuzzy ring on R and a nonempty set $\Re \subset 2^R - \{\phi\}$ is a HX ring. An intuitionistic fuzzy subset $\lambda^G = \langle A , \lambda^\mu(A) , \lambda^\eta(A) \rangle$ of a HX ring $\Re$ is said to be an intuitionistic anti fuzzy HX (IAFHXSR) subring of $\Re$ if the following conditions are satisfied. For all A , B ∈$\Re$,

i. λ^μ(A−B) ≤ max { λ^μ(A) , λ^μ(B) }, ii. λ^μ(AB) ≤ max { λ^μ(A) , λ^μ(B) },

iii. λ^η (A−B) ≥ min { λ^η (A) , λ^η (B) }, iv. λ^η (AB) ≥ min { λ^η (A) , λ^η (B) }

Where λ^μ(A) = min{ μ(x) / x∈A⊆R} , λ^η (A) = max{η (x) / x∈A⊆R}.

PROPERTIES OF AN INTUITIONISTIC ANTI FUZZY HX SUB MODULE

3.1. Definition

Let M be a HX module over a HX ring $\Re$.An intuitionistic fuzzy subset λ^G= { ⟨A, λ^μ(A), λ^η(A)⟩ / A∈M and 0≤λ^μ(A) + λ^η(A) ≤ 1} of M is called an intuitionistic fuzzy HX module if the following conditions are satisfied.

i. λ^μ (Q) =1, λ^η(Q) = 0 ii. λ^μ (A− B) ≥ min { λ^μ (A), λ^μ (B) } ,

iii. λ^η(A−B) ≤ max { λ^η(A) , λ^η(B) }, iv. λ^μ (ZA) ≥ λ^μ (A)

v. λ^η(ZA) ≤ λ^η(A) For all A,B∈M and Z∈$\Re$.

Where $\lambda^{\mu}(A) = \max\{ \mu(x) / \text{ for all } x \in A \subseteq M \}$ and $\lambda^{\eta}(A) = \min\{\eta(x) / \text{ for all } x \in A \subseteq M \}$.

3.2 Definition

Let M be a HX module over a HX ring $\Re$. An intuitionistic fuzzy subset $\lambda^G = \{ \langle A, \lambda^{\mu}(A), \lambda^{\eta}(A) \rangle / A \in M$ and $0 \le \lambda^{\mu}(A) + \lambda^{\eta}(A) \le 1\}$ of M is called an intuitionistic anti fuzzy HX module if the following conditions are satisfied.

i. $\lambda^{\mu}(Q) = 0, \lambda^{\eta}(Q) = 1$ ii. $\lambda^{\mu}(A - B) \le \max\{ \lambda^{\mu}(A), \lambda^{\mu}(B) \}$,

iii. $\lambda^{\eta}(A-B) \ge \min\{ \lambda^{\eta}(A), \lambda^{\eta}(B) \}$, iv. $\lambda^{\mu}(ZA) \le \lambda^{\mu}(A)$

v. $\lambda^{\eta}(ZA) \ge \lambda^{\eta}(A)$ For all $A,B \in M$ and $Z \in \Re$.

where $\lambda^{\mu}(A) = \min\{ \mu(x) / \text{ for all } x \in A \subseteq M \}$ and $\lambda^{\eta}(A) = \max\{\eta(x) / \text{ for all } x \in A \subseteq M \}$.

3.3. Theorem

Let $\lambda^G = \{ \langle A, \lambda^{\mu}(A), \lambda^{\eta}(A) \rangle / A \in M$ and $0 \le \lambda^{\mu}(A) + \lambda^{\eta}(A) \le 1\}$ be a IAFS in M, then λ^G is an IAFHXSM (intuitionistic anti fuzzy HX submodule) of M iff λ^G satiesfies the following conditions

i. $\lambda^{\mu}(Q) = 0, \lambda^{\eta}(Q) = 1$ ii. $\lambda^{\mu}(SA - TB) \le \max\{ \lambda^{\mu}(A), \lambda^{\mu}(B) \}$

iii. $\lambda^{\eta}(SA-TB) \ge \min\{ \lambda^{\eta}(A), \lambda^{\eta}(B) \}$. For all $A,B \in M$ and $S,T \in \Re$.

Where $\lambda^{\mu}(A) = \min\{ \mu(x) / \text{ for all } x \in A \subseteq M \}$ and $\lambda^{\eta}(A) = \max\{\eta(x) / \text{ for all } x \in A \subseteq M \}$.

Proof: Obvious from the definition.

3.4. Definition

Let $\lambda^G = \{ \langle A, \lambda^{\mu}(A), \lambda^{\eta}(A) \rangle / A \in M \}$ and $\gamma^H = \{ \langle A, \gamma^{\alpha}(A), \gamma^{\beta}(A) \rangle / A \in M \}$ and be any two intuitionistic fuzzy subsets of M. The intersection of λ^G and γ^H is defined as $\lambda^G \cap \gamma^H = \{\langle A, (\lambda^{\mu} \cap \gamma^{\alpha})(A), (\lambda^{\eta} \cup \gamma^{\beta})(A) \rangle / A \in M \}$.

3.5. Theorem

Let λ^G and γ^H be any two intuitionistic anti fuzzy HX sub modules of a HX module M then their intersection, $\lambda^G \cap \gamma^H$ is also an intuitionistic anti fuzzy HX sub modules of a HX module M .

Proof

Let $\lambda^G = \{ \langle A, \lambda^{\mu}(A), \lambda^{\eta}(A) \rangle / A \in M \}$ and $\gamma^H = \{ \langle A, \gamma^{\alpha}(A), \gamma^{\beta}(A) \rangle / A \in M \}$ be any two intuitionistic anti fuzzy HX sub modules of a HX module M. Then,

$\lambda^G \cap \gamma^H = \{ \langle A, (\lambda^{\mu} \cap \gamma^{\alpha})(A), (\lambda^{\eta} \cup \gamma^{\beta})(A) \rangle / A \in M \}$

Let $A,B \in M$ and $S,T \in \Re$.

i. $(\lambda^{\mu} \cap \gamma^{\alpha})(Q) = \min\{ \lambda^{\mu}(Q), \gamma^{\alpha}(Q) \}$

$= \min\{0,0\}$

$(\lambda^{\mu} \cap \gamma^{\alpha})(Q) = 0$

ii. $(\lambda^{\eta} \cup \gamma^{\beta})(Q) = \max\{ \lambda^{\eta}(Q), \gamma^{\beta}(Q) \}$

$= \max\{1,1\}$

$(\lambda^{\eta} \cup \gamma^{\beta})(Q) = 1$

iii. $(\lambda^{\mu} \cap \gamma^{\alpha})(SA-TB) = \min\{ \lambda^{\mu}(SA-TB), \gamma^{\alpha}(SA-TB)\}$

$\le \min\{ \max\{\lambda^{\mu}(A), \lambda^{\mu}(B)\}, \max\{ \gamma^{\alpha}(A), \gamma^{\alpha}(B)\}\}$

$= \max\{ \min\{\lambda^{\mu}(A), \gamma^{\alpha}(A)\}, \min\{\lambda^{\mu}(B), \gamma^{\alpha}(B)\}\}$

$(\lambda^{\mu} \cap \gamma^{\alpha})(SA-TB) \le \max\{ (\lambda^{\mu} \cap \gamma^{\alpha})(A), (\lambda^{\mu} \cap \gamma^{\alpha})(B)\}$.

iv. $(\lambda^{\eta} \cup \gamma^{\beta})(SA-TB) = \max\{ \lambda^{\eta}(SA-TB), \gamma^{\beta}(SA-TB)\}$

$\ge \max\{ \min\{\lambda^{\eta}(A), \lambda^{\eta}(B)\}, \min\{\gamma^{\beta}(A), \gamma^{\beta}(B)\}\}$

$= \min\{\max\{\lambda^{\eta}(A), \gamma^{\beta}(A), \}, \max\{\lambda^{\eta}(B), \gamma^{\beta}(B)\}\}$

$(\lambda^{\eta} \cup \gamma^{\beta})(SA-TB) \ge \min\{ (\lambda^{\eta} \cup \gamma^{\beta})(A), (\lambda^{\eta} \cup \gamma^{\beta})(B)\}$.

Hence, $\lambda^G \cap \gamma^H$ is an intuitionistic anti fuzzy HX sub module of a HX module M.

3.6 Definition

Let $\Re \subset 2^R - \{\phi\}$ be a HX ring of R. Let $\lambda^G = \{ \langle A, \lambda^\mu(A), \lambda^\eta(A)\rangle / A \in M \}$ and $\gamma^H = \{ \langle A, \gamma^\alpha(A), \gamma^\beta(A)\rangle / A \in M \}$ be any two intuitionistic fuzzy subsets of a HX ModuleM. The union of γ^G and λ^H is defined as, $\lambda^G \cup \gamma^H = \{ \langle A, (\lambda^\mu \cup \gamma^\alpha)(A), (\lambda^\eta \cap \gamma^\beta)(A)\rangle / A \in M \}$.

3.7 Theorem

Let λ^G and γ^H be any two intuitionistic anti fuzzy HX sub modules of a HX Module M then their union, $\lambda^G \cup \gamma^H$ is also an intuitionistic anti fuzzy HX sub module of a HX Module M.

Proof

Let $\lambda^G = \{ \langle A, \lambda^\mu(A), \lambda^\eta(A)\rangle / A \in M \}$ and $\gamma^H = \{ \langle A, \gamma^\alpha(A), \gamma^\beta(A)\rangle / A \in M \}$ and be any two intuitionistic anti fuzzy HX sub module of a HX Module M Then,

$\lambda^G \cup \gamma^H = \{ \langle A, (\lambda^\mu \cup \gamma^\alpha)(A), (\lambda^\eta \cap \gamma^\beta)(A)\rangle / A \in M \}$

Let $A,B \in M$ and $S,T \in \Re$.

i. $(\lambda^\mu \cup \gamma^\alpha)(Q) = \max\{ \lambda^\mu(Q), \gamma^\alpha(Q)\}$
$= \max\{0,0\}$
$(\lambda^\mu \cup \gamma^\alpha)(Q) = 0$

ii. $(\lambda^\eta \cap \gamma^\beta)(Q) = \min\{ \lambda^\eta(Q), \gamma^\beta(Q)\}$
$= \min\{1, 1\}$
$(\lambda^\eta \cap \gamma^\beta)(Q) = 1$

iii. $(\lambda^\mu \cup \gamma^\alpha)(SA-TB) = \max\{\lambda^\mu(SA-TB), \gamma^\alpha(SA-TB)\}$
$\leq \max\{ \max\{\lambda^\mu(A), \lambda^\mu(B)\}, \max\{\gamma^\alpha(A), \gamma^\alpha(B)\}\}$
$= \max\{\max\{\lambda^\mu(A), \gamma^\alpha(A)\}, \max\{\lambda^\mu(B), \gamma^\alpha(B)\}\}$
$= \max\{ (\lambda^\mu \cup \gamma^\alpha)(A), (\lambda^\mu \cup \gamma^\alpha)(B)\}$
$(\lambda^\mu \cup \gamma^\alpha)(SA-TB) \leq \max\{ (\lambda^\mu \cup \gamma^\alpha)(A), (\lambda^\mu \cup \gamma^\alpha)(B)\}$.

iv. $(\lambda^\eta \cap \gamma^\beta)(SA-TB) = \min\{\lambda^\eta(SA-TB), \gamma^\beta(SA-TB)\}$
$\geq \min\{ \min\{\lambda^\eta(A), \lambda^\eta(B)\}, \min\{\gamma^\beta(A), \gamma^\beta(B)\}\}$
$= \min\{\min\{\lambda^\eta(A), \gamma^\beta(A)\}, \min\{\lambda^\eta(B), \gamma^\beta(B)\}\}$
$= \min\{ (\lambda^\eta \cap \gamma^\beta)(A), (\lambda^\eta \cap \gamma^\beta)(B)\}$
$\lambda^\eta \cap \gamma^\beta)(SA-TB) \geq \min\{ (\lambda^\eta \cap \gamma^\beta)(A), (\lambda^\eta \cap \gamma^\beta)(B)\}$.

Hence, $\lambda^G \cup \gamma^H$ is an intuitionistic anti fuzzy HX sub module of a HX module M.

3.8 Definition

Let $\lambda^G = \{\langle A, \lambda^\mu(A), \lambda^\eta(A)\rangle / A \in M \}$ and $\gamma^H = \{ \langle A, \gamma^\alpha(A), \gamma^\beta(A)\rangle / A \in M \}$ be any two intuitionistic fuzzy subsets of a HX Module M, then an anti product of λ^G and γ^H is defined as $(\lambda^G \times \gamma^H) = \{\langle (A,B), (\lambda^\mu \cup \gamma^\alpha)(A,B), (\lambda^\eta \cap \gamma^\beta)(A, B)\rangle / (A, B) \in M_1 \times M_2\}$,

where, $(\lambda^\mu \cup \gamma^\alpha)(A, B) = \max\{\lambda^\mu(A), \gamma^\alpha(B)\}$, for all $(A, B) \in M_1 \times M_2$,
$(\lambda^\eta \cap \gamma^\beta)(A, B) = \min\{\lambda^\eta(A), \gamma^\beta(B)\}$, for all $(A, B) \in M_1 \times M_2$.

3.9 Theorem

If λ^G and γ^H are any two intuitionistic anti fuzzy HX sub modules of M_1 and M_2 respectively then, $\lambda^G \times \gamma^H$ is also an intuitionistic anti fuzzy HX sub module of a HX module $M_1 \times M_2$.

Proof

Let $\lambda^G = \{ \langle A, \lambda^\mu(A), \lambda^\eta(A)\rangle / A \in M_1 \}$ and $\gamma^H = \{ \langle A, \gamma^\alpha(A), \gamma^\beta(A)\rangle / A \in M_2 \}$ and be any two intuitionistic anti fuzzy HX sub modules of a HX module$M_1 \times M_2$respectively. Then,

$(\lambda^G \times \gamma^H) = \{\langle (A,B), (\lambda^\mu \cup \gamma^\alpha)(A,B), (\lambda^\eta \cap \gamma^\beta)(A, B)\rangle / (A, B) \in M_1 \times M_2\}$,

where, $(\lambda^{\mu}\cup\gamma^{\alpha})(A, B) = \max\{\lambda^{\mu}(A), \gamma^{\alpha}(B)\}$, for all $(A, B) \in M_1\times M_2$,

$(\lambda^{\eta}\cap\gamma^{\beta})(A, B) = \min\{\lambda^{\eta}(A), \gamma^{\beta}(B)\}$, for all $(A, B) \in M_1\times M_2$.

Let $A, B \in M_1\times M_2$, where $A = (C,D)$, $B = (E,F)$, $S,T \in \Re$

i. $(\lambda^{\mu}\cup\gamma^{\alpha})(SA-TB) = \max\{\lambda^{\mu}(SC-TE), \gamma^{\alpha}(SD-TF)\}$

$\leq \max\{\max\{\lambda^{\mu}(C), \lambda^{\mu}(E)\}, \max\{\gamma^{\alpha}(D), \gamma^{\alpha}(F)\}\}$

$= \max\{\max\{\lambda^{\mu}(C), \gamma^{\alpha}(D)\}, \max\{\lambda^{\mu}(D), \gamma^{\alpha}(E)\}\}$

$= \max\{(\lambda^{\mu}\cup\gamma^{\alpha})(C, D), (\lambda^{\mu}\cup\gamma^{\alpha})(E, F)\}$

$(\lambda^{\mu}\cup\gamma^{\alpha})(SA-TB) \leq \max\{(\lambda^{\mu}\cup\gamma^{\alpha})(A), (\lambda^{\mu}\cup\gamma^{\alpha})(B)\}$.

ii. $(\lambda^{\eta}\cap\gamma^{\beta})(SA-TB) = \min\{\gamma^{\beta}(SC-TE), \lambda^{\eta}(SD-TF)\}$

$\geq \min\{\min\{\gamma^{\beta}(C), \gamma^{\beta}(E)\}, \min\{\lambda^{\eta}(D), \lambda^{\eta}(F)\}\}$

$= \min\{\min\{\gamma^{\beta}(C), \lambda^{\eta}(D)\}, \min\{\gamma^{\beta}(E), \lambda^{\eta}(F)\}\}$

$= \min\{(\lambda^{\eta}\cap\gamma^{\beta})(C, D), (\lambda^{\eta}\cap\gamma^{\beta})(E, F)\}$

$(\lambda^{\eta}\cap\gamma^{\beta})(SA-TB) \geq \min\{(\lambda^{\eta}\cap\gamma^{\beta})(A), (\lambda^{\eta}\cap\gamma^{\beta})(B)\}$.

Hence, $\lambda^{G}\times\gamma^{H}$ is an intuitionistic anti fuzzy HX sub module of a HX module $M_1 \times M_2$.

3.10 Theorem

Let $\Re_1\subset 2^{R_1}-\{\phi\}$ and $\Re_2\subset 2^{R_2}-\{\phi\}$ be any two HX rings. If γ^{H} and λ^{G} are any two intuitionistic anti fuzzy HX sub modules of M_1 and M_2 respectively. Suppose that Q and Q^1 are identity elements of M_1 and M_2 respectively. If $\lambda^{G}\times\gamma^{H}$ is an intuitionistic anti fuzzy HX sub module of $M_1 \times M_2$, then atleast one of the following statements must hold.

i. $\gamma^{\alpha}(Q^1) \leq \lambda^{\mu}(A)$ and $\gamma^{\beta}(Q^1) \geq \lambda^{\eta}(A)$, for all $A \in M_1$

ii. $\lambda^{\mu}(Q) \leq \gamma^{\alpha}(B)$ and $\lambda^{\eta}(Q) \geq \gamma^{\beta}(B)$, for all $B \in M_2$.

Proof

Let $\lambda^{G} = \{\langle A, \lambda^{\mu}(A), \lambda^{\eta}(A)\rangle / A\in M_1\}$ and $\gamma^{H} = \{\langle A, \gamma^{\alpha}(A), \gamma^{\beta}(A)\rangle / A\in M_2\}$ be any two intuitionistic anti fuzzy HX sub modules of a HX module M_1 and M_2 respectively. Then,

$(\lambda^{G}\times\gamma^{H}) = \{\langle(A,B),(\lambda^{\mu}\cup\gamma^{\alpha})(A,B), (\lambda^{\eta}\cap\gamma^{\beta})(A, B)\rangle /(A, B) \in M_1\times M_2\}$,

where, $(\lambda^{\mu}\cup\gamma^{\alpha})(A, B) = \max\{\lambda^{\mu}(A), \gamma^{\alpha}(B)\}$, for all $(A, B) \in M_1\times M_2$,

$(\lambda^{\eta}\cap\gamma^{\beta})(A, B) = \min\{\lambda^{\eta}(A), \gamma^{\beta}(B)\}$, for all $(A, B) \in M_1\times M_2$.

Let $A, B \in M_1 \times M_2$, where $A = (C,D)$, $B = (E,F)$,

Let $\lambda^{G}\times\gamma^{H}$ be an intuitionistic anti fuzzy HX sub module of $M_1\times M_2$. By contraposition, suppose that none of the statements (i) and (ii) holds then we can find $A \in M_1$ and $B\in M_2$ such that

i. $\gamma^{\alpha}(Q') \leq \lambda^{\mu}(A)$ and $\gamma^{\beta}(Q') \geq \lambda^{\eta}(A)$, for all $A \in M_1$

ii. $\gamma^{\alpha}(Q) \leq \lambda^{\mu}(B)$ and $\gamma^{\beta}(Q) \geq \lambda^{\eta}(A)$, for all $B\in M_2$.

We have, $(\lambda^{\mu}\cup\gamma^{\alpha})(A, B) = \max\{\lambda^{\mu}(A), \gamma^{\alpha}(B)\}$

$> \max\{\lambda^{\mu}(Q),\gamma^{\alpha}(Q')\}$

$= (\lambda^{\mu}\cup\gamma^{\alpha})(Q, Q')$

$(\lambda^{\mu}\cup\gamma^{\alpha})(A, B) > (\lambda^{\mu}\cup\gamma^{\alpha})(Q, Q')$.

Also, $(\lambda^{\eta}\cap\gamma^{\beta})(A, B) = \min\{\lambda^{\eta}(A), \gamma^{\beta}(B)\}$

$< \min\{\lambda^{\eta}(Q), \gamma^{\beta}(Q')\}$

$= (\lambda^{\eta}\cap\gamma^{\beta})(Q, Q')$.

$(\lambda^{\eta}\cap\gamma^{\beta})(A, B) < (\lambda^{\eta}\cap\gamma^{\beta})(Q, Q^1)$.

Thus, $\lambda^{G}\times\gamma^{H}$ is not an intuitionistic anti fuzzy HX sub module of $M_1\times M_2$.

Hence, either $\gamma^{\alpha}(Q') \geq \lambda^{\mu}(A)$ and $\gamma^{\beta}(Q') \leq \lambda^{\eta}(A)$, for all $A \in M_1$

Or $\lambda^{\mu}(Q) \geq \gamma^{\alpha}(B)$ and $\lambda^{\eta}(Q) \leq \gamma^{\beta}(B)$, for all $B\in M_2$.

3.11 Definition

Let G be an intuitionistic fuzzy set of R. Let $\Re \subset 2^R - \{\phi\}$ be a HX ring. Let λ^G be an intuitionistic fuzzy set of a HX module M. We define the following "necessity" and possibility" operations:

$\Box\lambda^G = \{ \langle A, \lambda^\mu(A), 1-\lambda^\mu(A) \rangle / A \in M\}$

$\Diamond\lambda^G = \{ \langle A, 1-\lambda^\eta(A), \lambda^\eta(A) \rangle / A \in M\}$.

3.12 Theorem

Let λ^G be an intuitionistic anti fuzzy HX sub module of a HX module M then $\Box\lambda^G$ is an intuitionistic anti fuzzy HX sub module of a HX module M

Proof

Let λ^G be an intuitionistic anti fuzzy HX sub module of a HX Module M. Then for A,B∈M and S,T∈ℜ,

i. $\lambda^\mu(Q) = 0, \quad \lambda^\eta(Q) = 1$

ii. $\lambda^\mu(SA - TB) \leq \max\{\lambda^\mu(A), \lambda^\mu(B)\}$,

iii. $\lambda^\eta(SA - T B) \geq \min\{\lambda^\eta(A), \lambda^\eta(B)\}$.

We have, $\lambda^\mu(Q) = 0$

$1-\lambda^\mu(Q) = 1 - 0 = 1$

That is, $1-\lambda^\mu(Q) = 1$

Now, $\lambda^\mu(SA - TB) \leq \max\{\lambda^\mu(A), \lambda^\mu(B)\}$

$1 - \lambda^\mu(SA - TB) \geq 1 - \max\{\lambda^\mu(A), \lambda^\mu(B)\}$

$\geq \min\{1 - \lambda^\mu(A), 1 - \lambda^\mu(B)\}$.

That is, $1-\lambda^\mu(SA - TB) \geq \min\{1 - \lambda^\mu(A), 1 - \lambda^\mu(B)\}$.

Hence, $\Box\lambda^G$ is an intuitionistic anti fuzzy HX sub module of a HX module M.

3.13 Theorem

Let λ^G be an intuitionistic anti fuzzy HX sub module of a HX module M then $\Diamond\lambda^H$ is an intuitionistic anti fuzzy HX sub module of a HX module M

Proof

Let λ^G be an intuitionistic anti fuzzy HX sub module of a HX module M. Then,

i. $\lambda^\mu(Q) = 0, \quad \lambda^\eta(Q) = 1$

ii. $\lambda^\mu(SA - TB) \leq \max\{\lambda^\mu(A), \lambda^\mu(B)\}$,

iii. $\lambda^\eta(SA - TB) \geq \min\{\lambda^\eta(A), \lambda^\eta(B)\}$.

We have, $\lambda^\eta(Q) = 1$

$1-\lambda^\eta(Q) = 1 - 1 = 0$

That is, $1-\lambda^\eta(Q) = 0$.

Now, $\lambda^\eta(SA - TB) \geq \min\{\lambda^\eta(A), \lambda^\eta(B)\}$

$1 - \lambda^\eta(SA - TB) \leq 1 - \min\{\lambda^\eta(A), \lambda^\eta(B)\}$

$\leq \max\{1 - \lambda^\eta(A), 1 - \lambda^\eta(B)\}$.

That is, $1-\lambda^\eta(SA - TB) \leq \max\{1 - \lambda^\eta(A), 1 - \lambda^\eta(B)\}$.

Hence, $\Diamond\lambda^H$ is an intuitionistic antifuzzy HX submodule of a HX module M.

ACKNOWLEDGEMENT

The authors would like to express their sincere thanks to all our friends for their help to make this paper a successful one.

REFERENCES

1. Atanassov. K.T, intuitionistic fuzzy sets, Fuzzy Sets and System 20 (1986) ,pp 87-96.
2. Bing-xueYao and Yubin-Zhong, Upgrade of algebraic structure of ring, Fuzzy information and Engineering (2009)2:219-228.
3. Bing-xueYao and Yubin-Zhong, The construction of power ring, Fuzzy information and Engineering (ICFIE), ASC 40,pp.181-187,2007.
4. Li Hong Xing, HX group, BUSEFAL, 33(4), 31-37, October 1987.
5. Li Hong Xing, HX ring, BUSEFAL, 34(1) 3-8, January 1988.
6. Muthuraj. R, Muthuraman. M.S., Intuitionistic fuzzy HX ring, IOSR Journal of Mathematics (IOSR-JM) Volume 10, Issue 4,Ver II , 3-12 (Jul-Aug 2014).
7. Muthuraj. R and Muthuraman. M.S, Intuitionistic anti fuzzy semiprimary HX ideal in HX ring, International journal of applied engineering research, 2015, vol.10, no.39, pp.30050 – 30056.
8. Muthuraj. R and Muthuraman. M.S, Intuitionistic fuzzy HX k – ideal of a semi HX ring, SS International journal of pure and applied mathematics, 2015, pp. 31-41.
9. Paul Isaac, Pearly P. John, "On Intuitionistic Fuzzy Submodules of a Module",Int. J. of Mathematical Sciences and Applications, Vol. 1, No. 3, 2011, 1447-1454.
10. Rosenfeld. A., Fuzzy groups,J.Math. Anal., 35(1971), 512-517.
11. Zadeh. L.A., Fuzzy sets, Information and control, 8, 338-353.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

An Investigation of ATR-FTIR Compatibility Studies and Preformulation Studies of Tapentadol HCl to Design and Formulate Transdermal Proniosomal Gel

G.Sangeetha[1]*, M. Swamivel Manickam[2] and P. Sanil kumar[3]

[1]Department of Pharmaceutics, Krupanidhi College of pharmacy, Bangalore, Karnataka, India.
[2]Department of Pharmacy, Annamalai University, Chidambaram, Tamil Nadu, India.
[3]Accenture Pharmaceuticals, Bangalore, Karnataka, India.

Received: 21 Dec 2020 Revised: 27 Dec Accepted: 30 Dec 2020

***Address for Correspondence**
G. Sangeetha
Department of Pharmaceutics,
Krupanidhi College of pharmacy,
Bangalore, Karnataka, India.
Email: sange2008@gmail.com

ABSTRACT

Preformulation studies are the utmost substantial screening step for the development of drug products in the right therapeutic area and characterizing the physicochemical properties of drug substances; also it offers the crucial information for the candidate selection. The proposed formulation of the Tapentadol Hydrochloride-loaded proniosomal gel through transdermal drug delivery system helps to avoid poor oral bioavailability due to extensive first-pass metabolism. The present study was designed to assess the appropriateness of Tapentadol Hcl to design as proniosomal gel through transdermal delivery system.An experimental study was done to characterize the physicochemical aspects of Tapentadol Hydrochloride which entails spectrophotometric analysis, solubility analysis, melting point, partition coefficient and their compatibility with the selected excipients. Attenuated total reflectance-Fourier transform infrared (ATR-FTIR) technique was used to identify any interactions between the different non-ionic surfactants and the drug, both as individual components and as physical mixtures. The results obtained for solubility, melting point and partition coefficients of Tapentadol HCl studied were satisfactory. The IR spectra of Tapentadol HCl showed characteristic peaks at 3400 cm^{-1} (-OH stretching in alcohol), 1400-1600 cm^{-1} (C=C in an aromatic ring), 878 cm^{-1}, 797 cm^{-1} (C-H bending in an aromatic ring) and 1178 cm^{-1} (C-N stretching) and these characteristic peaks were typically shown in all physical mixtures studied. As the investigational spectra obtained corroborates no significant interaction in characteristic peaks of the drug after mixing, the strategy to formulate Tapentadol hydrochloride as a proniosomal drug delivery system will be promisingly successful.

Keywords: ATR-FTIR, Non-ionic surfactant, Partition co-efficient, Preformulation studies, Proniosomal gel, Tapentadol HCl

INTRODUCTION

Preformulation studies are intended to characterize the physical-chemical properties of the New chemical/molecule/biological entity that enables the formulation scientist to design an appropriate dosage form for those new drug substances. The development of dosage form is a multi-step process that involves complexity and are time consuming. An improper strategy in the development of dosage form would make the dosage form unsatisfactory in meeting the prerequisite safety, efficacy and stability, therefore it's essential to develop a reliable and accurate scientific strategy that ensures a coherent work flow in the experimentation of achieving the desired / set experimental objectives. The development and validation of a stable formulation is ensued upon the preformulation study in characterizing the suitability of the chosen drug candidate that reflects at all the subsequent stages of formulation development. Prior to the development of a final dosage form for any medicinal entity, preformulation studies are appraised to acquire cognizance on the physico-chemical properties of the medicinal entity that enables the formulation scientist in selecting the proper drug candidates and suitable excipients for the desired dosage form, by investigating the possible interactions between the chosen drug and excipients if any, and their compatibility in the development of a stable formulation. Preformulation studies also make an apparent outline pertaining to preparation, manufacture and storage conditions of the developing dosage form. The preliminary characterization of the drug substances serves to be a basic rationale in the formulation development. Preformulation study can be simply described as "learning before doing" [1].

The present study emphasizes on the preformulation studies involved in the design of pro-vesicular drug delivery system that included the identity and purity of the drug, solubility studies, melting Point, partition coefficient, compatibility studies, drug-excipient interactions, and development of a reliable, selective and precise spectroscopic method for measuring the drug concentration and purity [2].ATR-FTIR spectroscopy was involved to ascertain on any possible weak intermolecular interactions that can exist between any two substances when intimately mixed.Prior formulating a stable dosage form such interactions if any have to be ascertained in order to make a rational combination of drug and excipients.Tapentadol 3-[((1R, 2R)-3- (dimethylamino)-1- ethyl-2-methylpropyl) phenol] hydrochloride is a non-racemic molecule, approved by FDA on Nov 20, 2008 and is prescribed as analgesic in the relief of moderate to severe pain [3,4].Tapentadol hydrochloride (Figure 1) is a centrally acting analgesic, that adopts the dual mechanism, namely as an μ-opioid receptor agonist and as an noradrenaline reuptake inhibitor [5].Tapentadol hydrochloride is effective against inflammation, visceral, nociceptive and neuropathic conditions [6].According to WHO treatment guidelines, the three step pain ladder escalates the therapy from NSAIDs to opioid analgesics[7].The conditions of mild to moderate pain are not often supported with NSAIDs; in such conditions potent opioid analgesics were recommended amidst the loathe of the physicians to prescribe them since opioids prones for drug abuse, adverse effects, tolerance, withdrawal and liability [8].This experimental study is emphasized with an objective of formulation and development of Tapentadol hydrochloride loaded proniosomal gel to be delivered through skin portal for pain management. As a part to succeed in the successful formulation of a stable proniosomal gel, an investigation of the various preformulation parameters bound to the formulative aspects was studied.

MATERIALS AND METHODS

Tapentadol HCl was obtained as a gift from Symed Labs Ltd, Hyderabad. Cholesterol and lecithin were procured from Hi-Media Pvt Ltd, Mumbai. Span 40 and Tween 40 was received as a free sample from Mohini Organics Pvt Ltd, Mumbai. Span 60 and 80, Tween 60 and 80 were procured from S.D fine chemicals, Mumbai. Kolliphor RH 40, Capmul MCM and Labrafil M2125 CS were procured from BASF, India. All other chemicals used were of Analytical grade.

Analytical Method

Scanning of drug (Determination of λ_{max}): 10 µg/ml of Tapentadol Hydrochloride solution was prepared using both water and phosphate buffer pH 7.4 separately. These prepared drug solutions were scanned in the UV wavelength ranging between 200 to 400 nm (Shimadzu, UV-1800 spectrophotometer, Japan). The UV spectra obtained shows a maximum absorbance at 272 nm for Tapentadol Hydrochloride, irrespective of the media used in their dissolution.

Calibration curve for the estimation of Tapentadol Hydrochloride in water

Preparation of standard stock solution: A standard stock solution of concentration 1 mg/ml was prepared by accurately weighing 100 mg of Tapentadol HCl in a 100 ml volumetric flask. The drug was dissolved in a sufficient quantity of water and is diluted with further volumes of water up to the final volume.

Preparation of standard graph: The standard stock solution was appropriately diluted with water to obtain a series of dilutions to contain 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml of the drug. The absorbance of these dilutions was measured using a UV spectrophotometer at 272nm using water as blank. A graphical plot of the absorbance versus the concentration of Tapentadol Hydrochloride was constructed to yield a straight line by adopting linear regression analysis of the obtained absorbance data points. A straight line equation (Y = mx + c) was generated whose slope and intercept values are used to deduce the amount of the drug. Three standard samples (20, 60, and 100 µg/ml) were considered and is subjected to intra-day and inter-day precision and accuracy studies.

The calibration curve of Tapentadol HCl in Phosphate buffer pH 7.4

Preparation of Phosphate buffer 7.4 (PBS): 2.38 g of disodium hydrogen phosphate, 0.19 g of potassium dihydrogen phosphate and 8 g of sodium chloride was dissolved in distilled water and then final volume was made up to 1000 ml with distilled water. The desired pH was adjusted by using either hydrochloric acid or sodium hydroxide [9].

Preparation of standard stock solution: 100 mg of Tapentadol Hydrochloride was accurately weighed into 100 ml volumetric flask. The drug was dissolved and diluted to final volume with phosphate buffer pH 7.4. so as to get the concentration of 1 mg/ml.

Preparation of standard graph: The standard stock solution was appropriately diluted with Phosphate buffer pH 7.4 to obtain a series of dilutions to contain 10, 20, 30, 40, 50, 60, 70, 80, 90 and 100 µg/ml of the drug. The absorbance of these dilutions was measured using a UV spectrophotometer at 272nm using Phosphate buffer pH 7.4 as blank. A graphical plot of the absorbance versus the concentration of Tapentadol Hydrochloride was constructed to yield a straight line by adopting linear regression analysis of the obtained absorbance data points. A straight line equation (Y = mx + c) was generated whose slope and intercept values are used to deduce the amount of the drug. Three standard samples (10, 40, and 50 µg/ml) were considered and is subjected to intra-day and inter-day precision and accuracy studies.

Solubility Analysis

Saturation solubility in solvents: An excess amount of drug was dissolved in measured amounts of distilled water, ethanol and PBS, pH 7.4 separately in three separate wide mouthed test tubes until a saturated solution ensue. The solution was vigorously stirred for 24 h at a constant temperature to achieve equilibrium with the undissolved particles. The solution was filtered, the filtrate was diluted with respective solvents and concentration was measured using a UV spectrophotometer at 272 nm. An average of triplicate reading was taken.

Saturation solubility in surfactants: An excess amount of drug was dissolved in measured amounts of surfactants (Tween 40,Tween 60,Tween 80,Span 20, and Span 80) until a saturated solution ensue. The solution was vigorously stirred on rotary flask shaker for 24h at constant temperature (28±1 °C) until an equilibrium is attained with the undissolved particles. The solution was filtered, and the filtrate was diluted with the respective solvents. These solutions were scanned in a UV spectrophotometer at 272 nm, and the corresponding absorbance values are measured to determine the concentration of the solution. An average of triplicate reading was taken[10].

Determination of Melting Point: The melting point was determined by using the Microcontroller based Melting Point Apparatus (Chemi Line). A capillary tube filled with Tapentadol Hydrochloride is placed into the apparatus by accommodating the filled capillary tube inside the glass chamber containing the heating coil. When the apparatus is switched on, the heating coil works and the temperature of the glass chamber raises up. At a particular temperature the drug placed in the capillary tube melts up and this temperature was noted. The same procedure is repeated for four times in order to ascertain the precision in the measured melting point temperature.

Partition coefficient: The partition coefficient value of a drug accounts of the lipophilicity/hydrophilicity nature of the drug that can correlate the extent of permeability of the drug through the bio-membrane. Partition coefficient was determined using a separatory funnel containing a mixture of equal volume of 30 ml of each Octanol and PBS, pH 7.4. The Separatory funnel was shaken vigorously until a turbid appears. 10 mg of Tapentadol hydrochloride was added into the turbid mixture of the separatory funnel and were shaken continuously with frequent release of pressure for 24 h at room temperature. The mixture was allowed to stand for 3 h or until the two phases separates completely; both the phases were clarified by centrifugation and the drug content dispersed in each phase was measured using a UV spectrophotometer at 272 nm. The same experimental method was repeated using a mixture of equal volumes of Octanol and water as two phase system. The Experimental runs were made in triplicate[11].

Identification of drug: Tapentadol hydrochloride was analyzed in FTIR and the obtained IR spectrum was matched for the characteristic peaks in comparison with the reference spectrum of Tapentadol hydrochloride IP[12].

ATR-FTIR Compatibility studies: ATR-FTIR spectrum reveals information on the presence / absence of specific functional groups of the material studied, thereby accounts of interactions, if any, between the drug and other excipients. The drug, excipients such as lecithin, cholesterol, Span 60, Tween 80, Capmul MCM, Labrafil M2125cs, Kolliphore RH 40 and its mixtures prepared with an equimolar (1:1) ratio, were stored separately in a hermetically sealed container and examined physically for any deleterious changes throughout a period of one month. After a month the same samples were analyzed using ATR-FTIR (Shimadzu IR Spirit). The QATR-S single bounce ATR accessory has been designed specifically for the IR Spirit with easily user-swappable diamond and germanium crystals. The sample was on the prism (Diamond) and the spectrum was run in the wave number range of 400-4000 cm^{-1}. The spectra has been recorded using Lab Solutions, IR software [13].

RESULTS AND DISCUSSION

Calibration curve of Tapentadol Hydrochloride

Linearity: The linearity of Tapentadol hydrochloride was found in the range of 10-100 µg/ml in water and 5-50 µg/ml in phosphate buffer pH 7.4 with correlation coefficient 0.9981 and 0.9996 respectively. Results are shown in Table 1 and 2, Figures2 and 3.

Accuracy: The mean percentage recovery was 101.11% of phosphate buffer and 103.06% of water, as shown in Table 3.

Precision: Intra-day and inter-day precision were determined by analyzing three different solutions of Tapentadol HCl sampled within the same day and on three different days over a period of a week. Precision was calculated as intra and interday variations for Tapentadol hydrochloride in both water and phosphate buffer pH 7.4 (% RSD is less than 2), shown in Table4 and 5.

Limit of Detection (LOD) & Limit of Quantification (LOQ): LOD was found to be 1.485 for phosphate buffer and 1.375 for water. LOQ was found to be 4.5 and 4.2 for phosphate buffer and water respectively (Table 6).

Solubility Analysis: Saturation solubility of Tapentadol HCl in various solvents and surfactants was studied with an aim to choose the appropriate solvent or surfactant based on their solubility profiles in the respective media to formulate the proniosomal gel. The solvents used for solubility study include water, ethanol and phosphate buffer saline pH 7.4. Tapentadol HCl was freely soluble in almost all the solvents used for the study. The highest solubility was reported for ethanol as solvent, the solubility being 823 mg/ml. Solubility profiles for various solvents studied were illustrated in Figure 4. The surfactants used in solubility studies includes Tween 40, Tween 60, Tween 80, Span 20 and Span 80. The higher solubility of Tapentadol HCl was observed to be in correlation with higher grades of surfactants. The graphical representation of the solubility profile for surfactants has shown in Figure 5.

Partition co-efficient: The partition coefficient was found to be 2.96 (observed value) and the reported value, was 2.86 [14]. Hence, the drug was suitable for transdermal drug delivery.

Identification of drug by ATR-FTIR: The FTIR spectrum of Tapentadol hydrochloride was compared with a reference spectrum given in Indian Pharmacopoeia, 2018. The spectra obtained for the experimental sample matched similar to the reference spectra published in IP 2018. The characteristic peaks exhibited were of the same intensity and the corresponding wave numbers of the peak specific for the functional groups were observed to be the same as shown in the reference spectrum. The FTIR spectrum of pure Tapentadol hydrochloride is shown in Figure 6.

Drug – excipients compatibility studies by ATR-FTIR spectroscopy

The success of any formulation developed depends on the selection of the ideal excipients. The present work was attempted to screen the drug and excipients and to investigate the compatibility between the drug candidate and the excipients such as lecithin, cholesterol, Span 60, Tween 80, Capmul MCM, Labrafil M2125cs, Kolliphore RH 40, thereby develop the best ideal formulae to formulate proniosomal gel of Tapentadol Hcl intended for delivery via the skin portal. The FTIR study was conducted for the drug, the excipients individually [Figure 7] and for the physical mixtures of each excipient with the drug [Figure8]. The characteristic peak of individual compounds and physical mixtures were presented in Table 7 and 8.The significant peaks observed in pure Tapentadol Hcl that were characterized at 3400 cm^{-1} (-OH stretching), 1400-1600 cm^{-1} (C=C in an aromatic ring), 700 cm^{-1} (benzene derivative) were found to exhibit the significant peaks alike in all physical mixtures. The ATR-FTIR study disclosed that the

significant peak of the drug exhibited in the characteristic wave number was reproduced typically to be the same in all the physical mixtures without any identifiable shift in the characteristic peaks, thereby ensuing no interactions have occurred between the drug and excipients mixture studied.

CONCLUSION

The research work done in the preformulation perspective on the selected drug and excipients clearly disclosed on the feasibility of designing a proniosomal gel loaded opioid analgesic Tapentadol HCl. The fabrication of Tapentadol HCl loaded proniosomal gel as transdermal drug delivery system will be a step forward strategy in the effective management of pain. Preformulation studies such as solubility, Melting point and partition coefficient were ensued for Tapentadol Hcl to ensure on the outcome that is rationale in the development of a stable dosage form. The results obtained in these experimental trials were encouraging and also substantiates that the intended formulation could be devised successfully for the chosen drug and excipients. The spectrophotometric method of analysis developed for Tapentadol Hcl at λmax 272 nm was found to be reproducible, highly sensitive and also complies with all the method validation parameters ascribed viz., linearity, precision and accuracy. The correlation coefficient value obtained for Tapentadol HCl signifies that the proposed UV spectroscopy obey on Beer- Lambert's law. ATR-FTIR analysis of drug and excipients both as individual components and as physical mixtures in equimolar ratios was studied. The study authenticated that there wasn't any chemical interactions between the Tapentadol HCl and the other excipients chosen such as lecithin, cholesterol, span 60, tween 80, Capmul MCM, Labrafil M 2125cs and Kolliphore RH 40. Therefore, the present study adjudicates that the selected drug candidate and excipients were promising for the development of proniosomal gel loaded opioid analgesic fabricated as a transdermal drug delivery system.

ACKNOWLEDGEMENT

The authors are grateful to Symed labs, Hyderabad, for their generous contribution of drug.

CONFLICT OF INTEREST

There is no conflict of interest for this research work.

REFERENCES

1. Jones TM. Preformulation studies. In: Tovey GD, editor. Pharmaceutical Formulation: The Science and Technology of Dosage Forms. Croydon, UK: The Royal Society of Chemistry; 2018. p. 1-19.
2. Lundqvist T, Bredenberg S. Pharmaceutical Development. In: R G Hill, H P Rang, editors. Drug Discovery and Development- Technology in Transition. Edindburgh, Churchull Livnstone: Elsevier; 2013. p. 227-238.
3. http://drugcentral.org/drugcard/4283 (Last accessed on 7/6/2020)
4. https://www.accessdata.fda.gov/drugsatfda_docs/label/2010/022304s003lbl.pdf (Last accessed on 7/6/2020)
5. Knezevic NN, Tverdohleb T, Knezevic I, Candido KD. Unique pharmacology of tapentadol for treating acute and chronic pain. Expert Opin Drug Metab Toxicol. 2015;11: 1475-92.
6. Millan MJ. Descending control of pain. Prog Neurobiol. 2002; 66: 355-474.
7. World Health Organization. Cancer Pain Relief. Geneva, Switzerland: World Health Press; 1986.
8. Hatrick CT. Tapentadol immediate release for the relief of moderate-to-severe acute pain. Expert Opin Pharmacother. 2009;10:2687- 96.
9. Indian Pharmacopoeia 2014. Volume I. Ghaziabad: The Indian Pharmacopoeia Commission; 2014.Buffer solution; 760.
10. Jain A, Ghosh B, Rajgor N, Desai BG. Passive and iontophoretic permeation of glipizide. Eur J Pharm Biopharm. 2008;69: 958-63.

11. Ghosh S, Cramp GL, Coupland JN. Effect of aqueous composition on the freeze-thaw stability of emulsions. Colloids Surf A Physicochem Eng Asp. 2006; 272: 82-8.
12. Indian Pharmacopoeia 2018. Volume III. Ghaziabad: The Indian Pharmacopoeia Commission; 2018. Infra-red reference spectra; 676.
13. Khan MI, Madni MA, Ahmed S, Khan A. ATR-FTIR Based Pre and Post Formulation Compatibility studies for the Design of Niosomal Drug Delivery System Containing Non-ionic Amphiphiles and Chondro protective Drug. J Chem Soc Pak. 2015;37:527-34.
14. https://www.accessdata.fda.gov/drugsatfda_docs/nda/2011/200533Orig1s000ClinPharmR.pdf (Last accessed on 26/6/2020)

Table 1: Linearity of Tapentadol Hcl in phosphate buffer pH 7.4 and water

Phosphate buffer pH 7.4		**Water**	
Concentration (µg/ml)	Absorbance	Concentration (µg/ml)	Absorbance
5	0.102	10	0.097
10	0.134	20	0.166
15	0.163	30	0.225
20	0.204	40	0.293
25	0.233	50	0.335
30	0.253	60	0.415
35	0.283	70	0.484
40	0.316	80	0.541
45	0.353	90	0.604
50	0.379	100	0.645

Table 2: Results of linearity

Features	**Water**	**Phosphate buffer pH 7.4**
Regression equation	Y= 0.006x+0.039	Y= 0.006x+0.073
Correlation coefficient	R^2=0.997	R^2=0.997
Slope	0.006	0.006
Y-intercept	0.039	0.073
Beer's range	10-100 µg/ml	5-50 µg/ml

Table 3: Accuracy of Tapentadol Hcl in water and phosphate buffer pH 7.4

Phosphate buffer pH 7.4				**Water**			
Concentration (µg/ml)	Recovery	Mean % recovery	Mean (%)	Concentrations (µg/ml)	Recovery	Mean % recovery	Mean (%)
10	98.3	102.2	101.11	20	106.6	104.2	103.06
	103.3				105.0		
	96.7				100.8		
	108.3				104.1		
	101.7				105.8		
	105				102.5		
	101.1			60	104.4	104.1	
	98.9				103.3		
	98.3				103.8		

30	100.5	99.3			104.7		
	97.7				104.1		
	101.6				105.2		
50	103.3	102.05		100	101.3	100.9	
	99.3				101.0		
	100.6				100.7		
	102.0				101.5		
	105.3				100.3		
	101.7				100.8		

Table 4: Intraday precision analysis

Phosphate buffer pH 7.4		**Water**	
Intraday precision		Intraday precision	
Injection (30 mcg/ml)	Absorption	Injection (50 mcg/ml)	Absorption
1	0.232	1	0.337
2	0.231	2	0.335
3	0.234	3	0.334
4	0.229	4	0.336
5	0.237	5	0.331
6	0.232	6	0.338
Mean	0.2325	Mean	0.3351
CV	0.01178	CV	0.0074
SD	0.002739	SD	0.002483
%RSD	1.177898	%RSD	0.741056
% RSD was found to be <2			

Table 5: Interday precision analysis

Interday precision (phosphate buffer pH 7.4)										
	5 µg/ml	10 µg/ml	15 µg/ml	20 µg/ml	25 µg/ml	30 µg/ml	35 µg/ml	40 µg/ml	45 µg/ml	50 µg/ml
Day 1	0.105	0.135	0.162	0.203	0.233	0.251	0.281	0.318	0.354	0.383
Day 2	0.101	0.131	0.169	0.201	0.231	0.25	0.284	0.315	0.351	0.371
Day 3	0.103	0.132	0.163	0.205	0.234	0.254	0.283	0.311	0.353	0.375
Mean	0.103	0.133	0.165	0.203	0.233	0.252	0.283	0.315	0.353	0.376
SD	0.002	0.0020	0.0037	0.002	0.0015	0.0020	0.0015	0.0035	0.0015	0.0061
%RSD	1.9	1.5	2.0	1.0	0.6	0.8	0.5	1.1	0.4	1.6
Interday precision (water)										
	10 µg/ml	20 µg/ml	30 µg/ml	40 µg/ml	50 µg/ml	60 µg/ml	70 µg/ml	80 µg/ml	90 µg/ml	100 µg/ml
Day 1	0.097	0.167	0.225	0.292	0.337	0.415	0.487	0.541	0.602	0.647
Day 2	0.099	0.165	0.222	0.295	0.335	0.411	0.481	0.542	0.601	0.645
Day 3	0.096	0.162	0.225	0.291	0.334	0.413	0.485	0.538	0.605	0.643
Mean	0.097	0.164	0.224	0.293	0.335	0.413	0.484	0.540	0.602	0.645
SD	0.0015	0.0025	0.0017	0.0020	0.0015	0.002	0.0030	0.0020	0.0020	0.002
%RSD	1.6	1.5	0.8	0.7	0.5	0.5	0.6	0.4	0.3	0.3
% RSD was found to be <2										

Table 6: Limit of Detection and Limit of Quantification

Parameters	Phoaphate bufer pH7.4	Water
SD	0.0027	0.0025
Slope	0.006	0.006
LOD	1.485	1.375
LOQ	4.5	4.2

Table 7: Characteristics peaks of individual compounds

Individual compounds	Functional groups	Characteristic peaks (wave number)
Tapentadol Hydrochloride	-O-H stretching	3400 cm^{-1}
	-C=C in an aromatic ring	1400-1600 cm^{-1}
	-C-H bending in an aromatic ring	878 cm^{-1}, 797 cm^{-1}
	-C-O stretching	1178 cm^{-1}
	-C-N stretching	1259 cm^{-1}
	-CH_2 bending	1454 cm^{-1}
	-N-H stretching (tertiary amine)	2682 cm^{-1}
Lecithin	Symmetric CH_2	2854 cm^{-1}
	Asymmetric CH_2	2928 cm^{-1}
	CH_3 stretching	2956 cm^{-1}
	-C=O stretching	1736 cm^{-1}
	PO_2 vibration	1200 cm^{-1}
Cholesterol	-OH bond vibration	3421 cm^{-1}
	Acetyl group	2931 cm^{-1}
	Symmetric -CH3	2866 cm^{-1}
	Vinyl group	1770 cm^{-1}
	C=C vibration	1461 cm^{-1}
	-OH bending	1371 cm^{-1}
Span 60	-OH group	2916 cm^{-1}
	-CH3 group	1467 cm^{-1}
	Cyclic 5-membered ring	1734 cm^{-1}
	Aliphatic chain	1000-1200 cm^{-1}
Tween 80	-OH group	3488 cm^{-1}
	-CH3 group	1457 cm^{-1}
	-C-O-C- acyclic	1097 cm^{-1}
Capmul MCM	C-H stretch alkanes	2928 cm^{-1}
	C=O stretch esters	1735 cm^{-1}
	C-H bend	1466 cm^{-1}
	C-O stretch in alcohol	1150 cm^{-1}
Labrafil M2125cs	O-H bend carboxylic group	950 cm^{-1}
	N-H bend primary amine	1650 cm^{-1}
Kolliphore RH 40	C≡O stretch in esters	1735 cm^{-1}
	C≡C stretch in alkanes	1635 cm^{-1}
	C-H bend in alkanes	1466 cm^{-1}
	C-H rock in alkanes	1350 cm^{-1}
	C-O stretch in alcohol	1247 cm^{-1}
	=C-H bending	950 cm^{-1}

Table 8: Characteristics peaks of Physical mixtures

S.No	Physical Mixture	Components	Functional groups	Characteristic peaks (wave number)
1	PM1	Tapentadol hydrochloride +Lecithin	C-O stretching	1050-1100 cm^{-1}
			Benzene derivative	700 cm^{-1}
			C-H bending	1450-1465 cm^{-1}
			O-H stretching	3200-3300 cm^{-1}
2	PM2	Tapentadol hydrochloride + Cholesterol	O-H stretching	3250 cm^{-1}
			N-H stretching	2950 cm^{-1}
			C=C stretching	1550-1650 cm^{-1}
			C-H bending	1440-1460 cm^{-1}
			Benzene derivative	700 cm^{-1}
3	PM3	Tapentadol hydrochloride + Span 60	O-H stretching	3200 cm^{-1}
			H-C-H Asymmetric and Symmetric stretch	2800-3000 cm^{-1}
			N-H Bending	1450-1460 cm^{-1}
			C-O stretching	1150-1240 cm^{-1}
			Benzene derivative	670-700 cm^{-1}
4	PM4	Tapentadol hydrochloride + Tween 80	C-H stretching	2800-2990 cm^{-1}
			C-O stretching	1100 cm^{-1}
			Benzene derivative	700-730 cm^{-1}
5	PM5	Tapentadol hydrochloride + Capmul MCM	O-H stretching	3200 cm^{-1}
			C-H stretching	2960-2990 cm^{-1}
			C=O stretching	1730-1750 cm^{-1}
			C-C=C Asymmetric stretch	1450-1470 cm^{-1}
			C=C stretching	1160-1170
			Benzene derivative	650-700 cm^{-1}
6	PM6	Tapentadol hydrochloride + Labrafil M2125cs	C-H stretching	2850-2950 cm^{-1}
			C=O stretching	1750 cm^{-1}
			C-H bending	1440-1460 cm^{-1}
			Benzene derivative	700 cm^{-1}
7	PM7	Tapentadol hydrochloride + Kolliphore RH 40	C-H stretching	2850-2950 cm^{-1}
			C-O stretching	1060-1125 cm^{-1}
			$-CH_2$ bending	1450 cm^{-1}

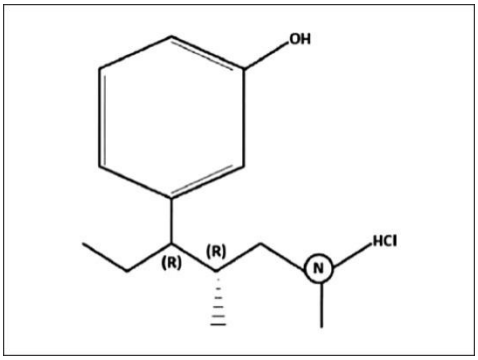


Figure 1. Chemical structure of Tapentadol HCl

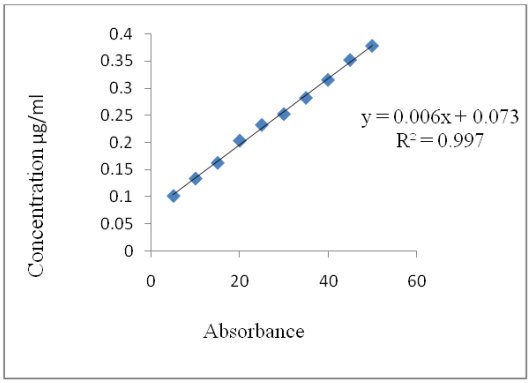


Figure 2: Linearity of Tapentadol Hcl in phosphate buffer pH 7.4

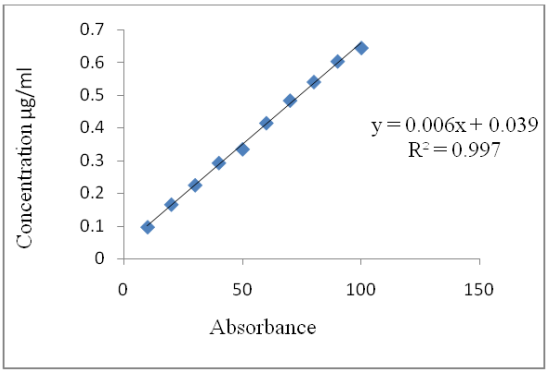


Figure 3: Linearity of Tapentadol Hcl in water

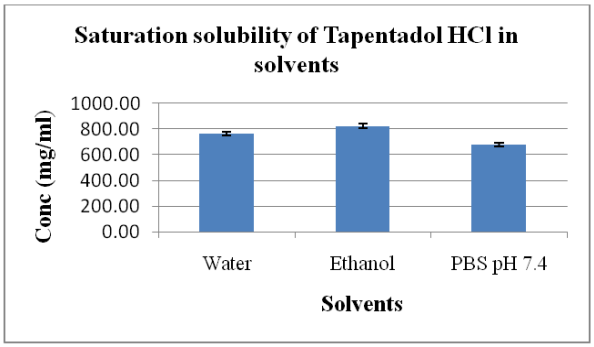


Figure 4: Saturation solubility of Tapentadol Hcl in solvents

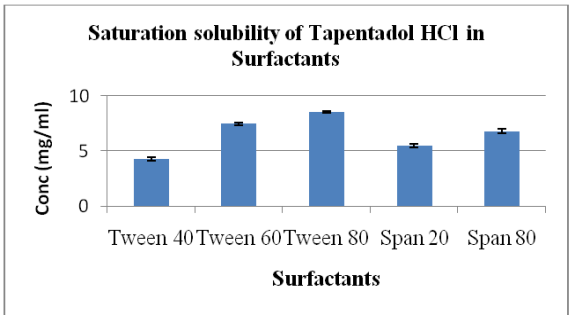


Figure 5: Saturation solubility of Tapentadol HCl in surfactants

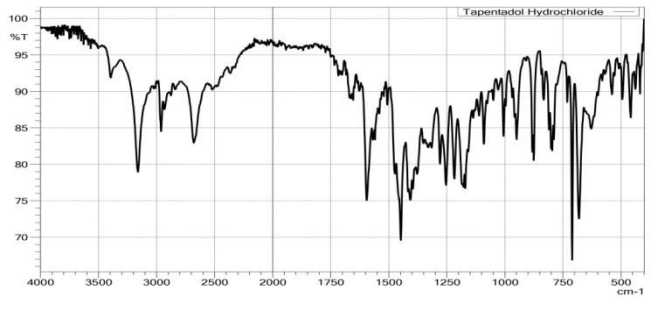


Figure 6: ATR-FTIR of Tapentadol Hydrochloride

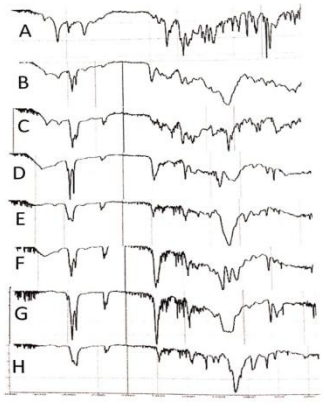


Figure 7: FTIR Spectra of Individual components: A. Tapentadol Hcl, B. Lecithin, C. Cholesterol, D. Span 60, E. Tween 80, F. Capmul MCM, G. Labrafil M 2125cs, H. Kolliphore H 40.

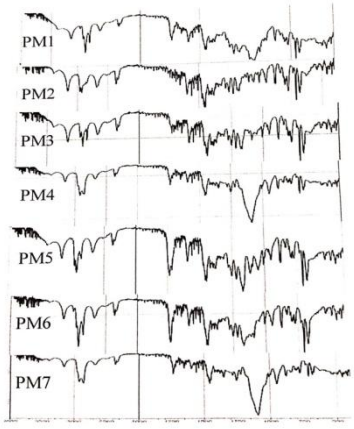


Figure 8: FTIR Spectra of Physical Mixtures

RESEARCH ARTICLE

An Empirical Study on Corporate Social Responsibility Laws and Business Practices in Industries: An Information Technology (IT) Framework Oriented Approach

K. Bhagyalakshmi* and S. Manimaran

Associate Professor, Department of Management Studies, PSNA College of engineering and Technology, Dindigul, Tamil Nadu, India.

Received: 18 Nov 2020 Revised: 21 Dec 2020 Accepted: 23 Jan 2021

***Address for Correspondence**
K. Bhagyalakshmi
Associate Professor,
Department of Management Studies,
PSNA College of engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: yakibha.ram@gmail.com

ABSTRACT

The profit maximization and shareholders wealth maximization is the supreme or primary objective of companies. They achieve their objective by framing certain set of rules and regulations that will confirm to the law and customs prevailing in the society. These rules and regulations that help in conducting the business with fair, transparent and responsible artificial person are regarded as the Corporate Social Responsibility (CSR). This CSR enabled organization may legally bound to create healthy business environment thereby directly or indirectly have greater impact on the financial performance of the corporate firms. Thus to develop the nation with high human rights, corporate practices and management inter-se the Australian Government has established the Australian Centre for Corporate Social Responsibility (ACCSR). This may ensure the application of CSR by major Companies in Australia and their contribution to the society as a whole. There exist various kinds of departments, research centre, third sector consultancy providing CSR ideas, prominent CSR entities offering conferences and workshops etc. This study will focus on in depth analysis of lawful consideration and empowerment of society with emerging CSR practices by the Australian industries. As such the supreme objective of the study would be to protect the human rights, legal formalities and the social environment through a CSR oriented product and services renders which will lead a CSR enabled Australian industry policies.

Keywords: Digital economy, Corporate Social Responsibility (CSR), Business practices, Transformation policy, Corporate laws

INTRODUCTION

According to a report a man who is illiterate does not know to read and write has been arrested for feeding Coca Cola to a child who is just 5 years old after spending welfare money for alcohol. (Deccan Chronicle, The largest circulated English daily in South India, Coimbatore, Saturday, 29, October 2018). This summons the necessity of Corporate Social Responsibility (CSR) policies in major industries with intervene of Legal formalities and regulation by the Government of Australia and other countries. Therefore, this study will provide an assessment on CSR concept adopted by Australian industry with reference to their product and service providers and the legitimacy to frame a lawful CSR.

Literature Review

CSR has become a part of the industry in Australia as said by firms or business entities and professional societies (Baker & McKenzie, 2007; Business Council of Australia, 2002; Group of hundred incorporations, 2003, Centre for Corporate public affairs, 2000). The Australian Government has also initiated significant contribution and motivation in implementing CSR concept which is proved through issue of award of Prime Minister's Business Community Partnership (PMCBP), enquiries by parliament into the CSR practices (Australian Government Department of Family and Community Services, 2005; Australian Government Department of the Environment and Heritage, 2005). There are separate department within the industry or corporate to monitor the CSR practices and to have clear idea about the CSR policy, strategy and position. National wide workshops, seminars and conferences are being encouraged on a regular basis in Australia with the objective of creating a awareness and eliminating the irregularities in the legitimacy, human rights protection and successful CSR practices for the society and corporate. Corporate Social Responsibility (CSR) as an approach to the forefront of business and financial based concerns because of the globalized establishment of trading and the so-called New Economy era, knowledge-driven, economic technology-driven settings that has, among other things, affected an increase in stakeholder's access to information[3]. The hypothesis of the corporate social responsibility progress is mode of implementing labor rights, work rights, and business standards, CSR functions has long been discussed as a feasible remedy to the inconsistency created and exacerbate by law of globalization. It means a corporation is not just an entity of profit-making, but that the company or business and its actions are also fundamental to the economy, civilization, culture, society and environment in which they occur. Financial directors and executives are becoming ever more conscious that CSR may provide work rights, labor rights and Safety environmental protections to the societies in which they live and to the people they employ. An approach taken to accomplish this objective is to enhance the mechanism that aims at keeping abuse and scam in checking verification. These include the responsibilities imposed on financial directors, the part of auditors, the establishment of auditing group, and disclosure requirements to name but a few. Accuracy, relevancy, systematic and timely prompt information is essential to establishing and preserving these techniques as well as to ensure their efficiency. Because the accessibility of data and information plays a guaranteed role, the increased exercise of (ICT) in information management has made a significant impact on these CSR mechanisms.

Research Questions

Nevertheless, CSR representation from all the firms or organization within a particular industry must help us to create an industry CSR IT domain by giving weight to each firms CSR representation in developing healthy environment and financial performance. For example a CSR policy cum practices for automobile industry will vary far than that of a CSR concept adapted by a beverage industry. Thus the legal considerations in practicing CSR by several firms within in an industry are highly influencial and raise various questions as to its reliability. As such there exist some remarkable questions as presented below:

- What requirement is basic to ICT enables CSR application in industry with certain relevant features as to legal compliance verses avoiding civil proceedings; profit maximization verses shareholders wealth maximization and so on.

- How for the CSR policies included in the usual business practices of firms are visible and apparently sighted by the Government of Australia?
- Whether the CSR practices reach massive environment residing in industry and business sector with defined domains?
- Are they providing information on participation to enhance proper awareness and knowledge on CSR practices through conference and seminars?
- Do employees and employer of the company shift from lawful, ethical and moral domain to another due to major CSR legal formalities or lawful consideration and lack of ethical CSR practices?
- How could a company deal most efficiently with CSR performance in co-ordinance with financial performance?

METHODOLOGY

This research paper focuses and aims to discuss the recent issues and challenges those business financial directors, investors and auditors are facing in adapting and utilizing lawful ICT to develop and enhance the legally framed technology in the principles of corporate authority and governance in gratifying the necessities of Corporate Social Responsibility. The project development will covenant to deal with providing a concise outline of the basic transformation brought about by digital law and ICT in the areas of storing financial data, confidential information, secured technical management, the processing of data dissemination, and information propagation. The Highlight of this paper will be discussing about how the changes can make an impact on the roles of financial directors, auditors, and investors in their respective domain of corporate social responsibility enhancement schemes and spot out few precise areas of prospective development and transforms. The emerging CSR concept in industry will be studied through a case study approach with empirical research design. As the primary step a pilot study will be conducted to identify the major players, Legal norms or statutory policies and industrial contribution towards CSR policies in Australia. A descriptive analysis to identify the need of the day in practicing technology driven CSR and legal requirements for further developing the adaption of CSR in Australia industry will be done. This research may thereby aim at developing an ICT enabled three-layer model of domain areas: LEGAL, ETHICAL and ECONOMIC.

DISCUSSION

Digital economy verses CSR laws

Nowadays, the paramount significance of "Digital transformation" which is disputable has become more contentious. Subsequently, the drastically increasing number of MNCs and business sectors having unique policies and programs explaining their commitment to the society has scintillating the importance this trend or scenario in CSR laws in recent years. The impact CSR laws in Australia pertain to reducing carbon footprints, charitable giving, volunteering in the community, improving labor policies like enacting Slavery Act, fair-trade practices, social and environment conscious investment. The digital economy is changing the CSR trends beckoning Metoo-movement embracing social injustice. As such, Australian government and companies infused digital transformation in view of CSR by operating a privately owned renewable energy technology or plant, investing in less carbon emission vehicle for logistics and supply chain, developing legal solar system greatly reducing use of electricity, 24 to 52 weeks of paid parental leaves as laid down by Listed Companies Act. Therefore, the overall digital transformation has changed the existing laws prevailing in the country into overall sustainable laws of the company through internal and external stakeholder's judgments.

Capgemini's business practices: A case study

Pragmatically, companies are responsible to incorporate sustainability through technology, ICT application and innovation for social development. Capgemini is one such company serving as a platform to strengthen Australian company's engagement with overwhelming workforce, clients or customers, partners, community helpers and the universe by pertaining to three fundamental domains which act as the pillars of successful leverage of CSR. The so

called pillars are Digital Inclusion, Environmental Sustainability, and Diversity along with inclusion. The dedication of this company is towards identifying diverse talents who can continuously thrive the work environment with exhilarating solidarity and environmental protection. This is known as the positive future architect that enables Support Employee Resource Groups (ERGs) recruited or joined Capgemini from all over the world to provide a sound and ergonomic workplace with fair act. The Capgemini team designs and develops business solutions to accelerate digital economy with people-centric approach in business. The actions built in organization culture streamlined to bridge the gap between the society and technology by offering environment oriented solutions at global, national and regional level for example the memorandum signed with United Nations (UN) Global Compact. To embark with, the first pillar diversity hitch hack gender disparities, cultural differences, ethnic and social disabilities. As a repercussion, they ensure deployment of two programs from 2018 such as high-potential women rights and enhancing role of women in digital and innovation. Secondly, digital inclusion drives integrity among society, technology and business by collaborating with Non-Governmental Organizations (NGOs), public and education institutes. As part of their digital CSR program they have implemented digital literacy programs and encouraged their employees to develop tech solutions serving health for all, alleviating poverty and protecting environment. Thirdly, environmental responsibility relying on fight against carbon emission by targeted clients. The architecture developed by Capgemini employees collect and analyzes over 10 million data that covers nearly 99 percentage of the Australia business thereby interpreting greenhouse gas emission and leading them to identify the best optimum resource to reduce the same. The summary of Digital Corporate Responsibility launched by Capgemini is given in Table 1.

Implication and suggestions

The suggested CSR Management Information System (CSRMIS) model articulates that the Australian law predominantly focuses on societal benefit in a broader perspective rather than focusing on financial benefit. Although there exists specific laws such as work safety and health law, Fair work, Bullying, Anti-discrimination, gender equality, Australian Consumer law, Anti-Competitive behavior, Marketing restrictions, Annual Reports, listed companies, Environmental protection and Green buildings the government may require a digital platform where business entrepreneurs and management people all over the nation collaborate together to exhibit profound CSR practice. Nevertheless, it is evident that CSR will not inculcate profit maximization. Therefore, with mutual benefit organizations must consider the stakeholders wealth and collectively work together to uplift the reputation of the whole nation. This is possible with the power of digital networking such as electronic gadgets and interoperability software application to enhance CSR policies. Rather than being commercial after all CSR must remain as a social well being application with strong insight into the laws governed by Australia. In this respect, as a repercussion the three conspicuous domains to develop a Corporate Social Responsibility Management Information System (CSRMIS) could be legal, ethical and economic. Adopting a CSR strategy with comply of legal, ethical and economic domains contribute less risk of declining economy and lower rate of consumer cynicism. Based on the conceptual and perceptual analysis it is the legal domain that can attribute to work health and safety, listed companies; Ethical domain may attribute to fair work, bullying, anti-discrimination, gender equality, environmental protection and green building; economic domain may attribute to anti-competitive behavior, marketing restrictions, annual reports, product disclosure statements.

CONCLUSION

The research findings herein provide an insight into the efficient and successful implementation of CSR laws in Australia industry with demonstrative Information Technology application. This may develop a nationwide economic benefit by means of protecting the human rights and financial reputation of companies by means of adapting legitimate CSR policies and practices. It is expected that there is possibility of monitoring and enhancing the CSR activities with participation of society, CSR communities, Companies or enterprises and Government, thereby reducing the complication and creating the ways to exactly categorize business entities and their activities

and performance within a CSR module or domain. Thus, the CSR module in a broader perspective ripples ethical legal and social or human responsible in align with the digital economy wherein each layer may overlap with each other. Since, Ethical concerns are interwoven such as fair work may lead to listed companies volunteering and protecting environment by providing good health. In a legal realm digital CSR can make it as simple as possible to guide education, job, and preservation, sustainable growth in income, product lifecycle, waste management, emissions and renewable energy.

REFERENCES

1. Aupperle, K. E. (1984). An Empirical Measure of Corporate Social Orientation. In research in Corporate Social Performance and Policy 6, ed. L. E. Preston (Greenwich, Conn.: JAI Press), 27 – 54.
2. Bollier, D., and S. Weiss, S. (1991). Merck & Co. Inc. The Business Enterprise Trust.
3. Carroll, A. B. (1979). A Three – Dimensional Conceptual Model of Corporate Performance. Academy of Management Review, 4(4): 497 – 505.
4. Mark S. Schwartz and Archie B. Carroll. Corporate Social Responsibility: A three- Domain approach.
5. Sethi, S. P. 2000. A Conceptual Framework for Environmental Analysis of Social Issues and evaluation of Business Response patterns. Academy of Management Review, 4(1): 63 – 74.
6. Fredrickson, B.L. (2004). The Broaden-and-Build theory of Positive Emotions. The science of well-being: integrating neurobiology, psychology and social science, 359, 1367–1377; doi:10.1098/rstb.2004.1512
7. Geiman, M (2016). A Multiple Case Study of the Influence of Positive Organizational Behavior on Human Resources. Doctoral thesis, Walden Dissertations and Doctoral Studies Collection at Scholar Works, Retrieved from http://scholarworks.waldenu.edu/dissertations
8. Levene, R.A.(2015). Positive Psychology At Work: Psychological Capital and Thriving as Pathways to Employee Engagement. Master of Applied Positive Psychology (MAPP) Capstone Projects, Retrieved from http://repository.upenn.edu/mapp_capstone/88
9. Luthans, F.(2002). The need and meaning of positive organizational behaviour. Journal of Organizational Behaviour, 23(6), pp. 695-706. doi 10.1002/job.165
10. Luthans, F. (2009). The Point of positive organizational behaviour. Journal of Organizational Behavior, 30, pp. 291-307; doi 10.1002/job.589
11. Mayman, M.M., Moghadam, N.S., & Farangi, A., & Rouholamini, M. (2016). Investigating the effect of POB on Innovation. International Business Management. 10(11), pp. 2282-2292
12. Nel, T., Stander, M.W., Latif, J. (2015). Investigating positive leadership, psychological empowerment, work engagement and satisfaction with life in a chemical industry. SA Journal of Industrial Psychology/SA Tydskrif vir Bedryfsielkunde, 41(1), Art. #1243, 13 pages. http://dx.doi.org/10.4102/ sajip.v41i1.1243
13. Paul, H., & Garg, P (2013). Healing HRM through positive psychology, ICTMS-2013. Procedia - Social and Behavioral Sciences 133 (2014) , pp. 141 – 150. Elsevier
14. Ramlall, S.J. (2008). Enhancing employee performance through positive organizational behaviour. Journal of Applied Social Psychology, 38**(**6), pp. 1580–1600
15. Seligman, M (2010). Flourish: Positive psychology and interventions, The Tanners lectures on human values. Retrieved from https://tannerlectures.utah.edu

Table 1. Digital Corporate Responsibilities launched by Capgemini

KEY PERFORMANCE INDICATORS*		2018	2019	TARGETS 2020
Improving parity in management teams	Percentage of women holding executive roles	14%	17%	20%
	Percentage of internal promotions and external hires	24%	259%	29%
Improving parity throughout the group	Percentage of staff who are women	31.9%	33.0	33.5%
Digital inclusion	Percentage of social impact projects	64%	74%	80%
Digital academies	Number of digital academy gradues	150	1,562	3,000
Digital literacy	Number of digital literacy program beneficiaries	-	27,300	1,00,000
Climate change	Rate of greenhouse gas emission reduction per business since 2015	20%	29%	20%

**Source: https://reports.capgemini.com/2019/files/ri/Capgemini*

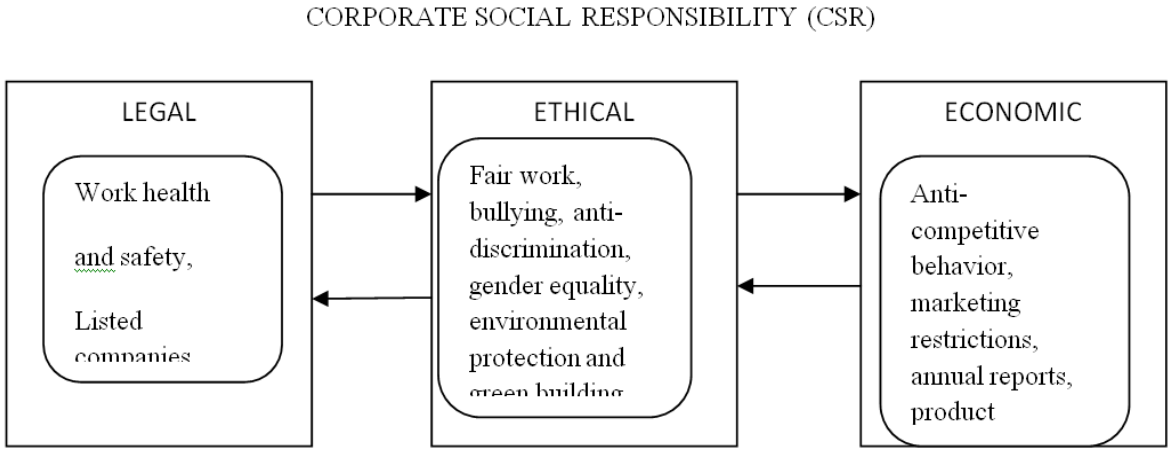


Figure 1. Corporate Social Responsibility Management Information System (CSRMIS) Model

Dr. Bhagyalakshmi Rajaram working as an Associate Professor and Research Supervisor, Department of Management Studies, PSNA College of Engineering and Technology, Dindigul, Tamilnadu, India. With over 12 years of academic experience a deterministic professional with the mission to deliver excellence in research and teaching. Research interest range from Management Information System (MIS), Healthcare IT, Electronic Health Records (EHR), Financial Management and Accounting. She completed Ph. D during 2014 in the area of HMIS with a profound knowledge and strong outcome in developing HMIS technology/Med Tech acceptance model for assessing the user's acceptance level in Rural Healthcare system of India. To her credit a Netherlands based peer reviewed, Science & Technology collection, Medline, Pubmed center, Scopus, EBSCO indexed and International journal titled "Technology and Health Care" published by IOS press with ISSN print 0928-7329 honored her as a SECTION EDITOR, public health, Health Information Systems.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

COVID 19 and its Impacts – A Case Study

R. Venkatesh[1]* and K. Jeyalakshmi[2]

[1]Assistant Professor, Department of Physics, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.
[2]Professor, Department of Physics, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.

Received: 18 Nov 2020 Revised: 20 Dec 2020 Accepted: 22 Jan 2021

***Address for Correspondence**
R. Venkatesh
Assistant Professor,
Department of Physics,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: lakatesh@gmail.com

ABSTRACT

Coronaviruses are a set of new viruses with non-segmented, single-stranded, and positive- sense RNA genomes. A severe acute respiratory syndrome coronavirus (SARS-CoV) and Middle East respiratory syndrome coronavirus (MERS-CoV) are zoonotic and highly pathogenic coronaviruses that have resulted in regional and global outbreaks coronavirus possess a distinctive morphology, the name being derived from the outer fringe, coronal of embedded envelope protein. Members of the family Coronaviridae cause a broad spectrum of animal and human diseases. Uniquely, replication of the RNA genome proceeds through the generation of a nested set of viral mRNA molecules. Human coronavirus (HCoV) infection causes respiratory diseases with mild to severe outcomes.

Keywords: COVID-19, Viruses, SARS, RNA, Economy

INTRODUCTION

Coronaviruses are a type of virus. There are many different kinds, and some cause disease. A newly identified coronavirus, SARS-CoV-2, has caused a worldwide pandemic of respiratory illness, calledCOVID-19. Coronaviruses are common in different animals. Rarely, an animal coronavirus can infect humans. There are many different kinds of coronaviruses. Some of them can cause colds or other mild respiratory (nose, throat, lung) illnesses. Other coronaviruses can cause more serious diseases, including severe acute respiratory syndrome (SARS) and Middle East respiratory syndrome (MERS). Coronaviruses are named for their appearance: Under the microscope, the viruses look like they are covered with pointed structures that surround them like a corona, or crown.

As of now, researchers know that the new coronavirus is spread through droplets released into the air when an infected person coughs or sneezes. The droplets generally do not travel more than a few feet, and they fall to the ground (or onto surfaces) in a few seconds — this is why physical distancing is effective in preventing the spread. VID-19 is the disease caused by the new coronavirus that emerged in China in December 2019. COVID-19 appeared in Wuhan, a city in China, in December 2019. Although health officials are still tracing the exact source of this new coronavirus, early hypotheses thought it may be linked to a seafood market in Wuhan, China. Some people who visited the market developed viral pneumonia caused by the new coronavirus. A study that came out on Jan. 25, 2020, notes that the individual with the first reported case became ill on Dec. 1, 2019, and had no link to the seafood market. Investigations are ongoing as to how this virus originated and spread.

COVID-19 symptoms include

- Cough
- Fever or chills
- Shortness of breath or difficulty breathing
- Muscle or body aches.
- Sore throat
- New loss of taste or smell
- Diarrhea
- Headache
- New fatigue
- Nausea or vomiting
- Congestion or runny nose

In rare cases, COVID-19 can lead to severe respiratory problems, kidney failure or death. If you have a fever or any kind of respiratory difficulty such as coughing or shortness of breath, call your doctor or a health care provider and explain your symptoms over the phone before going to the doctor's office, urgent care facility or emergency room.

Types

Coronaviruses (Fig.1) belong to the subfamily Coronavirinae in the family Coronaviridae. Different types of human coronaviruses vary in how severe the resulting disease becomes, and how far they can spread. Doctors currently recognize seven types of coronavirus that can infect humans.

Common types

1. 229E (alphacoronavirus)
2. NL63 (alphacoronavirus)
3. OC43 (betacoronavirus)
4. HKU1 (betacoronavirus)

Rarer strains that cause more severe complications include MERS-CoV, which causes Middle East respiratory syndrome (MERS), and SARS-CoV, the virus responsible for severe acute respiratory syndrome (SARS). In 2019, a dangerous new strain called SARS- CoV-2 started circulating, causing the disease COVID- 19.

Transmission of Virus

Droplet transmission occurs when a person is in close contact (within 1 m) with someone who has respiratory symptoms (e.g., coughing or sneezing) and is therefore at risk of having his/her mucosae (mouth and nose) or conjunctiva (eyes) exposed to potentially infective respiratory droplets. Transmission may also occur through fomites in the immediate environment around the infected person. Therefore, transmission of the COVID-19 virus can occur by direct contact with infected people and indirect contact with surfaces in the immediate environment or with objects used on the infected person (e.g., stethoscope or thermometer).

The National Institutes of Health (NIH) suggest that several groups of people have the highest risk of developing complications due to COVID-19. These groups include:

1. Young children
2. People aged 65 years or older
3. Women who are pregnant

Coronaviruses will infect most people at some time during their lifetime. Coronaviruses can mutate effectively, which makes them so contagious. To prevent transmission, people should stay at home and rest while symptoms are active. They should also avoid close contact with other people. Covering the mouth and nose with a tissue or handkerchief while coughing or sneezing canal so help prevent transmission. It is important to dispose of any tissues after use and maintain hygiene around the home.

COVID-19

In 2019, the Centers for Disease Control and Prevention (CDC) started monitoring the outbreak of a new coronavirus, SARS-CoV-2, which causes the respiratory illness now known as COVID-19. Authorities first identified the virus in Wuhan, China. More than 74,000 people have contracted the virus in China. Health authorities have identified many other people with COVID-19 around the world, including many in the United States. On January 31, 2020, the virus passed from one person to another in the U.S. The World Health Organization (WHO) have declared a public health emergency relating toCOVID-19.Since then, this strain has been diagnosed in several U.S. residents. The CDC have advised that it is likely to spread to more people. COVID-19 has started causing disruption in at least 25 other countries. The first people with COVID-19 had links to an animal and seafood market. This fact suggested that animals initially transmitted the virus to humans. However, people with a more recent diagnosis had no connections with or exposure to the market, confirming that humans can pass the virus to each other.

Corona virus life cycle Steps

1. Attachment and entry
2. Replicase protein expression
3. Replication and transcription
4. Assembly and release.

Coronaviruses are large, roughly spherical particles with unique surface projections. Their size is highly variable with average diameters of 80 to 120nm. Extreme sizes are known from 50 to 200 nm in diameter. The total molecular weight is on average 40,000 kDa. They are enclosed in an envelope embedded (Fig.2) with a number of protein molecules. The lipid bilayer envelope, membrane proteins, and nucleocapsid protect the virus when it is outside the host cell.

People can take several steps including

1. Resting and avoiding over exertion
2. Drinking enough water
3. avoiding smoking and smoky areas
4. Taking acetaminophen, ibuprofen, or naproxen for pain and fever
5. Using a clean humidifier or cool mist vaporizer
6. A doctor can diagnose the virus responsible by taking a sample of respiratory fluids, such as mucus from the nose, or blood.
7. Standard recommendations to prevent infection spread

Case Study

The Kerala state has confirmed (As on 23-06-2020) total 3,451 cases so far, of which 1,620 are active patients and 1,807 recovered. According to official data until 15 June, 95% of the state's active patients are expats who returned from foreign countries or other Indian states Kerala reported another coronavirus-related death and 141 new covid-19 patients on Tuesday, the highest daily-tally so far. This is the 23rd covid-19 related death in the state. Meanwhile, 60 persons recovered from the disease on the day. The state has confirmed total 3,451 cases so far, of which 1,620 are active patients and 1,807 recovered.

CONCLUSION

To overcome the crisis, central banks worldwide have made aggressive rate cut and infused liquidity. Though evaluation/conclusion on the efficacy of these policy measures, so early, would be hasty, available evidence do not indicate much success. It may be mentioned that liquidity enhancement through an external mechanism, like central bank intervention, is vitally necessary when internal dynamics are disrupted. Rate cut is, however, unlikely to remove the 'crisis of confidence' till the virus is put to rest. The monetary policy measures being pursued by the central banks do not appear to offer a comprehensive solution either. Considering that the crisis is a fallout of an unknown virus, an effective policy measure, in our view, is to increase spending on medical and health programmes, which would be directed not just at innovative cure (vaccine discovery), but also a widespread mission to increase testing centres (capacity) and quality so as to identify the infected early, and take quick remedial action. That could indeed be a confidence booster. Meanwhile, a well-thought-out programme for restoration of the supply chain, albeit in a limited manner, may also be thought of.

REFERENCES

1. "India: Measures in Response to COVID-19." KPMG. KPMG, April 13, 2020.
2. Mishra, Rajat. "COVID-19 And Its Impact on MSME In India." Outlook Money, April 24, 2020.
3. W.J. Mc Kibbin, R. Fernando The Global Macroeconomic Impacts of COVID-19: Seven Scenarios (2020).
4. Y. Okuyama Economic modeling for disaster impact analysis: past, present, and future Econ. Syst. Res., 19 (2) (2007).
5. International Energy Agency Global Energy Review 2020: The Impacts of the Covid-19 Crisis on Global Energy Demand and CO2 Emissions (2020).
6. Radhika Pandey, A. P. (2020). *Covid-19 and MSMEs: The 'identification' problem*. Ideas for India for More Evidence Based Policy.
7. The Economist. (2020, February 15). The new coronavirus could have a lasting impact on global supply chains.
8. World Health Organization Situation reports.
9. Coronavirus Outbreak. Available at: https://www.worldometers.info/coronavirus/. Accessed 23 Feb 2020.
10. Middle East Respiratory Syndrome Coronavirus. Available at: https://www.who.int/emergencies/mers-cov/en/. Accessed 16 Feb 2020.

Table 1: State/UT wise list of COVID confirmed cases in India

Source: Ministry of Health and Family Welfare, Government of India

State wise data: India (September 30) **As on : September 30, 2020, 08:00 IST (IST)**

S.No	State/UT	Confirmed Cases	Active Cases	Cured/ Discharged	Death
1	Andaman and Nicobar Islands	3821	181	3587	53
2	Andhra Pradesh	687351	59435	622136	5780
3	Arunachal Pradesh	9553	2794	6743	16
4	Assam	177221	32539	144002	680
5	Bihar	181285	12366	168025	894
6	Chandigarh	11816	2060	9598	158
7	Chhattisgarh	110655	31225	78514	916
8	Dadra and Nagar Haveli and Daman and Diu	3032	120	2910	2
9	Delhi	276325	27524	243481	5320
10	Goa	32777	4577	27781	419
11	Gujarat	135842	16676	115727	3439
12	Haryana	126974	14804	110814	1356
13	Himachal Pradesh	14747	3573	10991	183
14	Jammu and Kashmir	74095	17414	55517	1164
15	Jharkhand	82540	11942	69898	700
16	Karnataka	592911	107756	476378	8777
17	Kerala	187276	61869	124688	719
18	Ladakh	4195	1030	3107	58
19	Lakshadweep	0	0	0	0
20	Madhya Pradesh	126043	21317	102445	2281
21	Maharashtra	1366129	260789	1069159	36181
22	Manipur	10746	2642	8039	65
23	Meghalaya	5463	1476	3940	47
24	Mizoram	1986	410	1576	0
25	Nagaland	6040	1037	4986	17
26	Odisha	215676	33367	181481	828
27	Puducherry	27066	4933	21616	517
28	Punjab	112460	16824	92277	3359
29	Rajasthan	133119	20376	111272	1471
30	Sikkim	2937	667	2235	35
31	Tamil Nadu	591943	46281	536209	9453
32	Telangana	191386	29326	160933	1127
33	Tripura	25734	5765	19692	277
34	Uttar Pradesh	394856	52160	336981	5715
35	Uttarakhand	47995	9122	38282	591
36	West Bengal	253768	26064	222805	4899
	India	**6225763**	**940441**	**5187825**	**97497**

The details of corona virus affected, cured, and death cases are illustrated in the (Table. 1) with all states of India.

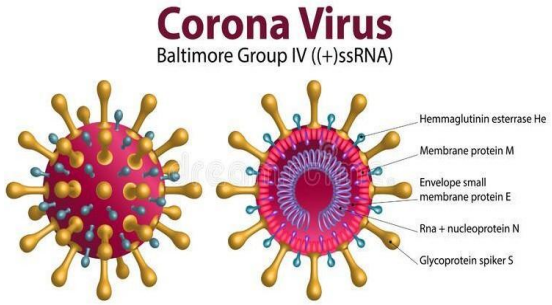


Fig. 1. Structure of Corona Virus

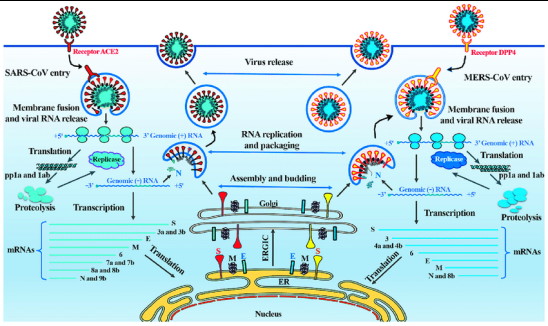


Fig. 2. Life cycle of Corona Virus

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Selected Biochemical Parameters Responses against Heavy Metal Mercury in White Leg Shrimp *Litopenaeus vannamei* (Boone, 1931)"

Arul Prasad S[1]* and Valarmathi V[2]

[1]Department of Zoology, Thiru.Vi.Ka. Govt. Arts College (Affiliated to Bharathidasan University, Thiruchirapalli), Thiruvarur, Tamil Nadu, India.
[2]Department of Zoology, A.D.M. College for Women (Autonomous) (Affiliated to Bharathidasan University, Thiruchirapalli), Nagapattinam, Tamil Nadu, India.

Received: 08 Nov 2020 Revised: 10 Dec 2020 Accepted: 13 Jan 2021

***Address for Correspondence**
Arul Prasad
Department of Zoology,
Thiru.Vi.Ka. Govt. Arts College
(Affiliated to Bharathidasan University, Thiruchirapalli),
Thiruvarur, Tamil Nadu, India.
Email : arulprasad.vkt@gmail.com

ABSTRACT

Fortunately or unfortunately we are in critical situation due to the problem of environment toxicants like heavy metals and pesticide. Heavy metals are very toxicant to any organism as well as human being even it may be low concentration. For that reason the present study focused on biochemical constituents against mercury toxicity in White leg shrimp *Litopenaeus vannamei*. The people doesn't thing about toxicity of heavy metal in aquatic organism. But the aquatic organisms got several influences by the toxicant used by us like heavy metals and pesticides. In the present study revealed that changes of biochemical constituents in different organs of *Liropenaeus vannamei* against mercury toxicity in different concentrations. The results showed that, among the selected organs, carbohydrate and protein were high in muscle tissues followed by hepatopancreas and gill. The lipid content was high in hepatopancreas followed by muscle and gills tissues of *Litopenaeus vannamei*. Among the different concentrations of mercury, 30% were significantly decreased level of carbohydrate, protein and total lipids in hepatopancreas, gill and muscle tissues of shrimp ($P>0.05$). Likewise, among the date of exposure periods 30 days of exposure periods was showed high declined of level of carbohydrate, protein and total lipids in three different organs of test organism ($P>0.05$). It indicated that exposure period is inversely proportional to the biochemical constituents of shrimp. Nevertheless, the results of the present investigation may be useful for assessing early warning signs of mercury poisoning and support the possibility to use *Litopenaeus vannamei* as biosensor of coastal marine and estuarine pollution by heavy metals. Nevertheless, the results of the present investigation may be useful for assessing early warning

signs of mercury poisoning and support the possibility to use *Litopenaeus vannamei* as biosensor of coastal marine and estuarine pollution by heavy metals.

Keywords: Mercury, Litopenaeus vannamei, Protein, Carbohydrate, Total lipids

INTRODUCTION

Heavy metals contamination is becoming a serious issue of concern around the world. As it has gained thrust due to the increase in the use and processing of heavy metals during various activities to meet the needs of the rapidly growing population. Soil, water and air are the major environmental compartments which are affected by heavy metals pollution [1]. It is also one of the exigent problems to the environmental biologists, as varieties of heavy metals have potentially harmful effects on the biological organisms [2]. It is easily mixed with surface water and ground water, and ultimately, they affect the aquatic environment. The heavy metals are slow poisoning and slow degradable substance and produce toxic effect to prolonged period. So it might be directly or indirectly affect the human population through the food chain or food web. They are also considered as a major source of pollution toxicity to the living organisms [3]. Among the heavy metal, the mercury (Hg) is a naturally occurring element that is toxic in nature. According to the US Environmental Protection Agency, the safe limit of mercury ion in drinking water is 10 nM to avoid the serious health problems to humans. Mercury is a pollutant of global concern. The Minamata Convention on Mercury entered into force from 2017, regarding the protection of human and environmental health. A recent review reported about the worldwide and regional time trends in total mercury levels in the human blood and breast milk and their associations with health effects [4]. *Litopenaeus vannamei* is a decapod crustacean which is native to the eastern Pacific coast of Central and South America from Tumbes, Peru in the south to Mexico in the north. It has been introduced widely around the world since the 1970s, but especially since 2000, as it has become the principle cultured shrimp species in Asia. The nutritional value of different species of fish and shellfish depend on their biochemical components such as protein, carbohydrate and lipids. These proximate components could serve as sensitive indicators for detecting potential adverse effects, particularly the early events of pollutant damage because their alterations appear before the clinical symptoms produced by the toxicant [5,6]. In the present investigation was made to record the changes of selected biochemical parameters against heavy metal Mercury in White leg Shrimp *Litopenaeus vannamei*. The selection of species for the particular study is due the economically and commercially important shrimp, readily available in good numbers throughout the year, easy to rear in laboratory conditions, and ability to respond against environmental pollution without showing prolonged stress due to handling.

MATERIALS AND METHODS

The White leg Shrimp, *Litopenaeus vannamei* of the carapace length of 3.0±0.5 cm and breath of 4.0 ± 0.5 cm were selected for the experiment and were collected from Muthupet mangroves (10° 20° N, 79° 35°E), Tiruvarur district. Shrimp were screened for any pathogenic infections. Plastic troughs were washed with 1% $KMnO_4$ to avoid fungal contamination and then sun dried. Healthy shrimps were then transferred to plastic troughs (20″ diameter) containing estuarine water (Temperature 29±3°C; DO 4.2 ± 0.5 mg/l; salinity 24.1±0.17 ppt and pH 8.6 ± 0.05). Shrimps were acclimated to laboratory conditions for 10 to 15 days prior to experimentation. They were regularly fed with natural food and the medium (estuarine water) was changed daily to remove faeces and food remnants. Toxicity studies were conducted to obtain reliable data regarding the effects of the toxicant on the test species. Static bioassay tests were conducted as per standards set by the American Public Health Association [7]. Based on acute toxicity test (96h LC50) sublethal concentrations (10% and 30%) were derived for mercury which was used as the experimental concentration of the mercury in the subsequent experiments. Ten shrimp were exposed to each concentration for a period of 10, 20 and 30 days. A control batch was maintained simultaneously and six trails were

run. The toxicant sample used possessed the following characteristics. The initial and final concentration of biochemical constituents, such as carbohydrate [8], total protein [9], and total lipid [10] were estimated in test organism in different exposure period. The period was 10 days, 20 days and 30 days and the selected organs (Hepatopancreas, gill and muscle) were chosen to analyses the above said parameters. One way ANOVA was performed to know the significance of the result carried out from the investigation by using PAST.

RESULTS

The present investigation was carried the biochemical constituents of hepatopancreas, gill and muscle tissues of shrimp *Litopenaeus vannamei* against different concentration mercury. Among the three selected organs, carbohydrate and protein were high in muscle tissues followed by hepatopancreas and gill. The lipid content was high in hepatopancreas followed by muscle and gills tissues of *Litopenaeus vannamei*.

Biochemical changes in the Hepatopancreas

In the normal untreated control, the level of carbohydrate content in Hepatopancreas of *Litopenaeus vannamei* showed the ranges from 13.31 to 13.61 mg/g wet weight of tissue during different periods of experiment. At sub-lethal concentration of mercury 10% treatment showed greater variance in 30 days (6.31 mg/g) followed by 20 (8.27 mg/g) and 10 days (10.55 mg/g). Likewise, the 30% sub lethal concentration of mercury treatment was showed greater difference from control groups as well as 10% SLC treated groups in all the three exposure periods. The protein content was showed the ranges from 54.2 to 55.0 mg/g wet weight tissue in control groups. In treated groups of 10% SLC was showed much variation in 30 days (36.5 mg/g) exposure period followed 20 (42.8 mg/g) and 10 days (46.6 mg/g). In 30% SLC of mercury was noticed much more variation from the control groups as well as 10% SLC groups in all the three exposure periods. The level of lipid content in hepatopancreas, untreated control groups was showed the range from 35.56 to 36.48 mg/g. The changes varied among the treated groups when compared with control groups. The 10% SLC of mercury treated shrimp was showed 23.61 mg/g in 30 days exposure period followed by 20 days (28.27 mg/g) and 10 days (31.16 mg/g). In 30% SLC of mercury treated groups were showed the greater reduction from control as well as 10% SLC groups in 30 days 17.16 mg/g) exposure period followed by 20 (22.12 mg/g) and 10 days (27.10 mg/g) (Table 1).

Biochemical changes in the Gill

In the normal untreated control, the level of carbohydrate content in gill tissues of *Litopenaeus vannamei* showed the ranges from 8.12 to 8.51 mg/g wet weight of tissue during different periods of experiment. At sub-lethal concentration of mercury 10% treatment showed greater variance in 30 days (5.32 mg/g) followed by 20 (5.52 mg/g) and 10 days (6.81 mg/g). Likewise, the 30% sub lethal concentration of mercury treatment was showed greater difference from control groups as well as 10% SLC treated groups in all the three exposure periods. The protein content was showed the ranges from 30.0 to 31.2 mg/g wet weight tissue in control groups. In treated groups of 10% SLC was showed much variation in 30 days (25.9 mg/g) exposure period followed 20 (26.3 mg/g) and 10 days (26.5 mg/g). In 30% SLC of mercury was noticed much more variation from the control groups as well as 10% SLC groups in all the three exposure periods. The level of lipid content in gill tisues, untreated control groups was showed the range from 17.16 to 17.61 mg/g. The changes varied among the treated groups when compared with control groups. The 10% SLC of mercury treated shrimp was showed 12.31 mg/g in 30 days exposure period followed by 20 days (14.12 mg/g) and 10 days (15.12 mg/g). In 30% SLC of mercury treated groups were showed the greater reduction from control as well as 10% SLC groups in 30 days 9.12 mg/g) exposure period followed by 20 (11.16 mg/g) and 10 days (14.28 mg/g) (Table 2).

Biochemical changes in the Muscle

In the normal untreated control, the level of carbohydrate content in muscle tissues of *Litopenaeus vannamei* showed the ranges from 10.41 to 10.81 mg/g wet weight of tissue during different periods of experiment. At sub-lethal concentration of mercury 10% treatment showed greater variance in 20 days (6.32 mg/g) followed by 30 (6.78 mg/g) and 10 days (8.32 mg/g). Likewise, the 30% sub lethal concentration of mercury treatment was showed greater difference from control groups as well as 10% SLC treated groups in all the three exposure periods. The protein content was showed the ranges from 80.2 to 80.6 mg/g wet weight tissue in control groups. In treated groups of 10% SLC was showed much variation in 30 days (54.2 mg/g) exposure period followed 20 (61.1 mg/g) and 10 days (71.5 mg/g). In 30% SLC of mercury was noticed much more variation from the control groups as well as 10% SLC groups in all the three exposure periods. The level of lipid content in muscle, untreated control groups was showed the range from 20.11 to 20.19 mg/g. The changes varied among the treated groups when compared with control groups. The 10% SLC of mercury treated shrimp was showed 11.38 mg/g in 30 days exposure period followed by 20 days (13.27 mg/g) and 10 days (17.11 mg/g). In 30% SLC of mercury treated groups were showed the greater reduction from control as well as 10% SLC groups in 30 days 7.26 mg/g) exposure period followed by 20 (10.12 mg/g) and 10 days (14.45 mg/g) (Table 3).

DISCUSSION

The present study showed that the hepatopancreas, gill, and muscle tissues of *Litopenaeus vannamei* treated with sub-lethal concentration of mercury (10% and 20%) showed a decreased level of carbohydrate, protein and lipid in all tissues. Increased date of exposure and concentration of mercury showed various declined level above biochemical constituents in test organism ($P<0.05$). The biochemical components are key biomarkers for the animal health. Biomarkers can be characterized as functional measures of exposure to stressors which are usually expressed at the biochemical, cellular, or tissue level. This present investigation is pioneer study to evaluate the biochemical constituents in different tissues of *Litopenaeus vannamei* against mercury toxicity.

Effect of mercury on Carbohydrate levels

The concentrations of the carbohydrate decreased in all the tissues significantly with the progress of exposure period irrespective of exposure concentrations. The order of percent decrease within the tissues was: muscle > hepatopancreas > gill. The carbohydrate level was decreased due to the stress of animal when it was introduced in toxic metals. Similar type of result suggest by several researchers with their findings in different aquatic organisms with different toxic elements. This is consistent with the previous observations that have been reported in the marine prawn, *M. monoceros* on exposure to methylparathion [11] and in the freshwater prawn, *M. malcolmsonii* following exposure to sublethal doses of endosulfan [12]. The depletion of the carbohydrate may be due to its rapid utilization to meet the reduced energetic efficiency under the impact of mercury. Carbohydrate metabolism is not considered to be a major energy source in fish [13], but its importance increases during hypoxia because of activation of anaerobic glycolysis. This may explain the observed depletion of the carbohydrate levels in test shrimps during the later stages of exposure as a result of increased demand of these molecules to provide energy for the cellular biochemical processes under hypoxic conditions induced by mercury. In fish, several findings were noted that, decreased level of carbohydrate due to the stress and unwanted movement of animal while toxic elements on the water. The animal was continuously using the glycogen for the survival during the stress condition as glucose molecules [14].

Effect of mercury on Protein levels

Protein is one of the important biochemical components and plays an important role in metabolic pathways and the biochemical reactions. Under tremendous stress conditions, protein supply energy in metabolic pathways and biochemical reactions. Therefore, an assessment of the protein content in different tissues could be used as a diagnostic tool for determining the physiological status of an organism [15]. The percent depletion progressively

increased with date of exposure irrespective of exposure concentrations (P< 0.05). A similar depletion in the total protein content in different tissues of crustaceans on exposure to various pesticides has been documented: in the freshwater prawn, *Macrobrachium kistensis* on exposure to pesticides by Nagabhushanam et al. [16]; in the white prawn, *F. indicus* on exposure to sublethal levels of phosphamidon and methylparathion by Reddy et al. [17]; in the marine edible crab, *Scylla serrata* on exposure to dimecron, an organochlorine pesticide, by Rao et al. [18]; in the freshwater field crab, *Paratelphusa hydrodromous* following exposure to malathion by Singaraju et al. [19]. A marked decrement in the concentrations of the total protein in the freshwater prawn, *M. malcolmsonii* [12] and two freshwater field crab species, *Oziotelphusa senex senex* [20], *Barytel phusa guerini* [21] on exposure to endosulfan have been reported. A reasonable explanation for such depletion of the protein levels in the tissues of gill and muscle of test shrimps might be due to the enhanced proteolytic activity in these organs under heavy metal stress. The depletion in the protein might be due to the diversification of energy to achieve the imminent energy demands when of animals are under toxic stress. The decreased protein concentrations might also be attributed to the destruction or necrosis of cells and resultant impairment in protein synthesis machinery [22]. Initiation of proteolysis as a result of elevated protease activity reflecting in the decrease of the protein levels of different tissues during a short-term exposure study (96 h) of endosulfan on *B. Guerini* has been documented by Reddy et al. [21]. Depletion of the protein content in the tissues of may constitute a physiological mechanism under heavy metal stress, to provide intermediates to the Kreb's cycle or to enhance osmolality, by retaining free amino acid content in the haemolymph, to compensate osmoregulatory problems encountered due to the leakage of ions and other essential molecule, during the pesticide stress [23]. Breakdown of various peptides in tissues and protein denaturation in freshwater prawn, *M. malcolmsonii* on exposure to endosulfan has been reported by Bhavan and Geraldine [24]. Suryavanshi et al. [25] revealed that, the levels of hepatic protein of test shrimps were found to be almost similar to that of control shrimps on 1- and 7 days of exposure but depletion was more prominent on 15- and 23 days of exposure of endosulfan. Moreover, the study explained the magnitude of depletion in the hepatic protein was directly proportional to the concentration of pesticide. The present investigation showed similar type of observations. Higher percent depletion in the hepatic protein was observed in test shrimps exposed to 60 ng/L compared to those exposed to 40 ng/L of endosulfan [25].
Protein depletion may be due to oxidative stress occurs when reactive oxygen species (ROS) overwhelm the cellular defences and damage proteins, cell membranes, and DNA. ROS are the by-products of electron transport chains, enzymes and redox cycling [26] and their production may be enhanced by xenobiotics [27]. The first effects of contaminants usually occur at the cellular or subcellular level and they can be good indicators of pollutant toxicity [28].

Effect of mercury on total lipid levels

The Total Lipid concentrations in different tissues mercury treated shrimps in the present study were found to be significantly lower than the concentrations in the same organs of controls (P < 0.05). Similar observations have been made in the freshwater prawn, *M. kistensis* on exposure to pesticides [16]; in the marine prawn, *M. monoceros* exposed to phosphamidon, methylparathion and lindane [29] and in the freshwater prawn, *M. malcolmsonii* exposed to endosulfan [12] and in the fishes, *Saratherodon (Oreochromis) mossambicus* and *Barbus conchonius* exposed to methylparathion and aldicarb, respectively [30, 31]. The decrement in the Total lipid levels may be due to the increased activity levels of lipase, the enzyme responsible for the breakdown of lipid into free fatty acids and glycerol. Lipids constitute the rich alternate energy reserves whose calorific value is twice that of an equivalent weight of carbohydrates and proteins and the mobilisation of lipid reserves may be due to the imposition of high energy demands to counter the toxic stress [29]. The concentrations of the total lipid decreased in all the tissues significantly with the progress of exposure period irrespective of exposure concentrations. The order of percent decrease within the tissues was: Hepatopancreas > muscle > gill. The considerable decrease in the total lipid in the HP and MU (>50%) on 30 days of exposure might be due to the drastic decrease in the glycogen levels in the same tissues. The hepatopancreas of crustaceans is analogous to the liver of vertebrates and is the centre of lipid metabolism [32]; higher levels of the total lipid could be expected in the hepatopancreas compared to other tissues. The evidence of relatively higher lipid deposition in the hepatic tissues has been reported in the penaeid prawn, *M. monoceros* exposed to phophamidon, methylparathion and lindane [29], in the freshwater prawn, *M. malcolmsonii*

exposed to endosulfan [12], in climbing perch, *A. testudineus* exposed to pesticides [33], in the fish, *B. conchonius* exposed to aldicarb [31]. In contrast, the concentrations of the total lipid decreased significantly in all the tissues of test shrimps. In conclusion, the study revealed that the sublethal doses of mercury significantly altered concentrations of biochemical constituents in the metabolically active tissues of hepatopancreas, gills, and muscles in *Litopenaeus vannamei*. The reduction in nutritive value, particularly the protein content in the shrimps exposed to sublethal doses of mercury warrants the need to regulate the pollution caused by industries in general and heavy metals in particular. Nevertheless, the results of the present investigation may be useful for assessing early warning signs of mercury poisoning and support the possibility to use *Litopenaeus vannamei* as biosensor of coastal marine and estuarine pollution by heavy metals.

ACKNOWLEDGEMENTS

The authors are thankful to The Principal, Thiru.Vi.Ka Government Arts College, Thiruvarur and The Principal, A.D.M College for Women, Nagapattinam for facilities and encouragement.

Conflict of Interest

The author(s) declare(s) that there is no conflict of interest.

REFERENCES

1. Masindi V, Muedi KL. Environmental Contamination by Heavy Metals, Intech Open. 2018; 115-133 http://dx.doi.org/10.5772/intechopen.76082.
2. Jagadeesan G, Kavitha AV. Recovery of phosphatase and transaminase activity of mercury intoxicated *Mus musculus* (Linn.) liver tissue by *Tribulus terrestris* (Linn.) (Zygophyllaceae) extract, Tropical Biomedicine, 2006; 23(1): 45–51.
3. Indra V, Gopinath. Effect of Cu and Cr compound to the freshwater fish *Channa punctatus*, J. Ecology Environ. Conser, 2004; 11(1): 8-13.
4. Sharma BM, Bharat GK, Šebková K, Scheringer M. Implementation of the Minamata Convention to manage mercury pollution in India: challenges and opportunities, Environ. Sci. Eur, 2019; 31: 96. https://doi.org/10.1186/s12302-019-0280-3.
5. Almeida JA, Diniz YS, Marques SFG, Faine IA, Ribas BO, Burneiko RC, Novelli EIB. The use of the oxidative stress responses as biomarkers in Nile tilapia (*Oreochromis niloticus*) exposed to in vivo cadmium contamination, Environ. Int. 2002; 27: 673–679.
6. Rao JV. Toxic effects of novel organophosphorus insecticide (RPR-V) on certain biochemical parameters of euryhaline fish, *Oreochromis mossambicus*. Pestic. Biochem. Physiol. 2006; 86: 78–84.
7. American Public Health Association (APHA), Standard Methods for the Examination of Water and Wastewater. 14th Edition, American Public Health Association, Washington DC, 1976; 1193.
8. Roe JR. The determination of sugar in blood and spinal fluid with anthrone reagent, Biol. Chem., 1955; 20: 335-343.
9. Lowry DH, Rosebrough NJ, Far AL, Randal RJ. Protein measurement with folin phenol reagent. J. Biol. Chem. 1951; 193: 265–275.
10. Folch J Lees M, Sloane Stanley GH. A simple method for the isolation and purification of total lipides from animal tissues, J Biol Chem. 1957; 226(1): 497-509.
11. Reddy MS, Rao KVR. Methylparathion induced alteration in the tissue carbohydrate catabolism of marine prawn, *Metapenaeus monoceros*, Bull. Environ. Contam. Toxicol. 1991; 47: 925–932.
12. Bhavan PS, Geraldine P. Alterations in concentrations of protein, carbohydrate, glycogen, free sugar, and lipid in the prawn, *Macrobrachium malcolmsonii* on exposure to sublethal concentrations of endosulfan, Pestic. Biochem. Physiol. 1997; 58: 89–101.

13. Walton MJ, Cowey CB. Aspects of intermediary metabolism in salmonid fish, Comp. Biochem. Physiol. 1982; 738: 59–79.
14. Paramanandham J, Kavitha AV, Ronald Ross P, Thenmozhi P, Muthulingam M. Effect of mercuric chloride and the influence of glutathione in liver tissue of fingerlings of fish *Labeo rohita* (Hamilton). Int. J. The Ecoscan. 2011; 1: 53- 57.
15. Prasath PMD, Arivoli S. Biochemical Study of Freshwater Fish *Catla catla* with Reference to Mercury Chloride, Iran. J. Environ. Health. Sci. Eng. 2008; 5 (2): 109-116.
16. Nagabhushanam R, Deshpande J, Sarojini R. Effects of some pesticides on the biochemical constituents of the freshwater prawn, *Macrobrachium kistensis*, In: Proceedings of the National Symposium on Ecotoxicology. 1972; 73–84.
17. Reddy MS. Rao KVR, Murthy BN. Changes in nitrogen metabolism of penaeid prawn, *Penaeus indicus*, during sublethal phosphamidon and methylparathion induced stress, Bull. Environ. Contam. Toxicol. 1988; 41: 344–351.
18. Rao KS, Nagabhushanam R, Sarojini R. Acute toxicity of some pesticides to the marine crab, *Scylla serrata*. Environ. Ecol. 1987; 5: 181–182.
19. Singaraju R, Subramanian M, Varadaraj A. Sublethal effects of malathion on the protein metabolism in the freshwater field crab *Paratelphusa hydrodromous*, J. Ecotoxicol. Environ. Monit. 1991; 1: 41–44.
20. Rajendra Prasad Naidu K. Impact of endosulfan on carbohydrate and protein metabolism of the freshwater field crab, *Oziotelphusa senex senex* (Fabricius). M.Phil. Dissertation, S.V. University, Tirupathi, India (1985).
21. Reddy AN, Venugopal BRK, Reddy LN. Effects of endosulfan 35 EC on certain aspects of protein metabolism in various tissues of a freshwater field crab, *Barytelphusa guerini*, Pestic. Biochem. Physiol. 1991; 39: 121–129.
22. Bradbury SP, Symonic DM, Costs JR, Atchison GJ. Toxicology of fenvalerate and its constituent isomers to the fathead minnow (*Pimephales promelos*) and blue gill (*Lepomis macrochirus*), Bull. Environ. Contam. Toxicol. 1987; 38: 727–735.
23. Yasmeen R, Tulasi SJ, Rao JVR. Metabolic changes in the air-breathing fish *Anabas scandens* on long term exposure to endosulfan, Pestic. Biochem. Physiol. 1991; 40: 205–211.
24. Bhavan PS, Geraldine P. Biochemical stress responses in the tissues of the prawn *Macrobrachium malcolmsonii* on exposure to endosulfan, Pestic. Biochem. Physiol. 2001; 70: 27–41.
25. Suryavanshi U, Sreepada RA, Ansari ZA, Nigam S, Badesab S. A study on biochemical changes in the penaeid shrimp, *Metapenaeus monoceros* (Fabricius) following exposure to sublethal doses of organochlorine pesticide (endosulfan), Chemosphere. 2009; 77(11): 1540–1550. doi:10.1016/j.chemosphere.2009.09.051
26. Kelly KA, Havrilla CM, Brady TC, Abramo KH, Levin ED. Oxidative stress in toxicology: established mammalian and emerging piscine model systems, Environmental Health Perspectives, 1998; 106(7) 375-384.
27. Slaninova A, Smutna M, Modra H, Svobodova Z. A review: Oxidative stress in fish induced by pesticides, Neuroendocrinology Letters. 2009; 30 (1): 2-12.
28. Overstreet RM. Aquatic pollution problems, Southeastern U.S. coasts: histopathological indicators, Aquatic Toxicology, 1988; 11 (3-4): 213-139.
29. Reddy MS, Rao KVR. In vivo modification of lipid metabolism in response to phosphamidon, methylparathion and lindane exposure in the penaeid prawn, *Metapenaeus mononceros*. Bull. Environ. Contam. Toxicol. 1989; 43: 603–610.
30. Rao JR, Rao KVR. Lipid derivatives in the tissue of freshwater teleost, *Saratherodon mossambicus*. Effects of methylparathion, Proc. Indian Natl. Acad. Sci. 1981; 47: 53–58.
31. Pant J, Tewari H, Gill TS. Effects of aldicarb on the blood and tissues of a freshwater fish, Bull. Environ. Contam. Toxicol. 1987; 38: 36–41.
32. Chang ES, O'Connor JD. Metabolism and transport of carbohydrates and lipids. In: Mantel, L.H. (Ed.), The Biology of Crustacea. Internal Anatomy and Physiological Regulation, vol. 5. Academic Press, New York, 1983; pp. 263–287.
33. Bakthavathsalam R, Reddy YS. Lipid kinetics in relation to the toxicity of three pesticides in the climbing perch, *Anabas testudineus* (Bloch). Proc. Indian Natl. Sci. Acad. 1981; B47: 670–676.

Table 1. Changes in the biochemical parameters (mg/g wet weight of tissue) in Hepatopancreas of *Litopenaeus vannamei* exposed to sublethal concentrations of mercury (n=6) (Mean±SD)

Biochemical parameters	Control			10% SLC			30% SLC		
Exposure Period	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Carbohydrate % COUTC	13.31±0.45	13.45±0.48	13.61±0.45	10.55±0.62 -20.73	8.27±0.69 -38.51	6.31±0.58 -53.63	9.31±0.46 -30.05	6.36±0.66 -52.71	4.16±0.55 -69.43
Total Protein % COUTC	55.0±0.72	54.2±0.69	54.6±0.48	46.6±0.46 -11.63	42.8±0.56 -21.03	36.5±0.52 -34.06	37.5±0.71 -36.00	32.3±0.47 -42.80	26.6±0.66 -54.21
Total lipid % COUTC	35.56±0.47	36.48±0.38	35.68±0.68	31.16±0.52 -12.37	28.27±0.32 -22.50	23.61±0.29 -33.82	27.1±0.36 -23.79	22.12±0.31 -39.36	17.16±0.31 -51.90

%COUTC- Percentage Change Over Untreated Control; SLC- Sub lethal concentration.
All the values are significant at 5% level of ANOVA

Table 2. Changes in the biochemical parameters (mg/g wet weight of tissue) in Gill tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of mercury (n=6) (Mean±SD)

Biochemical parameters	Control			10% SLC			30% SLC		
Exposure Period	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Carbohydrate %COUTC	8.12±0.67	8.31±0.85	8.51±0.78	6.82±0.61 -16.01	5.52±0.68 -23.94	5.32±0.38 -37.48	5.51±0.26 -32.94	4.28±0.61 -48.49	2.88±0.55 -66.15
Total Protein %COUTC	30.0±0.55	30.2±0.56	31.2±0.38	26.5±0.52 -11.66	26.3±0.38 -12.91	25.9±0.38 -16.98	26.4±0.65 -24.66	22.5±0.51 -39.37	18.2±0.72 -60.57
Total lipid %COUTC	17.16±0.35	17.61±0.36	17.21±0.62	15.12±0.32 -11.88	14.12±0.61 -19.81	12.31±0.32 -28.47	14.28±0.64 -16.78	11.16±0.38 -36.62	9.12±0.32 -47.01

%COUTC- Percentage Change Over Untreated Control; SLC- Sub lethal concentration.
All the values are significant at 5% level of ANOVA

Table 3. Changes in the biochemical parameters (mg/g wet weight of tissue) in Muscles tissues of *Litopenaeus vannamei* exposed to sublethal concentrations of mercury (n=6) (Mean±SD)

Biochemical parameters	Control			10% SLC			30% SLC		
Exposure Period	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days	10 Days	20 Days	30 Days
Carbohydrate %COUTC	10.41±0.78	10.81±0.91	10.62±0.68	8.32±0.89 -20.07	6.32±1.14 -24.51	6.78±0.47 -36.15	6.98±0.68 -20.61	5.12±0.65 -52.63	3.16±0.42 -70.24
Total Protein %COUTC	80.6±0.35	80.2±0.38	80.31±0.48	71.5±0.66 -11.29	61.1±0.46 -23.81	54.2±0.48 -32.51	61.6±0.58 -27.41	52.2±0.56 -33.90	42.1±0.59 -76.46
Total lipid %COUTC	20.11±0.38	20.19±0.35	20.15±0.51	17.11±0.55 -14.91	13.27±0.62 -34.27	11.38±0.38 -43.52	14.45±0.38 -28.14	10.12±0.38 -49.87	7.26±0.26 -63.97

%COUTC- Percentage Change Over Untreated Control; SLC- Sub lethal concentration.
All the values are significant at 5% level of ANOVA.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

In vitro Antioxidant and Anti-Inflammatory Properties of Aerial Parts of *Coldenia procumbens* Linn.

S. Babu[1]*, A.S. Shijila Rani[2], A. Anbukumaran[3] and V. Ambikapathy[1]

[1]Department of Botany, A.V.V.M. Sri Pushpam College (Auto) (Affiliated to Bharathidasan University), Poondi, Thanjavur, Tamil Nadu, India.
[2]Department of Microbiology, Marudupandiyar College (Affiliated to Bharathidasan University), Thanjavur, Tamil Nadu, India.
[3]Department of Microbiology, Urumu Dhanalakshmi College (Affiliated to Bharathidasan University), Trichy, Tamil Nadu, India.

Received: 16 Nov 2020 Revised: 18 Dec 2020 Accepted: 21 Jan 2021

***Address for Correspondence**
S. Babu
Department of Botany,
A.V.V.M. Sri Pushpam College (Auto)
(Affiliated to Bharathidasan University),
Poondi, Thanjavur, Tamil Nadu, India.
Email: selvababunvk@gmail.com

ABSTRACT

In the present investigation, the pharmacological studies of antioxidant and anti-inflammatory activities were evaluated from aerial parts of methanolic extract of *Coldenia procumbens* L. (Boranginaceae family) by *In vitro* methods. The pharmacological studies revealed that the aerial parts of methanolic extract of *Coldenia procumbens* may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical which is responsible for oxidation. The results suggested that satisfactory anti-inflammatory activity of aerial parts with methanolic extract of *Coldenia procumbens*.

Keywords: *Coldenia procumbens*, methanol, antioxidant activity, anti-inflammatory activity.

INTRODUCTION

The word herb was used in herbal medicine which is also known as botanical medicine or as phototherapy or phytomedicine which means a plant or plant part is used to make medicine to assist in the healing process during illness and disease (Satish Kumar, 2011). Herbal medicines are being used in 80% of the world population primarily in the developing countries for primary health care. The chemical constituents present in plants are a part of the physiological functions of living flora and compatibility with the human body (Zhang, 1998). Antioxidants, free radical scavengers prevent pathological conditions of human body namely its chemia, anaemia, asthma, arthritis,

inflammation, neurodegeneration and aging process (Polterait, 1997; Dimitrios Stagos, 2020). Oxidation is a natural metabolic process in cell, some time resulting in the formation of free radicals such as hydrogen peroxide (H_2O_2), hypochlorous acid (HOCl) and ozone (O_3) (Bandhopadhyay, 1999). Free radicals are also generated through cigarette smoke, automobile exhaust, radiation, pesticides and air pollution (Li and Trush, 1994). Free radicals play a crucial role in normal aging and neurodegenerative disorders (Beal, 1995). Free radicals damage the cell membranes, proteins, fats and cause heavy damage to the genetic material in the cell, this oxidative damage increase with age. Damaged protein and enzymes can result in premature wrinkling, aging and even cancerous growth on the damaged skin (Halliwell, 1999; Behl and Mosmann, 2001). *Coldenia procumbens* Linn. is an annual herb; common weed widespread throughout Africa, tropical Asia and Australia at altitudes up to 750 m. It belongs to family Boraginaceae and having 24 species of prostate (Mangeshrao and Nadkarni, 1995; CSIR, 1950). In India, it is found widely in South India on waste lands, common in dry rice grounds. *Coldenia procumbens* is only species of its genus has a place both in the *Hortus bengalensis* and Moon's Catalogue of ceylon plants (White Law Anisile, 1982). This plant is widely used in traditional medicines in India, Africa and Malaysia. Inflammation is a mandatory immune response which helps resist against infections and pathogens which maintains homeostasis. It is only when there is a prolonged presence harmful effects are more than beneficial effects (Ahmed, 2011). Long-term inflammation causes chronic inflammations associated along with atherosclerosis, diabetes, asthma and neurogenerative diseases. There are groups of clinical disorders due to inflammatory responses such as rheumatoid arthritis, obesity, and allergic inflammations known as inflammatory disorders (Chen *et al*., 2018). It has also proven that there is integrity between inflammation and cancer biology. Even DNA damage causes inflammation. This helps understand the importance toward curing anti-inflammatory disorders (Pahwa *et al*., 2020; Aru *et al*., 2005). Here in the present study the *Coldenia procumbens* have act as very good properties of free radical scavenging and anti-inflammatory activity.

MATERIALS AND METHODS

Plant collection and extraction

Coldenia procumbens plant is collected manually from harvesting rice field, N.V Kudikadu, Thanjavur district, Tamilnadu, India. The plant was authenticated by St. Josesph's College, Trichy. They were washed and shade dried. These dried materials are pulverized to attain a coarse powder. The whole plants of *Coldenia procumbens* powder was extracted by cold maceration using methanol for 7 days with proper intermediate shaking and the macerate was filtered into a container. The extract was concentrated in an evaporator. The dried residue was stored in a desiccator. This extract was used for *in vitro* antioxidant activity screening.

Determination of antioxidant activity

DPPH Free radical scavenging activity (Atiqur *et al*., 2008)

Different concentration (100 - 500µl/ml) of test sample and standard (BHA) were prepared. To 3 ml of 0.004% (w/v) of methanolic solution of DPPH was added and shaked well and then incubated at room temperature for period of 30 min. A blank was prepared in similar way, without plant extract and absorbance was measured at 517 nm.

Scavenging activity was expressed as the percentage inhibition calculated using formula:

Percentage Inhibition = {(Absorbance of control sample – Absorbance of test sample) /Absorbance of control sample} X 100

Ferric Reducing Antioxidant Power (FRAP) (Benzie and Strain, 1996)

Different concentration (100 - 500µl/ml) of test samples was added to 3.8 ml of FRAP reagent (0.3 M sodium acetate buffer pH 3.6, 10 mM TPTZ (2, 4, 6-TripyridylS-Triazine) solution in 40 mM HCL and 20 mM $FeCl_3$ solution) and incubated at 37°C for 30 min and increased in absorbance was measured at 593 nm. The antioxidant capacity based on the ability of the leaf extract to reduce ferric ions was expressed in terms of ascorbic acid equivalent (µl/ml).

ABTS radical cation decolourization assay (Re *et al.*, 1999)

In this assay, the oxidant was generated by persulfate oxidation of 2, 2,-azinobis (3-ethylbenzoline-6-sulfonic acid)-(ABTS2-). ABTS radical cation ($ABTS^{+}$) were produced by reacting ABTS solution (7mM) with 2.45 mM ammonium persulphate and the mixture was allowed to stand in dark at room temperature for 12-16 hrs before use. After 16 hrs, this solution was diluted with ethanol until the absorbance at 734 nm.

Superoxide Scavenging Assay (Nishimiki *et al.*, 1972)

About 1ml of Nitroblue Tetrazolium (NBT) solution containing 156µM NBT dissolved in 1.0 ml of phosphate buffer (100mM, pH 7.4) and 1ml of NADH solution containing 468 µM of NADH which was dissolved in 1ml of phosphate buffer (100 mM, pH 7.4) with 0.1 ml of various concentrations of plant extracts and the reference compounds (100 - 500µl/ml) were mixed and the reaction was started by adding 100 µl of Phenazine methosulphate (PMS) solution containing 60 µM of PMS 100 µl of phosphate buffer (100 mM, pH 7.4). The reaction mixture was incubated at 25°C for 5 min and the absorbance at 560nm was measured against the control samples.

Total phenolic content (Ikram *et al.*, 2009)

Various concentration of test sample (100 - 500µl/ml) and standard Gallic acid were prepared. 1.5 ml Folin ciocalteu's reagent was added to the volumetric flask containing the test sample and standard solution. After 5 min, 4 ml of sodium carbonate (7%) solution was added. Final volume was made up to 10 ml by using distilled water. Blank determination was done by using the methanol in place of test or standard solution. After 1 hr. measure the absorbance at 760 nm against the blank solution.

Determination of Anti-inflammatory activity

***In vitro* BSA denaturation method (Williams *et al.*, 2008)**

About 0.2% w/v of BSA solution was prepared and adjusted pH (tris buffer saline) to 6.8 using glacial acetic acid. Different concentrations (100, 200, 300, 400 and 500µl/ml) of methanolic extracts of *Coldenia procumbens* was used as the test and standard drug (Diclofenac sodium). The test tubes were heated at 72°C for 5 minutes and then cooled for 10 minutes. The absorbance of these solutions was determined by using UV spectrophotometer at 660nm. The percentage of inhibition of precipitation (denaturation of the protein) was determined on the percentage basis relative to the control using the following formula.

% of Inhibition = 100 X (At/Ac - 1)

Where, At = absorbance of the test sample, Ac = absorbance of the control

***In vitro* egg albumin denaturation method (Rahman *et al.*, 2015)**

About 0.2 ml of egg albumin mixture were taken and added 2.8 ml of Phosphate buffer (pH 6.4) and different concentrations (100, 200, 300, 400 and 500µl/ml) of methanolic extract of *Coldenia procumbens* was used as the test and standard drug (Diclofenac sodium). A similar volume of double distilled water served as control. The above mixtures were incubated at 37±2ºC, for 15 min and then heated at 70ºC for 5 min. The test was repeated 3 times. After cooling the absorbance was measured at 660 nm using multi-mode micro plate reader (Synergy Biotech, USA). The percentage inhibition of denaturation which is an index of anti-inflammatory activity was calculated using the following formula:

% of Inhibition = 100 × (At/Ac - 1)

Where, At = absorbance of the test sample, Ac = absorbance of the control

***In vitro* anti-inflammatory activity by HRBC membrane stabilization method (Oyedapo O. Famurewa, 1995)**

The assay mixture contains 1ml phosphate buffer (pH 7.4, 0.15 m), 2 ml hypo saline (0.36 %), 0.5 ml HRBC suspension (10 % v/v) with 0.5 ml of plant extracts of various concentrations (100, 200, 300, 400 and 500µl/ml) of

methanolic extract of *Coldenia procumbens* was used as the test and standard drug (diclofenac sodium) and control (distilled water instead of hypo saline to produce 100 % haemolysis) were incubated at 37°C for 30 min and centrifuged respectively. The haemoglobin content in the suspension was estimated using spectrophotometer at 560 nm.

% of Inhibition = 100 X (At/Ac - 1)

where, At = absorbance of the test sample, Ac = absorbance of the control

RESULT AND DISCUSSION

DPPH radical scavenging assay

In the present investigation, the DPPH assay showed that aerial parts of *C.procumbens* Linn. has high concentration dependent scavenging activity against DPPH free radicals. From Table 1 observed that methanol fraction showed maximum DPPH radical scavenging activity in 500 µl/ml compared to 200 µl/ml concentrations. Medpilwar *et al*. (2015) reported that the DPPH was a stable free radical that accepts an electron or hydrogen radical to become a stable diamagnetic molecule. The antioxidants reduce the stable DPPH radical to a yellow coloured diphenyl-picrylhydrazine with maximum absorption at 517 nm. The percent radical scavenging activity of ethanolic extracts of *Bougainvillea* species under study was found to be about 79 to 83%. Maajitha Begam *et al*. (2019) and Ara and Islam (2020) revealed that the plant extracts may have significant antioxidant effect which is probably mediated by inhibition of DPPH free radical, which is responsible for oxidation.

Ferric reducing power assay

Ferric reducing antioxidant power (FRAP) assay is based on the ability of antioxidants to reduce Fe^{3+} to Fe^{2+} in the presence of TPTZ complex forming an intense blue Fe^{2+} TPTZ complex with an absorption maximum at 593 nm (Benzie and Strain, 1996). Chappell and Hansford, (1972) reported that the yellow colour of the test solution changes to various shades of green and blue is depending upon the reducing power of each compound. A higher absorbance at 700 nm indicates a higher reducing power. In the present study Table 2 depicted reducing capacity of different fraction of *C.procumbens* Linn. and ascorbic acid. It was observed that absorbance of test sample and standard sample was increased with increase in concentration of test and standard. *C.procumbens* Linn. showed concentration dependant reducing capacity. This increased FRAP activity was found to correlate with the higher phenol content in the *C.procumbens* golden glow.

ABTS radical cation scavenging activity

The potential decrease in the concentration of ABTS radical was due to the scavenging ability of Gallic acid standard and *C. procumbens* methanolic extract showed significant ABTS scavenging activity at 500 µg/ml for Gallic acid standard and *C. procumbens* extract respectively (Table. 3).

Superoxide Scavenging Assay

The potential increase in the superoxide scavenging ability of BHT standard and *Coldenia procumbens* methanolic extract showed significant superoxide scavenging activity at 500 µg/ml concentration for BHT standard and *C.procumbens* methanolic extract respectively (Table. 4). Superoxide anion, which is a reduced form of molecular oxygen, is an initial free radical formed from mitochondrial electron transport systems. Superoxide anions serve as precursors to active free radicals that have the potential to react with biological macromolecules and thereby induce tissue damage (Robak and Gryglewski, 1988).

Total phenolic content

Phenolics are very important plant constituents because of their scavenging capability due to their hydroxyl groups. The various phenolic antioxidants such as flavonoids, tannins, coumarins and xanthones scavenge the radicals. The phenolic substance is known to possess ability to reduce oxidative damage and act as antioxidants (Sakat *et al*., 2017).

Table 5 showed the total phenolic content, the concentration was increasing and also the activity was increased. Similarly Vadnere *et al*. (2011) evaluated that the total phenolic content in the extract was determined by Folin-ciocalteu method. The total phenolic content in ethyl acetate fraction, benzene and methanol fraction of whole plant of *Cassia occidentalis* was expressed as gallic acid equivalent per mg of extract. Some workers are Malathi Suvarna *et al.* (2017) and Poulose and Mathew (2016) are recorded the antioxidant activity by using ethanol and methanol extract *C.procumbens*. These plants were good source for the antioxidant activity.

Anti-inflammatory activity of methanolic extract of *C.procumbens*

The effect of anti-inflammatory properties of *C.procumbens* with methanolic extract of 100, 200, 300, 400 and 500µl/ml was resulted as 23.5±0.7, 37.5±1.5, 53.5±1.2, 75.5±0.7 and 67.5±0.5% of activity were observed with diclofenac sodium was 48.9±0.8, 73.2±3.4 and 69.6±0.8 % of activity and 100 and 200µl/ml showed no activity on anti-inflammatory as well as IC_{50} value of 48.1 in diclofenac sodium and plant extract as 47.4% observed *in vitro* BSA denaturation activity recorded respectively (Table 6). In anti-denaturation assay conducted in denaturation of BSA is induced by heat treatment. The denatured BSA expresses antigens associated to Type III hyper-sensitive reaction which are related to diseases such as serum sickness, glomerulo nephritis etc., (Gell and Benacerraf, 1959; Ara and Islam *et al*., 2020).

The *invitro* egg albumin denaturation activity of methanolic extract of *C.procumbens* with different concentration of 100, 200, 300, 400 and 500µl/ml was 54.1±0.7, 63.1±1.3, 85.3±1.4, 88.8±1.4 and 75.5±0.8% of activity when compared with diclofenac sodium as a standard was 48.9±0.8, 73.2±3.4 and 69.6±1.8% of activity represented with respective concentration of plant extract (Table 6). Mikami *et al*. (1983) reported in earlier, the ethanolic extract of *C. procumbens* extract properties as an inhibitor of leukocyte migration and the formation of pleural exudates. Inflammation involves proliferation of macrophages, neutrophils and fibroflasts which are basic sources of granuloma formation. Hence, the decrease in the weight of granuloma indicated that the proliferative phase was effectively suppressed by the ethanolic extract of *C. procumbens* (Arul *et al*., 2005). As per the *In vitro* HBRC stabilization activity of methanolic extract of *C.procumbens* with different concentration of 100, 200, 300, 400 and 500µl/ml was 49.4±0.9, 62.2±1.1, 72.1±1.5, 79.9±0.8 and 63.2±1.1% of anti inflammatory activity results were observed and diclofenac sodium as a standard was 48.9±0.8, 73.2±3.4 and 69.6±1.8% in 300, 400 and 500µl/ml effect was observed and IC_{50} values of standard as 48.1% and plant extract results 46.7% were recorded respectively (Table 6). Similarly, Moulishree *et al*. (2020) reported that the anti-inflammatory activity increases as the concentration of the *C.procumbens* extract increases and proves to be significant on comparison with the activity of standard drug, diclofenac drug.

REFERENCES

1. Aazza S, Lyoussi B. and Miguel MG (2011). Antioxidant activity of some Morrocan hydrosols. *J. of Medi. Plant. Res*.; **5**(30):6688-6696.
2. Ahmed AU., (2011). An overview of inflammation: Mechanism and consequences. Front Biol; 6:274-81.
3. Ara J and Islam MS., (2020). Antioxidant and Anti-inflammatory Potential Evaluations of Methanolic Extract of *Tridax procumbens* (Linn.), a Phytochemical Screening. *Austin J Clin Trials Open Access*.; **2**(1): 1002.
4. Arul B, Kothai R, Suresh kumar K. and Christina AJ. (2005). Anti-inflammatory activity of *Coldenia procumbens* Linn. *Pak J. Pharm. Sci*.; 18:17-20.
5. Arulmozhi S, Mazumder P.M, Narayanan L.S. and Thakurdesai P.A., (2010). *In vitro* antioxidant and free radical scavenging activity of fractions from *Alstonia scholaris* Linn. *R.Br. Inter. J. of Pharma. Tech. Res*.; **2**: 18-25.
6. Atiqur R, Rahman M.M. and Sheik M.I., (2008). Free radical scavenging activity and phenolic content of *Cassia sophara* L. *Afri J. of biotec*.; **7**: 1591-1593.
7. Bandhopadhyay U, Das, D. and Banerjee, R.K., (1999). Reactive oxygen species: oxidative damage and pathogenesis. *Current Sci.;* 77:658-666.
8. Beal F., (1995). Aging, energy and oxidative stress in neurodegenerative diseases. *Annals of Neur*.; 383:357-366.
9. Behl C. and Mosmann, B., (2002). Antioxidant neuroprotection in Alzheimer's disease as preventive and therapeutic approach. *Free Radical Bio. and Medi*.; 33:182-191.

10. Benzie I.F. and Strain J.J., (1996). The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power" the FRAP assay. *Anal. Biochem*. 239:70–76.
11. Chappell J.B. and Hansford R.G., (1972). Preparation of mitochondria from animal tissues and yeasts. In: Birnie GD, editor. Subcellular components: preparation and fractionation. London: Butter worths: 77–91.
12. Chaulya, N.C., Haldar, P.K. and Mukharjee, A., (2010) *In vitro* Free radical scavenging activity of methanolic extract of rhizome of *Cyperus tegetum* Roxb. (Cyperaceae). *Inter. J. of cur. Pharma. Res*. 2,: 39-43.
13. Chen, L., Deng, H., Cui, H., Fang, J., Zuo Z. and Deng J., (2018). Inflammatory responses and inflammation-associated diseases in organs. *Oncotarget*;**9**:7204-18.
14. CSIR, (1950) The Wealth of India: A Dictionary of Indian Raw Materials and Industrial Products, Vol I, A-B, Council of Scientific & Industrial Research (CSIR), New Delhi, India: 307.
15. Dimitrios Stagos (2020). Antioxidant Activity of Polyphenolic Plant Extracts. *Antioxidants*; 9(19): 2-7.
16. Gell, P.G.H. and Benacerraf, B., 1959. Studies on hypersensitivity-II delayed hypersensitivity to denatured proteins in guinea pig. *Immun.;* 2: 64.
17. Halliwell B (1999). Establishing the significance and optimal intake of dietary antioxidants: the biomarker concept. Nutri. Revi. 57:104-113.
18. Ikram E.H.K., Jalil A.M, Ismail A., Idris A., Azlan A, Nazari H.M., Dilton N.A. and Mokhtar R.A., (2009). Antioxidant capacity and total phenolic content of Malaysian under utilised fruits. *J. of food. Comp. and Anal*.; 22: 388- 393.
19. Juntachote, T., Berghofer, E., Siebenhandl, S. and Bauer, F. (2006). The antioxidative properties of Holy basil and Galangal in cooked ground pork. *Meat sci*.; 72: 446-456.
20. Kadam, V.J., Joshi, Y., Sawant, H.P. and Jadav, T.A. (2010). Free radical scavenging activity of aqueous solution of black salt. *Inter. J. of pharm. and pharma. Sci*.; 2: 95-96.
21. Lavanya, R., Maheshwari, U.S, Harish, G. and Raj J.B., (2010) Investigation of in vitro antioxidant anti-inflammatory and anti-arthritic activities in the leaves of *Coldenia procumbens* Linn. *Res. J. of Pharma. Bio. and Chem. Sci*.; 1: 753-762.
22. Li Y. and Trush M.A, (1994) Reactive oxygen dependent DNA damage resulting from the oxidation of phenolic compounds by a copper redox cycle. *Cancer Res.;* 54:1895-1898.
23. Maajitha Begam, A., Muhammad Ilyas, M. H., Shijila Rani A.S and Ambikapathy, V., (2019). Phytochemical and Antioxidant Studies of *Tamarindus indica Extract*. *J. of Emerging Techno. and Innov. Res*.; **6**(1): 525- 532**.**
24. Malathi Suvarna C.H., Santosh, B., Sireesha, C.H. and Sarada Durga, C.H., (2017) Phytochemical screening and *in-vitro* anti-oxidant activity of methanolic leaf extract of *Coldenia procumbens* LINN. *Worl. J. of Pharma. Res*.; 6(14): 839-845.
25. Mangeshrao Nadkarni, K. and Nadkarni, A.K., (1955) Indian Materia medica, 3, Vol 1, Popular Book Depot, Bombay: 371.
26. Medpilwar, M. Darshil M., Meenakshi U., Neha L., Madhavi V. and Mugdha H., (2015) *In-vitro* Antioxidant and Anti-lipid Peroxidation Activity of Ethanolic Extracts of *Bougainvillea shubhra, Bougainvillea peruviana* and *Bougainvillea bhuttiana* Golden Glow: A Comparative Study. *J. of Nat. Rem*.; 15 (1): 43-48.
27. Mikami, T. and Miyasaka, E., 1983. Effects of several anti-inflammatory drugs on the various parameters involved in the inflammatory response in rat carrageenan induced pleurisy. *Eur. J. Pharmacol*.; **95**: 1-12.
28. Morgan K., (2002). Medicine of the Gods: Basic Principles of Ayurvedic Medicine.
29. Moulishree R., Gayathri R. and Vishnu Priya, V., (2020). Determination of anti-inflammatory potential of crude ethanolic leaf extract of *Coldenia procumbens. Drug Invention Today*; 14(2): 54-56.
30. Oyedapo, O.O. and Famurewa, A.J., 1995. Antiprotease and membrane stabilizing activities of extracts of Fagara. *Zanthoxyloids, Olax subscorpioides* and *Tetrapleura tetraptera*. *Pharmacol. Biol*.; **33**: 65-69.
31. Pahwa R., Goyal, A., Bansal P. and Jialal I., (2020). Chronic Inflammation. Treasure Island (FL): Stat Pearls Publishing.
32. Polterait O., (1997) Antioxidant and free-radical scavengers of natural origin, *Curr. Org. Chem*.; 1: 415-440.
33. Poulose, P. and Mathew, R., (2016) Study of antioxidant activity of monoolein based liquid crystalline nanoparticle of quercetin. *Asian Pacific Journal of Pharm. & Phytochemi.;* 1(04): 139-145.

34. Rahman, H., Eswaraiah, M.C. and Dutta, A.M., 2015. *In-vitro* anti-inflammatory and anti-arthritic activity of Oriza sativa Var. Joha Rice (An Aromatic Indigenous Rice of Assam). American-Eura. *J. of Agri. and Environ. Sci*.; **15**(1):115-121
35. Re, R., Pellegrini, N., Protoggenete, A., Pannala, A., Yang, M. and Rice Evans, C., (1999) Antioxidant activity applying an improved ABTS radical cation decolouration assay. *Free Radic. Biol. Med*.; 26:1231-1237
36. Robak, J. and Gryglewski, R.J., (1988). Flavonoids are scavengers of superoxide anions. *Biochem. Pharmacol*.; 37: 837-841.
37. Sakat, S., Juvekar A.R. and Gambhire, M.N., (2010) Antioxidant and anti-inflammatory activity of methanol extract of *Oxalis corniculata* Linn. *Inter. J. of Pharma. and Pharmace. Sci.*; 2: 146-155.
38. Saleem, A., Husheem, M., Harkonen, P. and Pihlaja, K., 2002. *J. Ethnopharmacol*; **81**:327-336
39. Satish Kumar B.N, (2011) Phytochemistry and Pharmacological Studies of *Pongamia Pinnata* (Linn.) Pierre. *Inter. J. of Pharma. Sci. Review. and Res*.; 9(2): 12-19.
40. Vadnere GP, Patil AV, Jain SK. and Wagh SS (2011) Investigation on *in-vitro* Antioxidant activity of whole plant of *Cassia occidentalis* Linn. (Caesalpiniaceae). *Inter. J. of Pharm. Tech. Res*.; 3(4): 1985-1991.
41. White Law Anisile (1826) Materia Indica, Vol II, Rees, Longman, Orme, Brown, and Green, London: 435-436.
42. Williams, L.A.D., Connar, A.O., Latore, Dennis, O., Ringer, S., Whittaker, J.A., Conard, J., Vogler, B., Rosner, H. and Kraus, W., 2008. The *invitro* anti-denaturation effects induced by natural products and non-steroidal compounds in heat treated (immunogenic) bovine serum albumin is proposed as a screening assay for the detection of anti-inflammatory compounds, without the use of animals, in the early stages of the drug discovery process. *West Indian Med J*.; **57**: 327-331.
43. Zhang X (1998). Regulatory Situation of Herbal Medicines. A Worldwide Review (WHO/trm/ 98.1), Geneva, World Health Organization.

Table 1: Antioxidant activity of methanolic extract of *C. procumbens* by DPPH method

Concentration of plant extract (µl/ml)	Percentage of activity (%)	
	Gallic acid (std)	Results
100	54.1±2.1	60.4±1.6
200	67.2±1.9	73.1±1.6
300	77.3±2.5	83.8±2.1
400	82.5±1.4	95.4±1.9
500	80.2±1.3	85.9±1.5
IC_{50}	46.4	46.0

Standard deviation ± standard error

Table 2: Antioxidant activity of methanolic extract of *C.procumbens* by Ferric reducing power assay

Concentration of plant extract (µl/ml)	Percentage of activity (%)	
	BHT (std)	Results
100	10.2±0.9	19.7±2.0
200	24.3±0.4	27.3±0.9
300	35.4±1.1	41.4±1.0
400	59.2±1.0	62.1±0.8
500	48.7±0.8	53.9±1.4
IC_{50}	48.2	47.9

Standard deviation ± standard error

Table 3: Antioxidant activity of methanolic extract of *C.procumbens* by ABTS radical cation scavenging method

Concentration of plant extract (µl/ml)	Percentage of activity (%)	
	BHA (std)	Results
100	11.3±0.4	31.8±0.8
200	21.5±0.8	52.1±0.9
300	33.7±0.7	64.2±1.2
400	59.8±0.6	88.1±2.5
500	54.8±0.9	72.5±2.4
IC_{50}	48.2	49.2

Standard deviation ± standard error

Table 4: Antioxidant activity of methanolic extract of *C.procumbens* by Superoxide scavenging method

Concentration of plant extract (µl/ml)	Percentage of activity (%)	
	Ascorbic acid (std)	Results
100	10.7±0.2	35.9±1.1
200	15.1±0.7	51.3±0.8
300	21.7±0.3	62.1±1.0
400	55.1±0.7	83.2±0.9
500	42.5±0.9	76.6±1.1
IC_{50}	48.5	46.9

Standard deviation ± standard error

Table 5: Antioxidant activity of methanolic extract of *C. procumbens* by Phenolic content

Concentration of plant extract (µl/ml)	Percentage of activity (%)	
	Ascorbic acid (std)	Results
100	0.5±0.1	0.8±0.3
200	0.7±0.2	1.2±0.1
300	1.1±0.1	1.4±0.5
400	2.2±0.4	4.1±0.4
500	1.5±0.3	1.9±0.2
IC_{50}	47.9	49.0

Standard deviation ± standard error

Table 6: *In vitro* egg albumin denaturation activity of methanolic extract of *C. procumbens*

S. No	Concentration of plant extract (µl/ml)	Percentage of activity (%)			
		Diclofenac Sodium (std)	Egg albumin	HBRC	BSA
1	100	-	54.1±0.7	49.4±0.9	23.5±0.7
2	200	-	63.1±1.3	62.2±1.1	37.5±1.5
3	300	48.9±0.8	85.3±1.4	72.1±1.5	53.5±1.2
4	400	73.2±3.4	88.8±1.4	79.9±0.8	75.5±0.7
5	500	69.6±1.8	75.5±0.8	63.1±2.1	67.5±0.5
6.	IC_{50}	48.1	46.3	46.7	47.4

Standard deviation ± standard error

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Markov Model of DNA Mutations

R. Sivaraman*

Associate Professor, Department of Mathematics, D. G. Vaishnav College, Chennai, Tamil Nadu, India.

Received: 05 Dec 2020 Revised: 13 Dec 2020 Accepted: 22 Dec 2020

***Address for Correspondence**
R. Sivaraman
Associate Professor,
Department of Mathematics,
D. G. Vaishnav College,
Chennai, Tamil Nadu, India.
Email: rsivaraman1729@yahoo.co.in

ABSTRACT

In this paper, I introduce Markov models and use it to analyze the mutations that occur in DNA molecules. It is very commonly known that DNA molecules contain four basic types of bases namely Adenine (A), Cytosine (C), Guanine (G), Thymine (T). In this paper, using Markov model assumptions, I have created a new model which will address mutation rates that occur in the four basic pairs of DNA, eventually proving the stability of mutations that occur in DNA base pairs.

Keywords: Markov models, Mutation rate matrix, Mutation rate, Associated Markov matrix, Diagonalization, Stability of mutation rates.

INTRODUCTION

A probabilistic model of a system is called a Markov model if the behavior of the system over any given time period depends only on the state the system is in at the beginning of that period. The earlier history of the system can affect what happens in the future only through having already affected the system's current state. More formally, in a Markov model the probability that a particular state change occurs given the system is in state *i* is the same as the probability of the same change, given any entire earlier history of states ending in state *i*. In particular, a `memory' of what state changes occurred during earlier times is useless for predicting future changes. We say the probabilities of state changes are independent of the earlier history. Moreover, I will be considering rooted binary tree for describing the model in this paper. Recall that a rooted binary tree is a binary tree in which the root *r* is the unique vertex of degree two and all other vertices have either degree three or one. In particular the vertices (or nodes) of degree one are often referred as leaves of the tree. The root of the tree represents the common ancestor of the tree and the whole tree is sometimes referred as Ancestral Tree and the root of the tree may also be called as Ancestral Node. The model I am going to describe in this paper regarding molecular evolution occurring through random base substitutions, satisfies the Markov assumption, because time proceeds from the root to the leaves, and the probabilities of the

various possible state changes on any given edge depend only on the state at the ancestral node on that edge and not on previous nodes.

Describing the Model

The simplest Markov model consisting of base substitution that I am going to describe here, apart from satisfying Markov model conditions adds several additional assumptions to the general Markov model. First, let us assume that the root distribution vector describes all bases occurring with equal probability in the ancestral sequence. Thus each of the four DNA bases will be assigned equal probability $\frac{1}{4}$. So the probability vector with respect to the root of the ancestral tree is given by $P_r = \left(\frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\right)$ (2.1)

Since any Markov model is considered as a continuous-time model we assume that the mutation rate matrix is given by $Q = \begin{pmatrix} -\lambda & \frac{\lambda}{3} & \frac{\lambda}{3} & \frac{\lambda}{3} \\ \frac{\lambda}{3} & -\lambda & \frac{\lambda}{3} & \frac{\lambda}{3} \\ \frac{\lambda}{3} & \frac{\lambda}{3} & -\lambda & \frac{\lambda}{3} \\ \frac{\lambda}{3} & \frac{\lambda}{3} & \frac{\lambda}{3} & -\lambda \end{pmatrix}$ (2.2)

Here λ is called as the mutation rate of DNA base pairs which varies according to the species chosen. Among four possible bases the base changes can occur in 4 choose 2 = $\frac{4\times 3}{1\times 2} = 6$ ways each with rate $\frac{\lambda}{3}$. In particular, from the rate matrix *Q* in (2.2), we notice that the rates of all pair base changes $\mathrm{A}\leftrightarrow\mathrm{T}, \mathrm{A}\leftrightarrow\mathrm{C}, \mathrm{A}\leftrightarrow\mathrm{G}, \mathrm{C}\leftrightarrow\mathrm{T}, \mathrm{C}\leftrightarrow\mathrm{G}, \mathrm{T}\leftrightarrow\mathrm{G}$ have same value $\frac{\lambda}{3}$. The total rate at which any specific base is changing to other three bases is therefore $\frac{\lambda}{3}+\frac{\lambda}{3}+\frac{\lambda}{3} = \lambda$.

The associated Markov matrix on an edge of length *t* can be calculated using the formula $M(t) = e^{Qt}$ (2.3).

Solving the Model

To proceed further, first we need to determine the eigen values and eigen vectors of the rate matrix *Q* defined in (2.2) and then diagonalize it. First, we note that the eigen values of the rate matrix *Q* in (2.2) are given by $0, \frac{-4\lambda}{3}, \frac{-4\lambda}{3}, \frac{-4\lambda}{3}$. The eigen vectors of these eigen values are given by $(1,1,1,1); (1,-1,1,-1); (1,1,-1,-1); (1,-1,-1,1)$ respectively.

Now from the matrix $R=\begin{pmatrix}1&1&1&1\\1&-1&1&-1\\1&1&-1&-1\\1&-1&-1&1\end{pmatrix}$ (3.1) (whose columns are eigen vectors of *Q*) we see that $|R|=16$.

Hence the matrix *Q* is diagonalizable using $Q=R^{-1}DR$ (3.2) where *D* is a diagonal matrix whose diagonal entries are eigen values of *Q*.

Thus the associated Markov matrix using (2.3) and (3.2) is given by $M(t)=e^{Qt}=R^{-1}e^{Dt}R=R^{-1}D_1R$ (3.3) where $D_1=e^{Dt}$ is a diagonal matrix whose diagonal entries (using diagonal entries of *D*) are $1, e^{\frac{-4\lambda}{3}t}, e^{\frac{-4\lambda}{3}t}, e^{\frac{-4\lambda}{3}t}$. Thus computing the matrix $M(t)$ in (3.3), using these information we have

$$M(t)=\begin{pmatrix}1-\mu&\frac{\mu}{3}&\frac{\mu}{3}&\frac{\mu}{3}\\\frac{\mu}{3}&1-\mu&\frac{\mu}{3}&\frac{\mu}{3}\\\frac{\mu}{3}&\frac{\mu}{3}&1-\mu&\frac{\mu}{3}\\\frac{\mu}{3}&\frac{\mu}{3}&\frac{\mu}{3}&1-\mu\end{pmatrix} \quad (3.4) \quad \text{where} \quad \mu=\mu(t)=\frac{3}{4}\left(1-e^{\frac{-4\lambda}{3}t}\right)$$

It is important to note that the model described above provide a stable base distribution at all vertices of the tree. To see this, we simply compute the effect of the substitution process as we proceed down an edge using the product of the matrices in (2.1) and (3.4) to give

$$P_r\times M(t)=\left(\frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\right)\times\begin{pmatrix}1-\mu&\frac{\mu}{3}&\frac{\mu}{3}&\frac{\mu}{3}\\\frac{\mu}{3}&1-\mu&\frac{\mu}{3}&\frac{\mu}{3}\\\frac{\mu}{3}&\frac{\mu}{3}&1-\mu&\frac{\mu}{3}\\\frac{\mu}{3}&\frac{\mu}{3}&\frac{\mu}{3}&1-\mu\end{pmatrix}=\left(\frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\ \frac{1}{4}\right) \quad (3.5)$$

This result is not very surprising because of the fact that the mutation rate matrix *Q* defined in (2.2) is symmetric, which in turn implies that for every substitution from state *i* to *j* we should expect a substitution back from *j* to *i*, so that the base distribution never changes.

CONCLUSION

By considering the concepts of rooted binary trees and four base pairs of DNA molecules, I have constructed a mutation rate matrix *Q* in (2.2). We see that *Q* it is a symmetric matrix and the total rate at which any specific base is changing to other three bases is always λ, the mutation rate of DNA base pairs. By determining the eigen values and eigen vectors of the mutation rate matrix *Q*, I have constructed a invertible matrix *R* which helps us to diagonalize *Q* through (3.2). The diagonalization process allows us to compute the associated Markov matrix $M(t)$ as in (3.4). By considering the product of probability vector of the root of ancestral tree and the associated Markov matrix $M(t)$, I have shown that the initial equal mutation rate probability distribution of the four base DNA pairs remain unchanged. Thus the model proposed in the paper, ensures the stability of the mutation rates of DNA base pairs. Further, the continuous time model accounts for all possible ways the final state could have been achieved from the initial one. We are likely, of course, to use a different value of μ for each edge of the tree, since the formula for μ depends on the edge length. We also note that from the expression for $\mu(t)$ in (3.4), that $\mu(t) \to \frac{3}{4}$ as $t \to \infty$ ensuring the stableness of mutation with equal probability of $1-\frac{3}{4}=\frac{1}{4}$ for all four DNA base pairs.. Thus the stability is maintained even in long span of time.

REFERENCES

1. J. E. Cohen and B. Singer. Malaria in Nigeria: Contrained continuous-time Markov models for discrete-time longitudinal data on human mixed-species infections. In *Some Mathematical Questions in Biology*, pages 69–133. Providence: American Mathematical Society, 1979.
2. Chih-Ming Liu, Kuo-Ming Wang, and Yuh-Yuan Guh. A Markov chain model for medical record analysis. *The Journal of the Operational Research Society*, 42(5):357–364, 1991.
3. S. I. Mc Clean, B. Mc Alea, and P. H. Millard. Using a Markov reward model to estimate spend-down costs for a geriatric department. *The Journal of the Operational Research Society*, 49(10):1021–1025, 1998.
4. L. Billard. Markov models and social analysis, *International Encyclopedia of the Social and Behavioural Sciences*, pages 9242–9250, 2004.
5. Amanda A. Honeycutt, James P. Boyle, Kristine R. Broglio, Theodore J. Thompson, Thomas J. Hoerger, Linda S. Geiss, and K. M. Venkat Narayan, A dynamic Markov model for forecasting diabetes prevalence in the United States through 2050. *Health Care Management Science*, 6:155–164, 2003.
6. Y. W. Tan. First passage probability distributions in Markov models and the HIV incubation distribution under treatment. *Mathematical and Computer Modelling*, 19(11):53–66, 1994.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

The Factors Determining HL7 Electronic Health Record (EHR) Functionality and Model usage: A Literature Review

S. Manimaran[1]* and K. Bhagyalakshmi[2]

[1]Head and Professor, Department of Management Studies, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.
[2]Associate Professor, Department of Management Studies, PSNA College of engineering and Technology, Dindigul, Tamil Nadu, India.

Received: 20 Nov 2020 Revised: 23 Dec 2020 Accepted: 25 Jan 2021

***Address for Correspondence**
S. Manimaran
Head and Professor,
Department of Management Studies,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.

ABSTRACT

Electronic Health Records are latest versions of electronic edition of patients' healthcare digital records. An electronic health record congregates, demonstrates, and stores the healthcare record electronically in systematic order. The electronic health record has been deliberated to be adopted by healthcare system providers. The electronic health record will certainly develop in maintaining clinical oriented documentations, quality concern, utilization factor of healthcare tracking, treatment billing and coding, and make ease of portability of health records. The foundation elements of an electronic health record includes the Medical administrative functions, computerized patient's history by physician array of entry, laboratory functioning systems, radiology functioning systems, pharmaceutical systems, and clinical care documentation. HL7 is the standard protocol of ICT technology that an electronic healthcare record utilizes. Implementation of these software and hardware, also implementation of IT networks are important for a successful electronic healthcare record project. As such this paper will elaborate the benefits of an electronic health record including an achievement in accessing healthcare efficiencies, ratio of large gains in excellence and security, and lesser costs of healthcare services to the patient' consumers. The social implication and Challenges of the Electronic health record might include understanding expensive software packages, IT security, confidentiality of patient, and unknown regulation in future government policies. It is evident from the study that future technologies for electronic health records include bar coding, RFI – Radio frequency identification, and speech recognition.

Keywords: Health Records, patient, IT, security, technology, HL7, Medical.

INTRODUCTION

Today's healthcare services around the world are inundated with very multifaceted external rules, not to mention the pressures from competition and the ability to deliver quality healthcare for their patients. This unstable healthcare situation has brought to the fore front the significance of Electronic Health Records. An international standards for the transfer of EHR is the clinical and administrative , accounting , finance, employment data's between software applications used by various healthcare providers to provide it fosters a collaborative community that is committed the service much quality in a fast way is HL7 - Health Level 7 to improving the health of people through technology around the globe. It permits the information and data that is in one format set-up to be translated when leaving an Information System and entering a different one in another format setup that the receiver from another-side identifies of ease of understandable. This is improving the Med Tech/healthcare experience and healthcare information system interoperability standards.

Impact of HL7

- To develop the care to patient and better service
- To optimize the cycle of healthcare workflow
- Successive development of interoperability between healthcare system providers.
- Enhance knowledge transformation among healthcare providers.

The CDDA – Clinical Document Design Architecture used to create a message of clinical data inputs , these clinical data messages from one clinical system were not suppose to be compatible with other clinical systems in existing methodology, for instance , the health record messages could not be interpret and inherent. To combat this issue an innovative standard of HL7 for messaging set-up format has been explored with transparent in conformance spot to support the data transformation compatibility. Predominantly, The Australian healthcare applications create and consume HL7 messaging, and many systems create and consume CDDA (Clinical Document Design Architecture) for transportation of Digital health records messaging set-up with technological factors indicated in the fig. 1.

The Primary function of HL7'S is to support a patient's medical record with the hospital and physician, it, in fact, supports all throughout the history of patient journey. It makes connection to physician to other physician, medical specialists, patients, government agencies, employers and patients with the transfer of care. It also reduces destructive errors which causes harm and assists in the medical record transfer between these parties. Even in different digital systems architecture works together of data standards in HL7. The CDDA ensures that all can access securely and make utilize the right health information's in all circumstances they required it. Existing document formats used within clinical systems in HL7 are digitally encrypted with end users resolved through associate's lookups from multiple sources of directories, including government-maintained directories and end to end vendor's subscriber directories. The ubiquitous advantage of HL7 is that users can switch over / transfer their confidential patient information securely across larger network architecture of healthcare providers to improve the Quality care Improvement shown in the fig. 2.

Also HL7 deals with pharmacy drugs records, laboratory test results records, doctors' orders and clinician remarks, discharge summaries, digital medical imaging results, medical research records and home health monitoring devices, etc. An existing system support of health care institutions that uses the HL7 protocol layer to exchange the key sets of data between different end – end computer application systems which has been active in Australia. While HL7 is addressing to the concerned immediate needs, there is a very strong focus on associated with other Standards development activities in United States of America and international HL7 initiatives in countries including developed countries like Canada, China, Europe, India, Japan, and the Netherlands.

The benefits of HL7

- It Promotes better routinely patient outcomes,
- Healthcare trends of Future-proof of better Service by staying ahead of standards
- It greatly helps in building Australia's National Health Information Network & Electronic Health Record more effectively,
- It helps in assisting the development of Health system interoperability,
- It aids in easy communications between end to end API's(Application Program Interfaces),
- It highly suits for the implements of cost-effective solutions pertaining to healthcare systems in Australia and also worldwide,
- With the help of HL7 standards, information is delivered in a consistent manner to patients and physicians though in the domain of Health management Information systems also referred as EHR(electronic health records),
- EHR and HL7 Standards generates best solution practices for the healthcare community in Australia which was shown in a 3 phase segments in the fig. 3.

Standards of HL7 Version 2.4

The HL7 layer protocol is a set of standard formats that states the implementation of interfaces between computer applications as we said earlier. It is not rigid; also Flexibility is built into the protocol to which permits compatibility for specific data sets that have facility-specific needs. One of HL7's strengths is its inbuilt flexibility and reliability. However, it's weaknesses in open to misinterpretation in its configuration and format. HL7 is based on the health environment in America. A common and consistent approach is required in the implementation of HL7 Version 2.4 Standard in the Australian health environment shown in the fig. 4 EHR mobile applications. The intended addressees for this HL7 Standards includes Public healthcare authorities, health service providers, healthcare institutions, vendors of health information technology, health information technology consultants and the healthcare informatics community. A basic understanding of HL7 is essential, as this Standard is based on and frequently refers to the HL7 Version 2.4 Standard.

In order to communicate the data and information of clinical setups, which is deeply context-dependent, it has been essential to use local extensions to the HL7 Version 2.4 Standard. The proposed extensions will be for inclusion in a later version of HL7 Version 2.x in future requirements and establishments. A medical appointment referral or shared concern messages may, under different circumstances truly, required including almost all data from a health record. This expands the capacity of such a message and requires more complex information contextual and relationship data's applying to the included sections. A message context is inferable from the starting -point event but this is inadequate for the more common information in ejection, Referral, Event outlines and Shared Care. This message has required including and combining the segments designed for use in simplest and more specific data messages where their context is implicit from the starting-point event. This applies mostly to Procedures, medical history, Observations, Medications, complex problems, event Goals and Pathways.

The message structure described in this configured Standard is proposed to make essential communication information from one clinical provider end or organization, institutes to another end potentially through a shared EHR and should be utilized wherever there is a absolute or partial transfer of data care message, as occurs on discharge from a hospital or other service care providers. The message will typically contain medical referral appointment details as well as a discharge or other summary or event history. Relevant message format definitions are included in Clause 4 of this Standard. Clinical management by cooperating healthcare providers, mandates healthcare service messaging built on fixed semantic exchange. The messaging protocols while described in this implementation Standard make use of a required level of coding as in the HL7 tables.

The Protocol Standard includes the data segments and data elements that are necessarily required, elective or conditional, and related usage comments in the Australian health environment. The Standard provides consistent use of data definitions as well as annotations and suggestions to the International Organization for Standardization (ISO), the Australia National Health Data Dictionary, the National Association of Testing Authorities Australia (NATA), and with the Commonwealth Department of Health and Ageing.

CONCLUSION

HL7 has not been extensively used for structured clinical data communications. When available, HL7 Version 3, Messaging and Clinical Document Design Architecture (CDDA) are estimated to be progressively more used for new applications from the health care domain. This Version 2 message is at present being used to inform the requirements and design of these future standards, this papers which covers the implementation of HL7 Version 2.4 for patient administration within Australia also provides a significant establishment for the construction of most clinical health care messages. Only those segments that have been identified as significant have been detailed relevant in the Australian implementation standards. Refer to the HL7 Version 2.4 protocol for all other message segments. Whereas segment is exclusively extended by the accumulation of new domains, these are added at the segment end and are to be considered as 'Local Usage'. It is to be proposed for addition in a later discharge of the HL7 Standards. A Specified terms and coding terminology is required for meaningful Healthcare digital data exchange, and this therefore forms part of this Standard in Australia in future.

REFERENCES

1. Aydin CE Forsythe DE. Implementing computers in ambulatory care: implications of physician practice patterns for system design. Proc AMIA Annu Fall Symp 1997:677-81.
2. Ash J. Organizational factors that influence information technology diffusion in academic health sciences centers. J Am Med Inform Assoc 1997;4(2):102-11.
3. Barnett, G.O., "The use of computers in clinical data management: the ten commandments. Presented at American Medical Association symposium on Computers in Medicine February, 1970.
4. "Implementation of E-health record systems in Australia", Jun Xu, Xiangzhu Gao, Golam Sorwar, Peter Croll-Medicine 2013.
5. **"**Current Status, Challenges, and Outlook of E-Health Record Systems in Australia**",** Jun Xu, Xiangzhu Gao, +1 author Peter Croll Published 2014 Medicine.
6. "This Australian Standard was prepared by Committee IT-014, Health Informatics. It was approved on behalf of the Council of Standards Australia" on 2 June 2020.
7. "The conceptual framework of interoperable electronic health record and ePrescribing systems" Hammerscdmidt, Reinhard, Karl Stroetmann, 2008.
8. EHDViz: clinical dashboard development using open-source technologies.
9. Hogg, M.A. (2016). Social identity theory. In Understanding peace and conflict through social identity theory (pp. 3-17). Springer, Cham.
10. Kim, H.S., Sherman, D.K., & Updegraff, J.A. (2016). Fear of ebola: The influence of collectivism on xenophobic threat responses. Psychological science, 27(7), 935-944.
11. Koh, H.H. (2003). On American Exveptionalism. Standford Law Review, 1479-1527. https://www.jstor.org/stable/1229556
12. Mottus, R., Sinick, J., Terracciano, A., Hrebickova, M., Kandler, C., Ando, J., …& Jang, K. L. (2019). "Personality characteristics below facets: A replication and meta-analysis of cross-rater agreement, rank-order stability, heritability, and utility of personality nuances". Journal of personality and social psychology, 117(4), e35.

13. Norris, F. H., & Wind, L. H. (2009). The experience of disaster: Trauma, loss, adversities, and community effects. In Y. Neria, S. Galea, & F. H. Norris (Eds.), Mental Health and Disasters (pp. 29-44). New York, NY: Cambridge Press.
14. "Patient-generated health data and electronic health record integration: protocol for a scoping review", Icahn School of Medicine at Mount Sinai, Mount Sinai Health System, New York City, New York, 2010.
15. Reinhart, C.M., & Mikels, J.A. (2014). Meta-analysis of the age-related positivity effect: age differences in preferences for positive over negative information. Psychology and aging, 29(1), 1. https://psycnet.apa.org/doi/10.1037/a0035194
16. Stewart, A.J., & Healy, J.M. (1989). Linking individual development and social changes. American Psychologist, 44(1), 30.

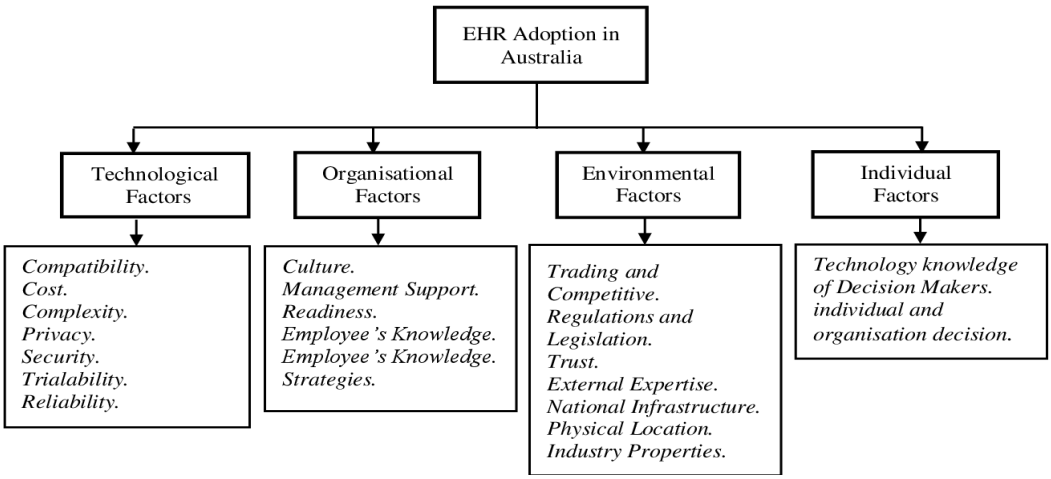


Figure 1. The modules of EHR adoption in Australia

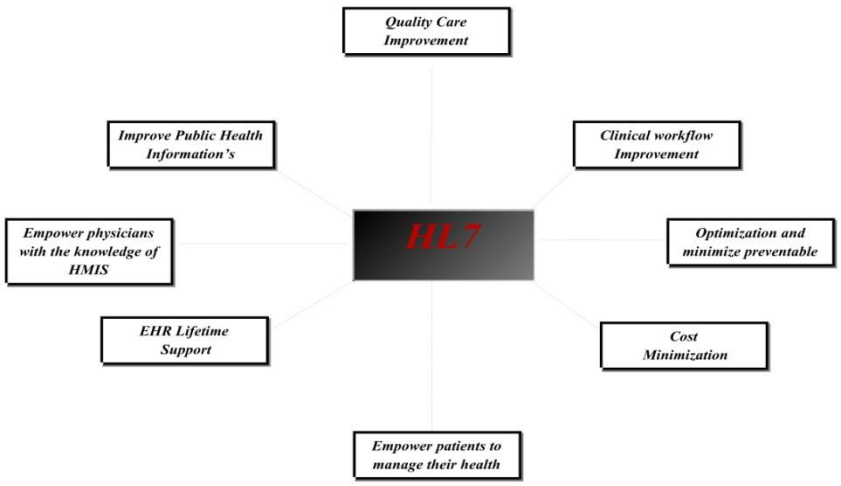


Figure 2. The HL7 Standard

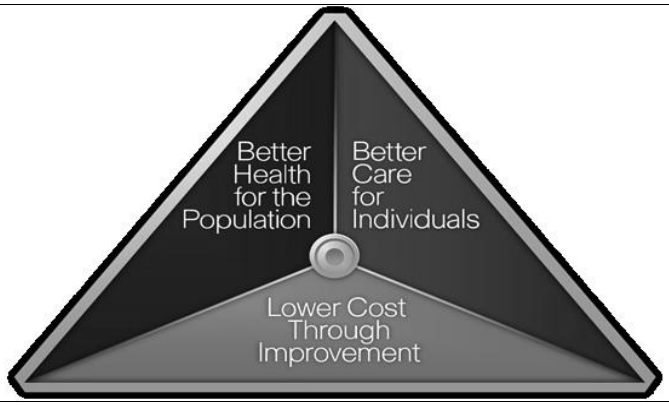


Figure 3. Aims of HL7 EHR in Australia

Figure 4. Mobile application model

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Comparative Study on Phytochemicals, Antioxidants and Antimicrobials Components in Leaf Extracts of *Curcuma caesia* Roxb. with Reference to Location

Jose Elseena[1]*, Anju George[2], Antony Mathew[2] and T.R. Neethu[2]

[1]Assistant Professor, Department of Pharmaceutical Chemistry, Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.
[2]Nirmala College of Pharmacy, Muvattupuzha, Ernakulam, Kerala, India.

Received: 02 Nov 2020 Revised: 10 Dec 2020 Accepted: 28 Dec 2020

***Address for Correspondence**
Jose Elseena
Assistant Professor,
Department of Pharmaceutical Chemistry,
Nirmala College of Pharmacy, Muvattupuzha,
Ernakulam, Kerala, India
Email : elseena_jose@yahoo.com

ABSTRACT

Medicinal plants are reported to possess various activities. The present study was designed to compare the antimicrobial and antioxidant potency of *Curcuma caesia* Roxb, Himalayan variety, and native Kerala variety. Methanol extracts of both varieties of *Curcuma caesia* Roxb were investigated for the comparison of antimicrobial activity by the disk diffusion agar plate method. The activity index was calculated which was more than 0.5. Himalayan variety exhibited more antimicrobial activity towards gram-positive and the Indian variety exhibited more activity in the gram-negative microorganism. We also investigated the antioxidant activity of *Curcuma caesia* Roxb, Himalayan variety, and native Kerala variety by FRAP method. The maximum percentage of inhibition by the Himalayan variety was found to be 98.76±0.2 % and that of Indian variety was found to be 81.81±0.32%. IC50 values were found to be 28.1µg/ml, 29.9µg/ml, and 39.9µg/ml for ascorbic acid, Himalayan variety, and Indian variety respectively. The study has provided a basis to explore the chemical constituents in *Curcuma caesia* Roxb.

Keywords: *Curcuma caesia* Roxb, FRAP method, disk diffusion agar plate method, antimicrobial and antioxidant potency.

INTRODUCTION

Medicinal plants rich sources of bioactive components and impart enormous biological activities. Novel bioactive components for several diseases have now been discovered from different medicinal plants. The major problem faced by antibiotic drugs is toxicity, lower potency, development of resistant bacterial strains, high cost of new generation antibiotics. Herbal medicine is having greater need and demands, all over the world [1,2]. A natural antioxidant biomolecule is available in many dietary foods and medicinal plants. Major plants show antioxidant properties due to the presence of polyphenols and carotenoids. Hence those drugs with significant antioxidant properties are used for the treatment of including anti-inflammatory, anti-aging, anti-atherosclerosis, and anticancer therapy. Curcuma species are well known for their antimicrobial activity and they are also been used as antioxidant drugs traditionally, shown health improvement and immunity achievement.[3] *Curcuma caesia* Roxb is commonly known as kali haldi and it belongs to the Family Zingiberaceae. Fresh and dried rhizome and leaves of *Curcuma caesia* Roxb. are used in treating leucoderma, asthma, tumors, pile, bronchitis. [4,5] The various medicinal activities of *Curcuma caesia* Roxb such as antimicrobial, antioxidant, rice seed germination, and anthelminthic activities of *Curcuma caesia* were already investigated [6-9]. The objective of this study was to perform the comparison of antimicrobial and antioxidant activities of two varieties of *Curcuma caesia* Roxb, native and Himalayan varieties.

MATERIALS AND METHODS

Collection and identification of plant

The varieties of *Curcuma caesia* were collected from Kottayam district. Kerala and was identified authentically by the Department of Botany, Nirmala College, Muvattupuzha. The leaves were collected in the month of September-October.

Extraction and preliminary Phytochemical Screening

The leaves were dried and 50gms of powdered leaves were subjected to soxhlet extraction using methanol as solvent. The percentage yield was calculated and recorded. The residue extract was stored in a refrigerator for further studies. The extracts of plant materials were subjected to phytochemical analysis using the methods mentioned [10].

Assessment of Antibacterial Activity

The antibacterial activity of the extracts was determined by Agar well diffusion method. Petri plates containing 20ml Muller Hinton Agar Medium were seeded with the bacterial culture of *Klebsiella Pneumoniae* and *Staphylococcus aureus* (growth of culture adjusted according to McFarland Standard, 0.5%). Wells of approximately 10mm was bored using a well cutter and different concentrations of the sample such as 50mg/mL and 100mg/mL were added. The plates were then incubated at 37°C for 24 hours. The antibacterial activity of the methanol extract of the leaves was assayed by measuring the diameter of the inhibition zone formed. Streptomycin and penicillin were used as a positive control and methanol were also kept as vehicle control [11,12].

Assessment of Antioxidant Activity

The antioxidant activity of the two varieties was determined by FRAP ((Ferric Reducing Antioxidant Power Assay) method. The reagent was freshly prepared in the lab. Different concentrations (10-50 µg/mL) of the methanolic extract (2.5ml each) were taken and added to 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of 1% potassium ferricyanide solution. The resulting mixture was vortexed well and then incubated for 20 min at 50°C. At the end of the incubation, 2.5 mL of 10% trichloroacetic acid was added to the mixture and centrifuged at 3,000 rpm for 10 min. The supernatant (2.5 mL) was mixed with 2.5 mL of deionized water and 0.5 mL of 0.1% ferric chloride. The colored solution was read at 700 nm against the blank using UV Spectrophotometer. Here, ascorbic acid was

used as a reference standard the reducing power of the samples was compared with the reference standard. % increase in Reducing Power = [absorbance of test solution / absorbance of control] - 1 X 100. [13,14]

RESULTS

The methanol extract of the Native variety and Himalayan variety of *Curcuma caesia* Roxb were investigated and compared for antimicrobial and antioxidant activities. The percentage yield of the extract of the Native variety and Himalayan variety of *Curcuma caesia* Roxb are shown in table 1.

Phytochemical Screening of the samples

The phytochemical analysis of both the extracts was performed to evaluate the presents of various constituents in *Curcuma caesia* Roxb. The results of the phytochemical screening are shown in Table 2.The phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and phenolic compounds.

Assessment of Antimicrobial Activity

In the evaluation of the antimicrobial activity of the Himalayan variety and native variety of *Curcuma caesia,* both gram-positive (*Staphylococcus aureus*) and gram-negative bacteria (*Klebsiella pneumoniae*) were used and results were compared. The antibacterial activity of both extracts was more promising for gram-positive organisms. Himalayan variety exhibited more significant antimicrobial activity in gram-positive bacteria than the Native variety. The activity of the extracts on the gram-negative organism was also evaluated. The Activity index was calculated using Penicillin and Streptomycin as standards for gram-positive and gram-negative organisms respectively [15]. Activity index more than 0.5 indicates that both varieties have a potential antibacterial effect on both gram-positive and gram-negative organisms [1]

Assessment of Antioxidant Activity

Antioxidant activity was measured using FRAP (Ferric Reducing Antioxidant Power Assay) method. Ascorbic acid was used as the Standard. FRAP assay is based on measuring the reducing ability of an antioxidant component. In this Assay Ferric tripyridyltriazine, complex react with antioxidant and converted to colored ferrous tripyridyltriazine. The absorbance of the resulting colored solution is used to calculate the activity [14]. The antioxidant activity of the methanolic extracts of leaves of the Himalayan and native variety of *Curcuma Caesia* was found to be increased with an increase in the concentration of active components. (Table 5) It was observed that a significant correlation exists between the concentration of the extract and % inhibition. (Correlation coefficient R2: Standard ascorbic acid -0.997, Himalayan variety-0.992 and Native variety-0.996) (Figure 5, Figure 6 and Figure 7) respectively. IC 50 values were calculated to establish a relationship between the antioxidant activities of both extracts. (Table 5**).** *Curcuma caesia* Himalayan Variety extract showed comparable antioxidant activity with the that of the standard Ascorbic acid (IC 50 value 29.9±0.23)

DISCUSSIONS

The present study was carried out to compare the antimicrobial and antioxidant activity of *Curcuma caesia* Himalayan Variety and native variety. The preliminary phytochemical analysis confirmed the presence of alkaloids, flavonoids, tannins, and phenolic compounds in both the varieties. The in-vitro antimicrobial activity study was carried out with different concentrations of methanol extract of leaves of the Himalayan variety and Indian variety of *Curcuma caesia* Robx . and Himalayan variety exhibit more significant antimicrobial activity in gram-positive and the Indian variety exhibit more significant activity in the gram-negative microorganism. The *In vitro* antioxidant activity were carried out with different concentrations of extract for the Himalayan variety and Indian variety of *Curcuma caesia* Robx using the FRAP method and IC 50 values indicate that the Himalayan variety

has more antioxidant potency (IC50-29.9(µg/ml)) when compared to Native variety (IC50-39.9(µg/ml). It is concluded that both the Himalayan variety and Native variety of *Curcuma caesia* Robx are good sources of antimicrobial and antioxidant constituents. Further research is needed to identify and characterize the active principles of *Curcuma caesia* Robx.

ACKNOWLEDGMENT

Authors are thankful to the management and faculty of Nirmala College of Pharmacy, Muvattupuzha, Kerala, India for providing invaluable support and for providing research facilities

REFERENCES

1. Pandey D and Gupta AK: Antibacterial efficacy of *Curcuma caesia* from the Bastar district of Chhattisgarh, India. Indian Journal of Pharmaceutical Science and Res 2014; 5(6): 2294-01.doi: 10.13040/IJPSR.0975-8232.5(6).2294-01
2. G. Singh, I.P. S K apoor, et.al; Comparative study of chemical composition and antioxidant activity of fresh and dried rhizomes of turmeric (Curcuma longa Linn).Food and Chemical Toxicology 2010 481026-1031.
3. HeisanamPushparani Devi P.B. Mazumder Laishram Priyadarshani Devi. Antioxidant & antimutagenic activity of *Curcuma caesia* Roxb rhizome extracts. Toxicology reports 2015; 2: 423-428.
4. Nair T.S; Beegam Rasiya A; Mohanan N, Rajkumar. G. Flowering plants of Kerala. Tropical Botanical Garden and Research Institute, Thiruvananthapuram.
5. Cooke, Theodore, C.I.E. Flora of the Presidency of Bombay. Vol II
6. Baghel SS, Baghel RS, SharmaK, Sikarwar I. Pharmacological activities of *Curcuma Caesia*. International Journal of Green Pharmacy 2013;7:1-5
7. Randeepkaur, Barinderjit Kaur. The comparative assessment of in vitro antimicrobial activity of *Curcuma caesia* & *Curcuma Amada*. Asian Journal of pharmaceutical and clinical research2018;11(2).
8. C.R Laltha, K. Raveendran & Nabeesa Salim, effect of *Curcuma caesia* leaves on rice seed germination and seedling establishment, the ancient science of life1995; vol no.XV2: 115-118
9. Soundarya Budala, Vineela Chadalavada .Study of anthelmintic activity of *Curcuma caesia*. Indo American Journal of pharmaceutical research 2017:7(07)
10. Trease, G.E., and Evans, W.C. Pharmacognosy. 13th ed. ELBS/Bailliere Tindall, London 1995. 345-6, 535-6, 772-3.
11. Chandrakanth Kokare. Textbook of pharmaceutical microbiological experiments and techniques 4th ed. 71
12. https://repository.seafdec.org.ph/bitstream/handle/10862/1615/Laboratory-Manual-Antimicrobial.pdf
13. Maruthamuthu Vijayalakshmi and Kandasamy Ruckmani. The ferric reducing antioxidant power assay in the plant extract. Bangladesh J Pharmacol. 2016;11:570-572.
14. Nilima S. Rajurkar and S. M. Hande. Estimation of Phytochemical Content and Antioxidant Activity of Some Selected Traditional Indian Medicinal Plants. Indian Journal Of Pharmaceutical Sciences 2011.73(2):146-151
15. Jaishree Sharma, Padma Kumar.Comparative Study of Antimicrobial Activity and Phytochemical Screening of Serial Extracts from Leaves and Fruit of Aegle Marmelos and Carica Papaya. International Journal of Pharmacy and Pharmaceutical Sciences 2017.9(12):119-123

Table 1: Percentage Yield of Extract

Extract	Percentage yield
Himalayan variety	15.5%w/w
Native variety	14.6%w/w

Table 2: Phytochemical Screening *Curcuma caesia* Roxb.

Si no	Chemical Constituents	Himalayan variety	Native variety
1	Alkaloids	+	+
2	Carbohydrates	-	-
3	Flavonoids	+	+
4	Tannins and phenolic test	+	+
5	Glycoside	-	-
6	Saponins	-	-
7	Protein and amino acids	-	-

Table 3: Antibacterial Activity of *Curcuma caesia* Against Gram Positive and Gram Negative Bacteria

Concentration (mg/ml)	Zoneof inhibition (mm,mean±SD)	
	Staphylococcus aureus	*Klebsiella pneumoniae*
C. caesia (Himalayan variety)		
50	16±0.01	NA
100	20±0.06	13±0.03
200	24±0.01	15±0.66
C.caesia (Native variety)		
50	14±0.02	12±0.03
100	16±0.13	14±0.13
200	18±0.12	16±0.22
Standards		
Penicillin(100 µg/ml)	28±0.37	NA
Streptomycin (100 µg/ml)	NA	26±0.06
Blank	methanol	NA

Table 4: Activity Index of the Extracts of C*urcuma caesia* with Respect to Standards

Plant Used	*Staphylococcus aureus*		*Klebsiella pneumoniae*	
	Zone of Inhibition in mm	Activity Index(AI)	Zone of Inhibition in mm	Activity Index (AI)
C. caesia (Himalayan variety)	20	0.71	13	0.5
C.caesia (Native variety)	16	0.57	14	0.54
Penicillin	28	-	NA	NA
Streptomycin	NA	NA	26	-

Table 5: Antioxidant Activity of *Curcuma caesia* Against Gram Positive and Gram Negative Bacteria

samples	Parameters observed	Concentration (µg/ml)					IC 50 value (µg/ml)
		10	20	30	40	50	
Ascorbic Acid	% inhibition (mean+ SD)	12.2±0.32	30.3±0.2	53.1±0.31	75.7±0.26	99.01±0.21	28.16±0.26
Himalayan variety		7.79±0.31	23.37±0.17	48.05±0.23	72.73±0.27	98.76±0.20	29.9±0.23
Native variety		2.59±0.22	19.18±0.27	40.85±0.11	57.84±0.24	81.81±0.32	39.9±0.23

Figure 1: Himalayan variety

Figure 2: Native variety

Antibacterial activity of methanol extracts of leaves of *C. caesia* against gram negative bacteria(*Klebsiella Pneumoneae)*

Figure 3: Zone of inhibitions shown by standard drug, Himalayan Variety and native variety respectively

Antibacterial activity of methanolic extracts of leaves of *c. caesia* against gram positive bacteria (*Staphylococcus aureus)*

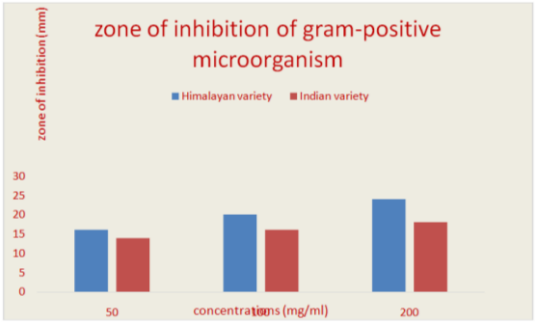


Figure 4: Zone of inhibition produced by the extracts on gram- positive microorganism

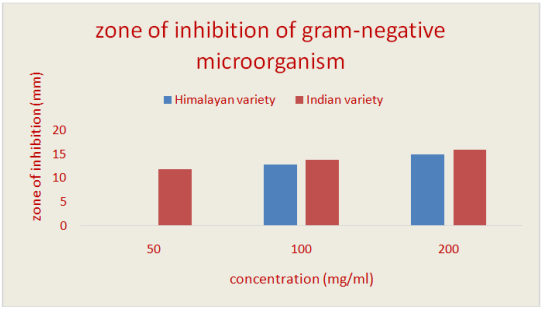


Figure 5: Zone of inhibition produced by the extracts on gram- negative microorganism

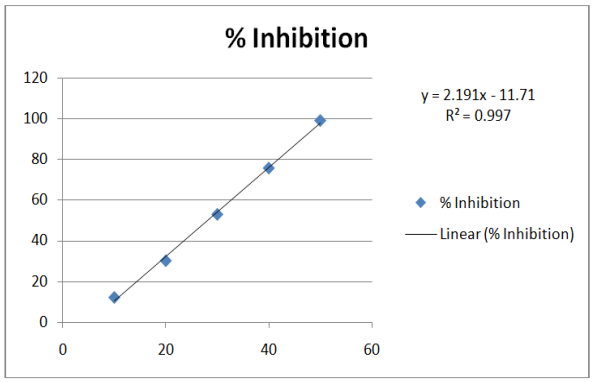


Figure 6: FRAP determination of Ascorbic Acid

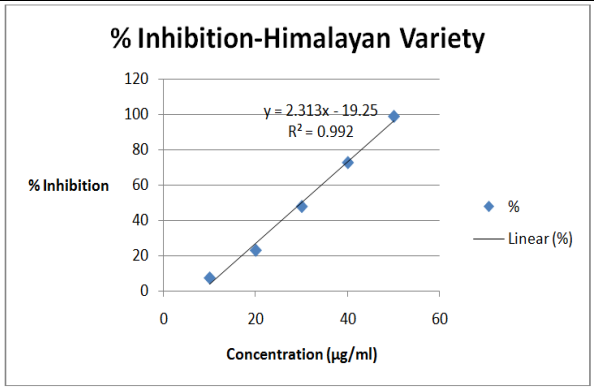


Figure 7: FRAP determination of *Curcuma Caesia* (Himalayan variety)

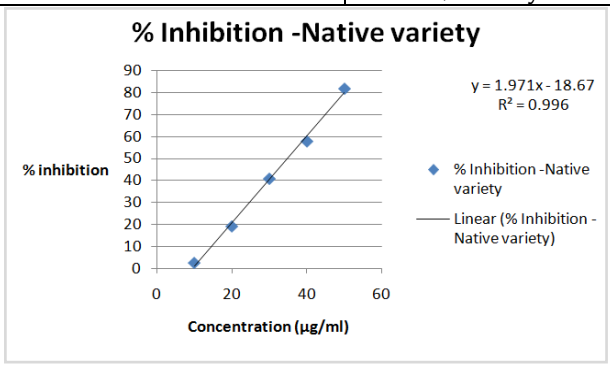


Figure 8: FRAP determination of *Curcuma Caesia* (Native variety)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

SHORT COMMUNICATION

Conditions for Superconductivity at Room Temperature

B. Jose Pandian*

169 – 4 Sri Meenakshi Amman Nagar, Surya Nagar, K. Pudur, Madurai, Tamil Nadu, India.

Received: 15 Dec 2020 Revised: 26 Dec 2020 Accepted: 05 Jan 2021

***Address for Correspondence**
B. Jose Pandian
169 – 4 Sri Meenakshi Amman Nagar,
Surya Nagar, K. Pudur,
Madurai, Tamil Nadu, India.
Email: josepandian49@gmail.com

ABSTRACT

We are now in the electricity age. The main problem is the resistance of the electricity conducting material. More than twenty percent (20%) of the electricity produced in the world is wasted due to the resistance of the conducting material. For this problem, the overall solution is the superconductivity and superconductors. The main thing should be considered that the difference between normal conductor and superconductor. The main difference is the reaction of the lattice ions when electricity is passed in the normal conductor and the superconductor.

Keywords: electricity, superconductor, material.

INTRODUCTION

In a normal electrical conductor, when electric current is passed the electrons starts to flow. The lattice ions start to vibrate and move randomly. When the normal conductor is cooled below the critical temperature (Tc) the lattice ions vibrations are very very low and they does not move randomly. In an electric current passed normal conductor the electron flow is disturbed by the randomly moving lattice ions. The electrons also collide with the randomly moving lattice ions [1]. The similarity between normal conductor and superconductor is the flow of electrons when the electric current is passed. The difference between normal conductor and superconductor when electric current is passed is listed in Table 1.

Short Communication

In a normal conductor, the vibration and random movement of the lattice ions disturbs the electron flow which is the electrical energy flow. In a superconductor, the lattice ions stay at fixed points and their vibrations are very very low, so there is no disturbance in electron flow. The lattice ions vibration and random movement is the property of the normal electrical conducting material. In an electrical conducting material if the lattice ions vibrations are very very low and the lattice ions stay at fixed points when electric current is passed then this material is known as

superconductor and this phenomenon is known as superconductivity. If an electrical conducting material having the above phenomenal property at normal room temperature then that material is the superconductor at room temperature. The very low vibrations of the lattice ions and the fixation of the lattice ions at fixed points at room temperature, when electric current is passed, are the conditions for superconductivity at room temperature.

REFERENCE

1. Bardeen, J.; Cooper, L.N.; Schrieffer, J.R.; (April 1957) "Microscopic Theory of Superconductivity". Physical Review. 106(1) : 162 – 164

Table 1: The difference between normal conductor and superconductor

Sl. No.	Normal Conductor	Super Conductor
1.	The lattice ions vibrations are high.	The lattice ions vibrations are very very low.
2.	The lattice ions move randomly.	The lattice ions stay at fixed points.
3.	The randomly moving lattice ions disturbs the electron flow.	There is no disturbance in the electron flow.
4.	The electrons collide with the randomly moving lattice ions.	The electrons flow freely.
5.	There will be loss of electrical energy.	There will be no loss of electrical energy.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Green Technology in Tailoring Rice Husk Ash with Epoxy as (GRHA-EP) Nanocomposites for Persisting UV Rays in Aerospace Applications

K. Kanimozhi[1]*, M. Alagar[2], V. Selvaraj[3], P. Prabunathan[4] and S. Thirumoorthi[5]

[1]Assistant Professor, Department of Chemistry, University College of Engineering (BIT Campus), Tiruchirappalli, Tamil Nadu, India.
[2]Former Professor, Chemical Engineering, Anna University, Chennai, Tamil Nadu, India.
[3]Assistant Professor, Department of Chemistry, University College of Engineering, Villupuram, Tamil Nadu, India.
[4]Assistant Professor, Department of Chemistry, PSG Research Institute & Technology, Coimbatore, Tamil Nadu, India.
[5]Assistant Professor, Department of Chemistry, Indian Institute of Handloom Technology, Salem, Tamil Nadu, India.

Received: 02 Nov 2020 Revised: 29 Nov 2020 Accepted: 21 Dec 2020

***Address for Correspondence**
K. Kanimozhi
Assistant Professor,
Department of Chemistry,
University College of Engineering (BIT Campus),
Tiruchirappalli, Tamil Nadu, India.
Email: kanirams47@gmail.com

ABSTRACT

Refuse what you do not need; Reduce what you do need; Reuse what you consume; Recycle what you cannot refuse, reduce or reuse; and rot (compost) the rest. –Bea Johnson. This famous quote made us to work in this platform that the rice husk ash which is not degraded in the environment as it is, made to functionalize in the green technique process and reuse as novel composites. GRHA-EP synthesized composites a reuse material of rice husk ash can be utilized in the preparation of composite materials for many applications specially here aerospace industry. Surface functionalized rice husk ash reinforced epoxy composites have been prepared by chemical blending method. The rice husk was treated by acid bleaching followed by functionalization using 3-glycidoxypropoyltrimethoxysilane. The glycidyl functionalization in the rice husk was confirmed by FT-IR, TGA and XRD analysis. Different weight percentages of surface functionalized rice husk ash were (0.5, 1.0 and 1.5 wt % GRHA) reinforced separately into epoxy matrix and were cured using DDM. From TGA and DSC analysis it has been proved that the surface functionalized rice husk ash reinforced epoxy nanocomposites (GRHA-EP) exhibits higher thermal stability than that of neat epoxy matrix. SEM and TEM observations indicated that the uniform and homogenous dispersion of surface functionalized rice husk ash into epoxy matrix.

From mechanical studies, it was confirmed that the GRHA-EP nanocomposites have improved tensile and flexural properties with high impact strength and hardness when compared to those of neat epoxy matrix. The lower water absorption and higher degree of contact angle values for GRHA- EP surely gives an idea for withstanding in the UV rays and to enhance the aircraft applications with longevity. The dielectric constant values of the synthesized new matrix composite are lower than that of neat epoxy matrix which also favours the coating application property.

Keywords: Epoxy, 3-Glycidoxypropoyltrimethoxysilane (GPTMS), Glycidyl functionalized rice husk ash (GRHA), morphology, mechanical properties and thermal properties.

INTRODUCTION

Epoxy resin possesses very good thermo mechanical properties and excellent processing behaviour and hence they are widely used in engineering and industrial applications. However the shrinkage during cure and brittle behaviour restricts its utility for high performance applications. Hence modification of epoxy resins to improve fracture toughness has been the subject of intense research interest. One such attempt is to form a covalent bond between organic polymers and inorganic components through chemical blending to enhance the compatibility as hybrid materials [1-6]. Many works have been put forward by incorporating inorganic fillers into the epoxy polymeric network. By choosing the inorganic fillers it is necessary to consider the environmental impact too without compromising the overall properties. Rice husk is a by-product obtained from rice processing. Rice husk ash is a solid obtained after burning of rice husk. Among the various biomasses, rice husk has a hard surface, high silica content (80- 90 %), tough, insoluble in water, possess chemical stability, high mechanical strength, abrasive in nature, inherent resistance behaviour, possess small bulk density, non-toxicity, low cost, and stable to bacteria [7-11]. Due to the high content of silica moiety in the rice husk ash it is expected to function as OMMT-clay with epoxy polymer and it has been chosen as the inorganic reinforcement in the present work. Rice husk is a fibrous material and has a variable range of aspect ratio. Thus, it can be used as reinforcing filler for the development of lightweight polymer composites [12-14]. Rice husk ash is an alternative source of silica derived from natural content, which is different from conventional inorganic fillers. The present work is carried out with GPTMS functionalized rice husk ash followed by reinforcing with DGEBA epoxy and cured using DDM. The resulting hybrid organic-inorganic composites were characterised and reported.

EXPERIMENTAL AND MATERIALS

Rice husk, GPTMS, ethanol, diaminodiphenylmethane (DDM), were obtained from SRL (India) and were used as received. The matrix resin used in the present study was diglycidyl ethers of bisphenol – A (DGEBA epoxy resin) (LY556) was received from Ciba-Geigy Ltd. Rice husk ash was synthesized as per the reported procedure [15].

Preparation of Rice husk ash (RHA)

Acid treatment is one of the most useful routes used to remove the lignin, wax and oils covering the external surface of the fiber cell wall of natural fibers. Rice husk ash is a solid obtained after burning of rice husk and was washed with distilled water, dried in an oven at about 60°C for 2h. Then bleached with Conc. HCl to remove the dirt and other contaminants present in it and subsequently washed with water till the pH become neutral, then dried in oven at 60°C for 4h. Sample of rice husk was heated at 500°C for 5h in a muffle furnace, to obtain rice husk ash.

Surface functionalization of rice husk ash (GRHA)

3-Glycidoxypropyltrimethoxysilane (GPTMS) was used as coupling agent to functionalize the rice husk ash. 4ml of GPTMS was mixed with 95% absolute ethanol and 5% deionised water and the resulting solution was sonicated for

15 min. The pH of the solvent was initially adjusted to 4.5 using acetic acid and subsequently sonicated for 1h in order to get complete hydrolysis of GPTMS. Then 10g of rice husk ash was added and the resulting mixture was sonicated for 2h. Then the mixture wasrefluxed for 24h at 80°C and centrifuged with addition of water followed by ethanol and hexane. The rice husk ash thus functionalized was further dried in hot air oven at 100°C in order to remove the moisture (Fig.1).

Preparation of surface functionalized rice husk ash reinforced epoxy nanocomposites (GRHA-EP)

The DGEBA epoxy resin and desired amount of glycidyl functionalized rice husk ash (0.5 %, 1.0% and 1.5% GRHA) were mechanically stirred at 50°C for 24h. A stoichiometric amount of DDM corresponding to epoxy equivalents was also added. The resulting product was poured into a pre-heated mould. The mould was pre heated at 120°C for an hour in order to remove the moisture and trapped air. The samples were cured successively at 120°C for 2h and post cured at 180°C for 3h and finally removed from the mould and characterized (Scheme 1).

Instrumentation

Fourier transform infrared (FT-IR) spectra for the samples were recorded on a Perkin Elmer 6X FT-IR spectrometer. The glass transition temperature (Tg) of the samples was determined, using DSC 200 PC differential scanning calorimeter (DSC) (Netzsch Gerateban GmbH). Thermogravimetric analysis (TGA) was carried out, using the DSTA 409 PC analyzer (Netzsch Gerateban GmbH). The tensile (stress–strain) properties were determined, using INSTRON (Model 6025 UK) as per ASTM D 3039. The flexural properties were measured by the INSTRON (Model 6025 UK) as per ASTM D 790. The un-notched Izod impact strength of each sample was studied as per ASTM D 256. The water absorption behaviour of the samples was tested as per ASTM D 570. The percentage of water absorbed by the specimen was calculated, using the following equation:

$$\% \text{ Water absorption} = (w_2 - w_1) \times 100 / w_1 \quad \ldots\ldots (1)$$

where w_1 is the initial weight of the sample and w_2 is the weight of the sample after immersion in distilled water for 48 h at 30°C. The dielectric studies of the neat epoxy and GRHA-EP nanocomposites were determined with the help of an impedance analyser. Contact angle measurements were carried out using 210 a Rame-hart Inc. goniometer (Succasunna, NJ, USA) with 5µl of deionised water and diiodomethane (DIM). X-ray diffraction patterns were recorded at room temperature, by monitoring the diffraction angle 2θ from 10 to 70° as the standard, on a Rich Seifert (Model 3000) X-ray powder diffractometer. The surface morphology of the fractured surface of the samples was examined, using a scanning electron microscope (SEM; JEOL JSM Model 6360). A JEOL JEM-3010 analytical transmission electron microscope, operating at 80 kV with a measured point-to-point resolution of 0.23 nm, was used to characterize the phase morphology of the developed nanocomposites. TEM samples were prepared by dissolving polymers in ethanol mounted on carbon-coated Cu TEM grids and dried for 1h at 70°C to form a film of < 100 nm.

RESULTS AND DISCUSSION

FT-IR Spectra

Fig. 2 showed the FT-IR spectra of neat rice husk ash (RHA) and glycidyl functionalized rice husk (GRHA). The FT-IR observed for neat rice husk ash (RHA) shows the peak for OH at 3434cm^{-1}. The disappearance of peak at 2338 cm^{-1} after functionalization is due to the chain lengthening with GPTMS and confirms the surface functionalization of rice husk ash. The appearance of peak at 965 cm^{-1} in GRHA confirms the glycidyl functionality has been introduced into RHA. The presence of broad peak at 3468 cm^{-1} shows the stretching vibration of O-H bond. The peaks appeared at 1093 and 800 cm^{-1} indicate the presence of Si-OH bond due to the glycidyl functionality in the rice husk. Peaks appeared at 2351 and 2930 cm^{-1} showed the CH_2 symmetric and asymmetric stretching respectively in GRHA.

The FT-IR spectra of rice husk ash reinforced DGEBA epoxy nanocomposites (GRHA-EP) are shown in Fig.3. The disappearance of oxirane ring peak at 965 cm^{-1} confirms that the GRHA-EP, epoxy and DDM have under gone a complete curing reaction. The cleavage of oxirane ring and the formation of –OH linkage were also confirmed by the occurance of polymeric network structure. The peaks appeared at 2863, 2964 cm^{-1} (aliphatic CH_2), 842 cm^{-1}& 1026 cm^{-1} symmetric and asymmetric stretching vibrations of (Si-O-Si), 3458 cm^{-1} (OH) confirm the curing of epoxy resin system. This is due to the complete polymerization of the surface activated rice husk ash within the epoxy matrix thereby isolating the hydroxyl group. This clearly confirms the ring opening polymerization between the epoxy, DDM and the functionalized rice husk ash has occurred during curing process.

Thermo gravimetric analysis

The thermal degradation behaviour of surface functionalized rice husk ash reinforced epoxy (GRHA-EP) composites were analysed using TGA and the data are presented in Fig.4 and Table 2. The 20 % weight loss for neat epoxy, 0.5, 1.0 and 1.5 wt % GRHA-EP nanocomposites were occurred at the temperature of 372˚C, 380˚C, 385˚C and 387˚C respectively. Similarly it was noticed that the 40 % loss of the composites occurred between the temperature from 393˚C and 441˚C. When the percentage weight of surface functionalized rice husk ash was increased it enhances the degradation temperature (Table 2). This may be explained that the surface functionalized rice husk ash reinforcement tightly holds the organic polymeric backbone and make the composites in a state of cross linked network structure. The initial degradation temperature of surface functionalized rice husk ash reinforced epoxy system is higher than that of neat epoxy system, due to the influence of silica component in the system. The organic component degrades faster when compared to neat epoxy system. The degradation temperature for 40 and 60 wt % losses of the GRHA-EP composites were increased with increase in percentage content of surface functionalized rice husk ash. The char yield of neat epoxy matrix and varying weight percentages of surface functionalized rice husk ash (0.5, 1.0 and 1.5 wt %) reinforced epoxy composites are 0, 17.48, 18.30 and 19.02 percentages at 700˚C respectively. As the weight % of surface functionalized RHA content increases in the epoxy matrix, the system prolongs the degradation temperature of the composites. Thus the thermal stability of the composites was found to increase due to the incorporation of surface functionalized rice husk ash into the epoxy network, which provides both thermal stability and strength properties [19, 20].

Differential scanning calorimeter

The value of glass transition temperature (Tg) of the neat epoxy matrix and the surface functionalized rice husk ash reinforced epoxy composites were obtained from DSC and data are presented in Table 2. The Tg values recorded from the experimental range of 30 to 400˚C. In the present work the surface functionalized rice husk ash reinforced epoxy composites exhibited significantly higher Tg values than that of the neat epoxy matrix. The Tg value of neat epoxy matrix is 162˚C, whereas for varying weight percentages reinforced (0.5, 1.0 and 1.5 wt. %) GRHA-EP nanocomposites exhibit 173˚C, 176˚C and 179˚C respectively. The enhanced values of Tg may be explained due to the introduction of silica network through hydrogen bonding interaction, which restricts the mobility of polymer chain. The increase of glass transition temperature with incorporation of GRHA was attributed due to the chemical interaction of GPTMS with the rice husk ash with the formation of an entangled three-dimensional rigid structure [21, 22].

Mechanical properties of neat epoxy and GRHA-EP nanocomposites

Table 1presents the mechanical properties of neat epoxy matrix and the glycidyl functionalized rice husk ash reinforced nanocomposites. An incorporation of varying weight percentages (0.5, 1.0 and 1.5 wt. %) of surface functionalized rice husk ash reinforcement increases the values of tensile and flexural strength. For neat epoxy matrix the value of tensile strength is 61.3 MPa. 0.5 wt. % of GRHA reinforcement enhances the tensile values to 64.1

MPa. The higher value of tensile strength obtained as 75.7 MPa for 1.5 wt % of surface functionalized rice husk ash reinforcement. The silica present in the rice husk ash forms a strong covalent linkage with the GPTMS which results an inter-crosslinked network structure in the reinforced composites and thus improves the value of tensile strength. The curative DDM also open its NH-OH linkage and forms an entanglement molecular structure. The homogenous dispersion of GRHA reinforcement into the epoxy matrix supported the stress transferred from polymer matrix. It was also clearly observed from the SEM analysis that no void growth formation or crack propogation occurred in the resulting reinforced composites. Moreover, the addition of rice husk ash to the polymer matrix enhances the values of tensile modulus significantly. The increase in modulus value is showing the stiffness of the epoxy rice husk ash reinforced composites. This improvement is attributed to the relatively lower strain rates of the composites. An observation similar to this was noticed also in the case of behaviour of hardness. Due to the increment of the loading, the values of hardness enhanced from 84 to 133. Further it is observed that the reinforced composites have resistant to penetration, scratching and indentation of other foreign agents. However, it was ascertained that similar wt. % of functionalized rice husk ash reinforced epoxy composites possess higher values of tensile and flexural strength, tensile modulus and hardness [23, 24]. The amount of energy absorbed per unit area is calculated by the impact strength. The values of impact strength of neat epoxy matrix and GRHA-EP nanocomposites are presented in Table 1. An incorporation of GRHA into the epoxy matrix improves the impact behaviour according to their percentage content. The oxirane ring in the DGEBA molecule and the silanol group functionalization with the –OH surface of the reinforcement induces to form hydrogen bond with the curing agent. The primary long-chain rigid structure and possible secondary hydrogen bond formation is expected to form a chain-entangled network structure [18]. An incorporation of rice husk ash reinforcement into the DGEBA epoxy enhances the value of impact strength to a significant extent when compared to that of neat epoxy matrix due to the flexibility and plasticizing effect imparted by the silica moiety [25, 26].

Water absorption behaviour

Water absorption characteristics of the neat epoxy matrix and varying weight percentages of rice husk ash reinforced epoxy composites are presented in Table 1. The water absorption test was carried out by immersing a specimen of appropriate dimension (1×1 cm) in deionized water at 30°C for 48 h. The experiments showed volume change without any clustering or micro voiding. An increasing concentration of rice husk ash reinforcement into the epoxy reduced the polarity of the polymer since the water uptake was mainly related to the polarity of the polymers and hence to the amount of bound water, rather than the free water trapped in micro-voids of free volume in the composites. Low water absorption is a very important property for a material used for insulation applications, especially those requiring stable high performance. In general, the absorbed water will affect the properties of the original material including thermal, mechanical and dielectric properties, etc. The results suggest that those materials could be used as efficient dielectrics [27, 28].

Dielectric Constant

The values of dielectric constant of the composite samples are presented in Table 1. As there is an increment of surface functionalized rice husk ash into the epoxy matrix there is a reduction in the values of dielectric constant. Neat epoxy matrix possesses the values of dielectric constant of 4.50 at 1 MHz whereas 0.5, 1.0 and 1.5 wt. %. GRHA-EP is 3.48, 3.11 and 3.07 respectively. The low k value is one of the most desirable properties for the next generation of electronic devices. The reduction in the value of dielectric constant for GRHA-EP composites may be explained by the formation of inter cross linked network imparted by the chemical interaction of surface functionalized reinforcement and the matrix. The signal propagation delay time of integrated circuits is proportional to the square root of the dielectric constant of the matrix, and the signal propagation loss is proportional to the square root of the dielectric constant and dissipation factor of the matrix. Thus, a material with low dielectric constant and low dissipation factor will reduce the signal propagation delay time and the signal propagation loss and in turn improve the effective and efficient functioning of micro-electronic devices [27-29].

Contact angle

The variation of contact angle against blend composition is given in Fig.5 and in Table 3. It can be observed that the composites possess higher values of contact angle when compared to that of neat epoxy matrix. The values of contact angle of GRHA-EP composites are increased from 93.0 to 102 and from 57.5 to 72.3 for water and DIM respectively. An increase in the value of contact angle of water and diiodomethane is noticed for all composite samples. The lower affinity of the GRHA-EP composites indicates the improvement in the hydrophobic behaviour contributed by the reinforcement to organic matrix. This may be due to the existance of non-polar nature of the toughened rice husk ash which may ultimately increases the roughness of the polymer surface. The acidic treatment and the glycidyl functionalization of the rice husk ash is an important modification disrupts the molecular motion of the polymer and achieved a crosslinked network structure of the composites, thereby increasing the surface roughness. The roughness of polymer surface was also supported by the SEM micrograph, (Fig.6). The surface free energies (┌) of the organic–inorganic hybrid nanocomposites were calculated according to the geometric mean model.

Cosθ = 2/γL [(γLd γsd)1/2 + (γLp γsp)1/2 -1 ……………………………………………………………………………..(2)

γs = γsd + γsp ……………………..……………………………………………………………………………………….(3)

Where θ is the contact angle and γL is the liquid surface tension; and γsd and γsp are the dispersive and polar components of γL, respectively. The additional free energy at the surface of the composites is known as surface free energy. High-energy absorption received by the activated curing agents of –NH bonds strike the DGEBA epoxy which interacted with the toughened surface of rice husk reinforcement and thereby forming a network structured of composites. Consequently, the developed GRHA-EP composites reduced the surface energy and enhance the substrate surface roughness and make the material surface become super hydrophobic [30].

MORPHOLOGY

Scanning electron microscopy (SEM)

The morphology of the neat epoxy matrix and the surface functionalized rice husk ash reinforced epoxy composites were analysed using SEM micrograph and are presented in Fig.6. From Fig.6 the neat epoxy matrix shows a smooth glassy fractured surface in different places. The brittle behaviour was overcome by incorporating the glycidyl functionalized rice husk ash into the epoxy matrix. The homogenous dispersion of rice husk ash into epoxy enhances the impact behaviour. There is no observance of crack formation or pulled out flaws in the SEM image which clearly provides an idea about the exfoliation of reinforcement into the matrix. Further there is no chance for agglomeration of reinforcement in the epoxy matrix due to the addition of inorganic component rice husk ash. The SEM micrograph (Fig.6) clearly proves that the existence of homogenous and molecular level dispersion of rice husk ash into epoxy matrix which contributes to the improvement of values of tensile and flexural strength. This also confirms the efficient interaction of covalent linkage between the functionalized rice husk ash, DGEBA matrix and the curative DDM [31].

XRD for 0.5 % and 1.5 % GRHA-EP nanocomposites

The XRD patterns of 0.5 % GRHA-EP and 1.5 % GRHA-EP reinforced epoxy composites are presented in Fig 7. From the XRD analysis, the spacing between the silicate layers in rice husk ash reinforced epoxy composites can be evaluated. From Fig 7, it is evident that there is no diffraction peak observed for epoxy composites. The absence of the diffraction peak in the case of reinforced composites is due to the complete exfoliation of the rice husk ash into the epoxy network structure. The observance of broad amorphous peak at 20° for 2θangle showed the uniform level dispersion and also confirms the efficient and effective compatibility between the reinforcement and the epoxy matrix. The rice husk ash disperses homogenously in the form of individual layers within the polymer matrix and

leads to form exfoliated composites which attributes to the improvement of the thermal and mechanical properties of the resulting nanocomposites.

TEM

The TEM micrograph for 1.0 wt% GRHA-EP nanocomposites was shown in Fig. 8. The nanosized distribution of the GRHA reinforcement can be viewed from the TEM micrograph and was ascertained that the dark portion of the pictures are due to the homogenous dispersion of rice husk ash reinforcement dispersed in the epoxy matrix. At smaller magnifications TEM image contrast because of absorption of electrons in the material and is due to the thickness and composition of the material. No accumulation mode was observed in the particle number size distribution, the particles on the left corner were clearly visible which indicates the presence of rice husk ash reinforcement.

Atomic force microscopy

The surface morphology of the hybrid materials is of great importance for many technical applications. The compatability between the organic polymer and inorganic rice husk ash filler had a great significant in the thermal and mechanical properties. The surface topology of 1.0 wt. % GRHA-EP in 2-D and 3-D was characterized using the AFM techniques and are shown in Fig. 9. The surface of the hybrid thin film is quite smooth and has few visible defects, indicating that the synthesized hybrids have a molecular level dispersion of organic and inorganic networks, and the silica particles in GRHA-EP are distributed uniformly at the in the organic phase. The phase images also indicate that the GRHA domains were uniformly dispersed throughout the matrix. This is further supported by SEM analysis that the hybrid exhibits excellent structural uniformity, with no cracks or flaws.

CONCLUSION

Varying weight percentages of surface functionalized rice husk ash reinforced epoxy composites (0.5, 1.0 and 1.5 wt. % GRHA-EP) were developed and their mechanical, thermal, dielectric and surface morphology were studied. An incorporation of the surface functionalized rice husk ash reinforcement into DGEBA epoxy matrix has improved the thermo-mechanical properties. The percentage increment of reinforcement enhances the values of glass transition, delay in degradation of the composites and higher char yield which showed that the composite materials can withstand at higher temperature than those of neat epoxy matrix. The homogenous level of dispersion and exfoliation of the composites were confirmed by the SEM, TEM, AFM and XRD analysis. These surface functionalized rice husk ash reinforced epoxy composites can be used in the form of coatings, adhesives and matrices used for the fabrication of advanced composite components in the place of conventional epoxy for high performance applications. The roughness of the surface also increased with which provides an efficient interaction between GRHA-EP and DGEBA epoxy matrix. The hydrophobic nature of the composite samples is well understood from the increasing value of contact angle than that of neat epoxy matrix. All the above strengthen statements of GRHA-EP clearly shows its performance in higher degree and can be used in the panel boards, coating material, UV-absorption, Resistant material, aerospace applications.

ACKNOWLEDGEMENTS

The authors thank the Department of Mechanical Engineering and the Department of Ceramic Technology, Anna University, Chennai for providing to characterization facility. The authors also thank Dr. MR. Vengatesan and Dr. S. Devaraju for providing characterization facility and their moral support.

REFERENCES

1. Balamurugan, R., Kannan, P.: 1,3,4-Oxadiazole epoxy resin-based liquid crystalline thermo sets and their cure kinetics. J of Mat. Sci 45, 1321-1327 (2010)
2. Xinghong, Z., Shang, C., Tao, C., Xue, K.S., Fei, L., Guorong Qi.: Synthesis of a Soluble Azomethine-Containing Bisphenol and the Properties of Its Modified Epoxy Thermosets. J of App. Polym. Sci.106, 1632–1639 (2007)
3. Qingming, J., Shaoyun, S., Yaming, W., Lili, G., Jinbo, L.: Tribological performance and thermal behaviour of epoxy resin nanocomposites containing polyurethane and organoclay. Polym. Adv. Technol 19,859–864 (2008)
4. Bagherzadeh, MR., Mahdavi, F.: Preparation of epoxy–clay nanocomposite and investigation on its anti-corrosive behavior in epoxy coating. Progress in Organic Coatings 60, 117–120 (2007)
5. Sea-Fue, W., Yuh-Ruey, W., Kuo-Chung, C., Shih-Hsin, C.: Physical and electrical properties of polyimide/ceramic hybrid films prepared via non-hydrolytic sol–gel process, J of Mat Sci Mat Electron21,104–110 (2010)
6. Farhadyar, N., Rahimi, A., Langroudi, A.E.:, Iranian Preparation and Characterization of Aromatic Amine Cured Epoxy-Silica HybridInorganic-Organic Coating via In Situ Sol-gel, Process Polym. J14, pp. 155-162 (2005)
7. Krishnarao, RV., Mahajan, YR., Kumar, T.J.: Conversion of Raw Rice Husks to Sic by Pyrolysis in Nitrogen Atmosphere, J of EurCeram Soc 18, 147-152 (1998)
8. Li, Y., Ding, X., Guo, Y., Rong, C., Wang, L., Qu, Y., Ma, X., Wang, Z.: A new method of comprehensive utilization of rice husk. Journal of Hazardous Materials186,2151–2156 (2011)
9. Yue, C., Yanchao, Z., Zichen, W., Ying, L., Lili, W., Lili, D., Xiaoyan, G., Yuejia, M., Yupeng, G.: Application studies of activated carbon derived from rice husks produced by chemical-thermal process—A review. Advances in Colloid and Interface Science 163, 39–52. (2011)
10. Qiang, Z., Jian, T., Richard, C.M.Y., Albert, C.K.M., Robert, L.K.Y., Cunjiang, S.: Biodegradation behaviour of polycaprolactone / ricehusk composites in simulated soil medium. Polym. Degrad. and Stability 93, 1571–1576 (2008)
11. Hanafi, I., Nizam, J.M., Abdul Khalil, H.P.S.: The effect of a compatibilize on the mechanical properties and mass swell of white rice husk ash filled natural rubber/linear low density polyethylene blends. Polymer Testing 20, pp.125–133(2001)
12. Navin Chand, Fahimb, M., Prabhat Sharmaa., Bapat, M.N.: Influence of foaming agent on wear and mechanical properties of surface modified rice husk filled polyvinylchloride Jour: Wear (elseiver) 278, 83-86 (2012)
13. Kumagai, S., Sasaki, J.: Carbon/silica composite fabricated from rice husk by means of binderless hot-pressing. Bioresource Technology 100(13),3308–3315 (2009)
14. Khalf, A.I., Ward, A.A.: Use of rice husks as potential filler in styrene butadiene rubber/linear low density polyethylene blends in the presence of maleic anhydride. Materials & Design 31 (5), 2414–2421 (2009)
15. Bhagiyalakshmi, M., Yun, L.J., Anuradha, R., Jang, H.T.: Utilization of rice husk ash as silica source for the synthesis of mesoporous silicas and their application to CO_2 adsorption through TREN/TEPA grafting. Journal of Hazardous Materials 175, 928–938 (2010)
16. Adam,F., Kasim Mohammed, H., Tammar Hussein Ali.: Solvent free liquid-phase alkylation of phenol over solid sulfanilic acid catalyst. Applied catalysis A 399,42-49 (2011)
17. Adam, F., Farook, A., Jimmy Nelson., Mohamed Iqbal., Mohammad Anwar.: The utilization of rice husk silica as a catalyst: Review and recent progress. Catalysis Today 190, 2–14 (2012)
18. Chiang, C.L.,Chang, R .C.: Synthesis, characterization and properties of novel self-extinguishing organic–inorganic nanocomposites containing nitrogen, silicon and phosphorus via sol–gel method. Comp Sci and Tech 68,2849–2857 (2008)
19. Devaraju, S., Vengatesan, M.R., Alagar, M.: Studies on thermal and dielectric properties of ether linked cyclohexyl diamine (ELCD)-based polyimide POSS nanocomposites (POSS-PI). High Performance Polymers23(2), 99–111 (2011)

20. Vengatesan, M.R., Devaraju, S., Alagar, M.: Studies on thermal, mechanical and morphological properties of organoclay filled azomethine modified epoxy nanocomposites. High Performance Polymers 23(1) 3–10, (2011)
21. Silvia, L.F., Milena, S.L., Alberto, G.V., Eduardo, R.: Chemical, morphological, and mechanical analysis of rice husk/post-consumer polyethylene composites, Composites: Part A, 41, 154–160 (2010)
22. Arunkumar, R., Alok, S.:, Study on mechanical and tribo-performance of rice-husk filled glass–epoxy hybrid composites. Materials and Design 41, 131–141(2012)
23. Hanafi, I., Jaffri, R.M.: Material Properties Physico-mechanical properties of oil palm wood flour filled natural rubber composites, Polymer Testing18, 381–388 (1999)
24. Chandramohan, A., Dinkaran, K., Ashok Kumar, A., Alagar, M.: Synthesis and characterization of epoxy modified cyanate ester POSS nanocomposites. High Performance Polymers, 1–13 (2012)
25. Nagendiran, S., KarikalChozhan, C., Alagar, M., Hamerton, I.: Inorganic/Organic Hybrid Nanocomposites involving OMMT Clay and Cyanate ester–Siloxane-modified Epoxy Resin: Thermal, Dielectric and Morphological Properties. High Performance Polymers 20, 323–347, (2008)
26. Lin, C.H., Feng, C.C., Hwang, T.Y.: Preparation, thermal properties, morphology, and microstructure of phosphorus containing epoxy/SiO_2 and polyimide/SiO_2 nanocomposites. Eur Polym J43, 725–742 (2007)
27. .Nagendiran, S., Alagar, M., Hamerton, I.: Octasilsesquioxane reinforced DGEBA and TGDDM epoxy nanocomposites: characterization of thermal, dielectric and morphological properties. Acta Materialia 58, 3345–3356 (2010)
28. Zulfiqar, S., Ahmad, Z., Sarwar, M.I.: Soluble aromatic polyamide bearing ether linkages: synthesis and characterization. Colloid Polym Sci285, 1749–1754 (2007)
29. Hameed, N., Thomas, S. P., Abraham, R., Thomas,S.: Morphology and contact angle studies of poly(styrene-co-acrylonitrile) modified epoxy resin blends and their glass fibre reinforced composites eXPRESS Polymer Letters 1, (6) 345–355 (2007)
30. Devaraju, S., Vengatesan, M.R., Selvi, M., Ashok Kumar, A., Hamerton, I., Alagar, M.: Low surface free energy cyanate ester–silica hybrid (CE–SiO_2) nanomaterials for low k dielectric applications . RSC Advances (2013)
31. Silvia Luciana, F., Milena Savioli, L., Alberto, G.V., Ricardo, R.S., Eduardo Radovanovic.: Chemical, morphological, and mechanical analysis of rice husk/post-consumer polyethylene composites. Composites: Part A 41, 154–160 (2010)

Table 1: Mechanical properties of neat epoxy and GRHA-EP nanocomposites

Sample	Tensile strength (MPa)	Tensile Modulus (MPa)	Flexural strength (MPa)	Impact strength (J/m²)	Hardness HV1.2	Dielectric constant	Water absorption
Epoxy	61.3	2721	106.0	101.0	84.00	4.50	0.1232
0.5 % GRHA-EP	64.1	2725	110.2	105.0	105.0	3.48	0.0239
1.0 % GRHA-EP	69.8	2922	126.9	119.1	110.0	3.11	0.0193
1.5 % GRHA-EP	75.7	3325	134.5	132.2	133.0	3.07	0.0188

Table 2: Thermal properties of neat epoxy and GRHA-EP nanocomposites

Sample	Tg (˚C)	Temperature at characteristic weight loss (˚C)			Char Yield at 700˚C (%)
		20 %	40 %	60 %	
Neat epoxy	162	372	393	420	0
0.5 % GRHA-EP	171	380	400	425	17.48
1.0 % GRHA-EP	176	385	410	465	18.30
1.5 % GRHA-EP	179	387	441	471	19.02

Table 3: Contact angle and surface energy of GRHA-EP nanocomposites

Sample name	Contact angle		Surface energy		
	Water	Diiodomethane	γ^d	γ^p	Γ
0.5 % GRHA-EP	93.0	57.5	30.7	1.5	32.2
1.0 % GRHA-EP	100.3	63.8	26.4	1.1	27.5
1.5 % GRHA-EP	102.1	72.3	21.4	1.0	22.4

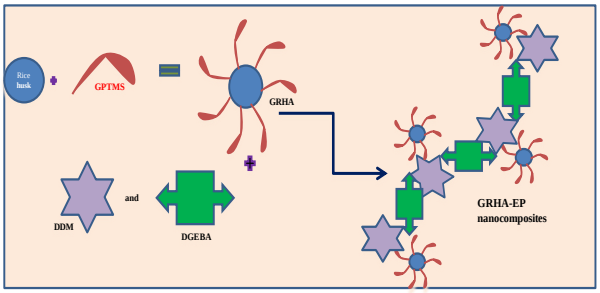


Fig.1: Preparation of surface functionalized rice husk ash reinforced (GRHA-EP) nanocomposites (Graphical abstract)

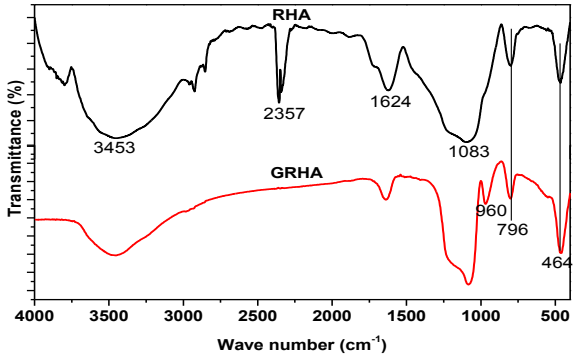


Fig.2: FT-IR spectra of neat rice hush ash (RHA) and functionalized rice husk ash (GRH)

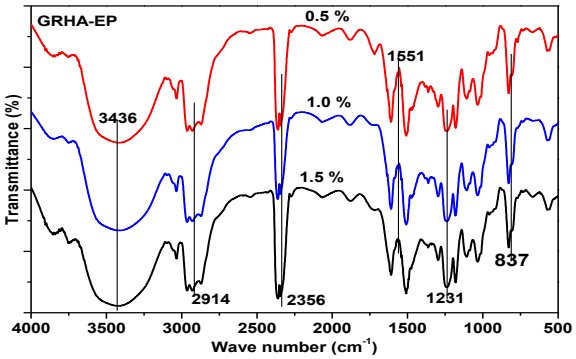


Fig.3: FT-IR spectra of glycidyl functionalized rice husk ash reinforced epoxy nanocomposites (0.5%, 1.0%, 1.5 % GRHA-EP)

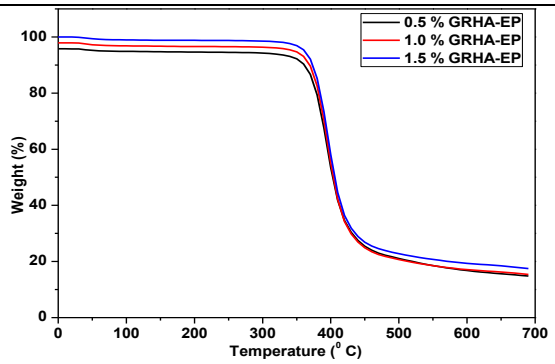


Fig.4: TGA of (0.5%, 1.0%, 1.5 % GRHA-EP) nanocomposites

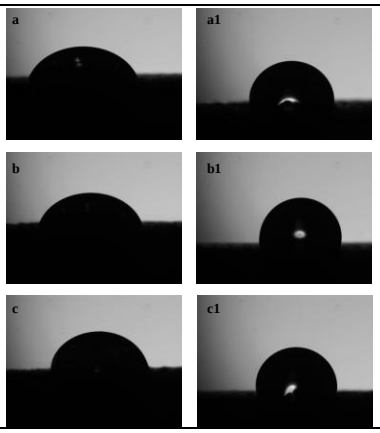


Fig.5: Contact angle of (a-0.5%, b-1.0%, c-1.5 % GRHA-EP) for diiodomethane and water (a1-0.5%, b1-1.0%, c1-1.5 % GRHA-EP)

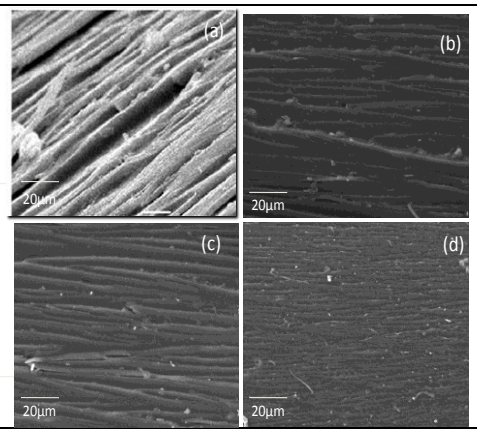


Fig.6: SEM micrograph of (a) neat epoxy, (b) 0.5% GRHA-EP, (c) 1.0% GRHA-EP, (d) 1.5 % GRHA-EP, nanocomposites

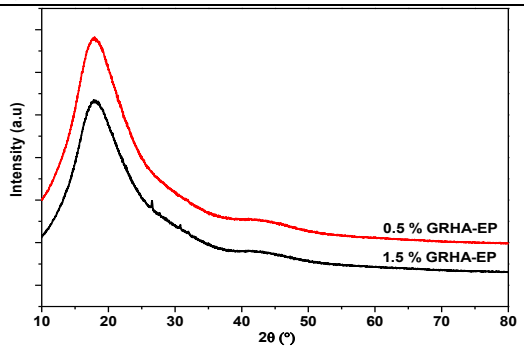


Fig. 7: XRD of 0.5% GRHA-EP and 1.5 % GRHA-EP nanocomposites

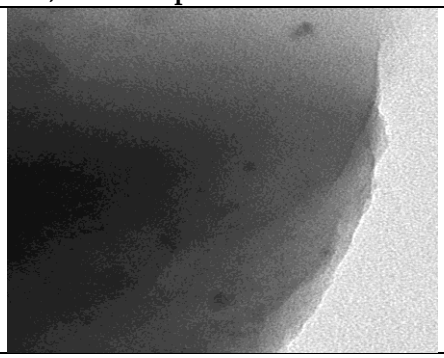


Fig. 8: TEM micrograph of 1.0 % GRHA-EP nanocomposites

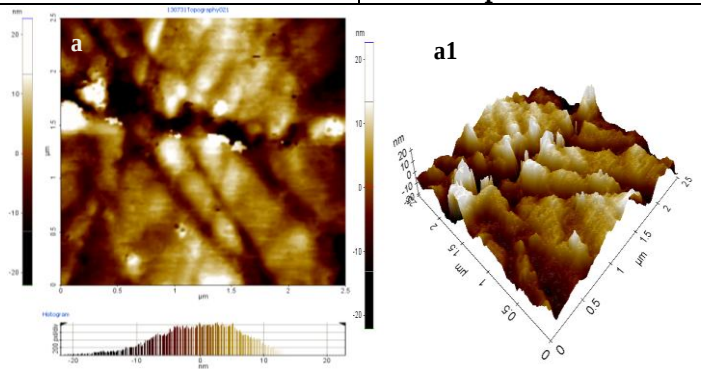
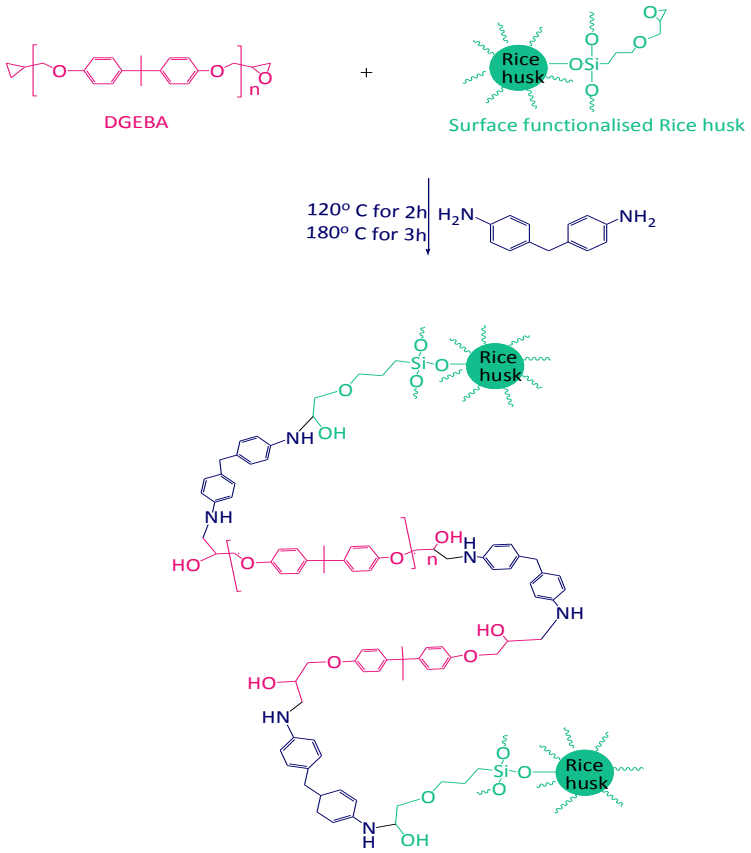


Fig. 9: AFM microphotographs of 1.0 % GRHA-EP nanocomposites (a) 2D image (a1) 3D image



Scheme 1: Schematic representation of GRHA-EP nanocomposites.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of Anti Atherogenic Effect from Flower Part of *Rhododendron arboreum* Linn. Fed With High Fat Diet

Swamidasan R[1]*, Sanil Kumar R[2] and Manasa Deepa R[3]

[1]Noori College of Pharmacy, Andersonpet, KGF, Kolar District, Karnataka, India.
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[3]East West College of Pharmacy, Yelahanka, Bengaluru, Karnataka, India.

Received: 15 Nov 2020 Revised: 17 Dec 2020 Accepted: 19 Jan 2021

***Address for Correspondence**
Swamidasan R
Noori College of Pharmacy,
Andersonpet, KGF,
Kolar District, Karnataka, India.
Email : samsamm1985@gmail.com

ABSTRACT

To investigate the evaluation of various extract from flower part of *Rhododendron arboreum* Linn. in rats fed with high fat diet (HFD). The various extract of *Rhododendron arboreum* Linn. was administered in doses of 100 and 200 mg/kg/day to rats fed with atherogenic diet to assess its possible lipid-lowering potential. There was a recognize increment in the body weight in HFD fed group ($p<0.001$), which was reduced by administration of higher dose of ethyl acetate and ethanolic extract of *Rhododendron arboretum* (200 mg/kg), lower dose of ethyl acetate and ethanolic extract of *Rhododendron arboretum* (100 mg/kg). Evaluate the plasma lipid profile and plasma lipoprotein such as HDL, VDL, VLDL and total cholesterol. The effect of tissue lipid content was free cholesterol, ester cholesterol, phospholipid, triglyceride and free fatty acid. Then *In vivo* enzymatic antioxidant study of SOD, CAT, GPx, glutathione reductase, glutathione-S-transferase, and non-enzymatic antioxidant of glutathion. The elevated levels of TBARS and conjugated denies were observed. The ethanolic extract of *Rhododendron arboreum*could protect against atherosclerosis and decrease the atherogenic index and cardiac risk ratio. This finding provides some biochemical basis for the use of various extract of flower part of *Rhododendron arboretum*as hypolipidemic agent having preventive and therapeutic effect against hyperlipidemia.

Keywords: High fat diet, hyperlipidemia, *Rhododendron arboreum,* wistar rats.

INTRODUCTION

Atherosclerosis is a rheumatoid heart disease in which the inside of an artery narrows are buildup of plaque. Initially, there are generally no symptoms. When severe, it can result in coronary artery disease, stroke, peripheral artery disease or kidney problems, depending on which arteries are affected. Symptoms, if they occur, generally do

not begin until middle age. Atherosclerosis generally starts when a person is young and worsens with age. Almost all people are affected to some degree by the age of 65. It is the number one cause of death and disability in the developed world. Though it was first described in 1575, there is evidence that the condition occurred in people more than 5,000 years ago. *Rhododendron arboreum* Linn. is an evergreen shrub or small tree with a showy display of bright red flowers. The name 'RHODODENDRON' is derived from the Greek word 'RHODO' means rose & 'DENDRON' means tree. Rhododendron is the national flower of Nepal & is known as (Laligurans) & the state tree of Uttarakhand. It is called 'Burans, Bras, Buras or Barahke-phool' in local dialect. It is widely popular for the processed juice of its flowers which have gained market popularity as rhodojuice / sharbat. The plant is found in the Himalayas from Kashmir eastwards to Nagaland. Various parts of the plant exhibited medicinal properties & it is used for the treatment of various ailments. *Rhododendron arboreum* Linn. plant belongs to genus *Rhododendron*and family *Ericaceae.* Commonly it is known as "Billi" and "Buransh" in hindi. The flowers of *R. arboreum* range in color from a deep scarlet, to red with white markings, pink to white. It is reported that the bright red forms of this rhododendron are generally found at the lower elevations **[20]**. Flowers are showy, red in dense globose cymes **[3]**. Calyx- fine cleft, Corolla-tube spotted funnel shaped, Stamens-hypozygnous declining, Filaments filiform, Anthers-ovate, Style-capitate **[5]**. It is one of the most traditional system of medicine in Ayurvedic and Siddha.

Sub species

- *Rhododendron arboreum* spp. Arboreum (red or rose red flowers) found in Western Himalayas.
- *Rhododendron arboreum* spp. Cinnamomeum (white, pink or red flowers) found in Central Himalayas.
- *Rhododendron arboreum* spp. Delavayii (red flowers) found in Eastern Himalayas.
- *Rhododendron arboreum* spp. Nilagiricum (red flowers) found in Nilgiri.
- *Rhododendron arboreum* spp. Zeylancium (orange red flowers) found in Sri Lanka.

Petals are eaten for their sour-sweet taste. Flowers are offered to deities in almost all religious functions. Fresh leaves are burnt along with leaves of species of Juniper/Thuja/ Pinus, for making smoke that is believed to be sacred and help in purifying air in West Kameng and Tawang districts of Arunachal Pradesh. **Young leaves are applied to the forehead for alleviating headache. Flowers and bark are used to cure digestive and respiratory disorders.**

MATERIALS AND METHODS

Collection and identification of *Rhododendron arboreum* (Linn)

The flower part of *Rhododendron arboreum* was collected from Himachel Pradesh, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamil Nadu, India. The plant powdered materials were put away in a hermetically sealed holder. The flowers were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Preparation of extract

The flowers powdered materials were successively extracted with pet.ether, ethyl acetate and ethanol (40 to 60°C) by hot continuous hot percolation method in Soxhlet apparatus (Harborne, 1984) for 24 h. Then the extract was concentrated by using a rotary evaporator and subjected to freeze drying in a lyophilizer till dry powder was obtained.

Animal diet

The compositions of the two diets were used as follows **[11]**

Control diet

22.5% of wheat flour, 60% of roasted bengal gram powder, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% of starch with salt mixture, 0.5% of choline mixture and vitamin.

Atherogenic diet

20.5% of wheat flour, 52.6% of roasted bengal gram, 5% of skimmed milk powder, 4% of refined oil, 4% of casein, 4% starch with salt mixture, 9% of coconut oil, 0.5% of choline mixture and vitamin & 0.4% of cholesterol.

Experimental design

Totally 42 number of rats were utilized for this experiment. The rats were divided into seven groups of six each.

S. No	Groups	Treatment
1.	Group I	Normal Saline (Normal control, Orally)
2.	Group II	High fat diet Control (HFD- 9% Coconut Oil and 0.4% Cholesterol; Orally)
3.	Group III	HFD + Atorvastatin (10 mg/kg; p.o.)
4.	Group IV	HFD + Ethyl acetate extract of *Rhododendron arboreum*(100 mg/kg, p.o.)
5.	Group V	HFD + Ethyl acetate extract of *Rhododendron arboreum*(200 mg/kg, p.o)
6.	Group VI	HFD + Ethanolic extract of *Rhododendron arboreum*(100 mg/kg, p.o.)
7.	Group VII	HFD + Ethanolic extract of *Rhododendron arboreum*(200 mg/kg, p.o)

Rats of groups IV and V were orally fed with the ethyl acetate extracts of *Rhododendron arboreum*, rats of group VI and VII were fed with the ethanolic extracts of *Rhododendron arboreum*and rats of group III were fed with standard drug atorvastatin. Both the ethyl acetate and ethanolic extracts and atorvastatin were suspended in 2% of tween 80 independently and sustained to the particular rats by oral intubation. Animals were sacrificed toward the end of 63 days (9weeks) every one by cervical dislocation after during the night fasting. Blood was gathered in heparinised tubes and plasma was isolated. Aorta, heart and liver were cleaned up adhering fat, measured precisely and utilized for the preparation of homogenate. Animals were sufficiently given consideration according to the Animal Ethical Committee's suggestions.

Biochemical analysis

Plasma samples were estimated for total cholesterol, HDL-cholesterol and triglycerides utilizing Boehringer Mannheim kits by Erba Smart Lab analyzer United States of America. Friedwald method [25] was used to determine the LDL-cholesterol and VLDL-cholesterol. Extract was utilized for the estimation of free cholesterol as cholesterol digitonide. Ester cholesterol extracted with petroleum ether (30-60°C BP) was selectively. Both the ester cholesterol and free cholesterol were then quantitation subjected to Liebermann-Burchard reaction [14]. Free fatty acids [19], Phospholipids [17]and Triglycerides were estimated by the method of [7].Segments of liver tissues, heart tissues and aorta tissues were blotted, measured and homogenized with 3 volumes of methanol and the lipid extracts were gotten by the method [22]. Plasma total cholesterol: HDL-cholesterol ratio and LDL-cholesterol: HDL-cholesterol ratio was also calculated to access the atherogenic risk [28], cardiac risk ratio [10] and Atherogenic coefficient [9].The tissue lipid peroxidation of estimation of thiobarbituric acid reactive substances (TBARS) [27] and estimation of conjugated dienes [16].

Analytical methods

A known measure of the tissue was homogenized with - 2.5 mL (3:1 v/v) ethanol-ether mixture and processed for around two hours at 60-65^{o}C and the supernatant was collected. Three mL of ethanol-ether mixture and the residue was mixed, processed further for a time of two hours at 60-65^{o}C and the supernatant was gathered. Chloroform-methanol mixture- 1 mL (1:1 v/v) was then mixed to the residue. It was repeat processed for 1 hours at 50-55°C and

the supernatant was collected. The upper layer was pooled and made up to a specified volume. The plasma was also treated with extraction of lipids for similarly. This lipid extract was finally used for the estimation of free cholesterol, ester cholesterol, triglycerides, free fatty acids and phospholipids. [11], [22].

Statistical analysis

The results were expressed as mean ± standard deviation of 6 rats in each group. The statistical significance between the groups was carried out by using one way ANOVA as in standard statistical software package of social science (SPSS).

RESULTS AND DISCUSSION

Effect of various extracts of flower of *Rhododendron arboreum* on body weight changes in rats

The average body weight of rats in all the seven groups increased after 63 days of experimental period. Atherogenic diet feeding rats (group II) had a significantly increased body weight compared with control group rats (group I). The body weight significantly reduced in the ethanolic extract of *Rhododendron arboreum* treated groups.

Effect of various extracts of flower of *Rhododendron arboreum* on plasma lipid in high fat diet rats

The concentration of free cholesterol, ester cholesterol, triglyceride and phospholipid were significantly increased in plasma and tissues of rats fed HFD (group II) as compared with the control groups of rats (group I). Treatment of ethanolic extract of *Rhododendron arboreum*at the dose of 200mg/kg body weight along with HFD significantly reduced the plasma and tissues free cholesterol & ester cholesterol and triglyceride & phospholipid levels in as compared to rats fed with HFD (group II).

Atherogenic index

Atherogenic index was elevated on treatment with HFD rats, but it was reduced markedly by ethanolic extract of *Rhododendron arboreum.*

Effect of various extracts of leaves of *rhododendron arboreum* on plasma lipoprotein in high fat diet fed rats

The LDL, VLDL cholesterol concentration increased in rats fed with HFD (group II) compared to control rats (group I). Rats treated with ethanolic extract of *Rhododendron arboreum* at the dose of 200mg/kg body weight significantly reduced the levels of LDL and VLDL cholesterol as compared to HFD rats (group II). The HDL cholesterol concentration significantly reduced in HFD rats (group II) compared to the control group of rats (group I). HDL cholesterol level significantly increased in rats treated with ethanolic extract of *Rhododendron arboreum* at the dose of 200mg/kg body weight (group V) and it was comparable with standard drug atorvastatin (group III).

Effect of various extracts of flower part of *Rhododendron arboreum* on tissue lipid content in high fat diet rats

Effect of various extracts of flower part of *Rhododendron arboreum* on tissues lipid peroxidation in high fat diet rats

The TBARS and conjugated diene levels significantly elevated in aorta, heart and liver of rats fed with HFD (group II) as compared to control rats (group I). However, the treatment of ethanolic extract of *Rhododendron arboreum* (200mg/kg body weight) significantly reduced the levels of TBARS and conjugated diene as compared with HFD rats (group II).

Effect of various extracts of flower part of *Rhododendron arboreum* on tissue enzymatic antioxidants in high fat diet rats

The activities of the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), glutathione reductase (GRX) and Glutathione-S-transferase (GST) and non-enzymatic antioxidant

glutathione levels in tissues significantly decreased in HFD rats (group II) when compared with control rats (group I).

Effect of various extracts of flower part of *Rhododendron arboreum* on tissue non – enzymatic antioxidants

Treatment of ethanolic extract of *Rhododendron arboreum* (200mg/kg body weight) with HFD rats significantly elevated both enzymatic and non-enzymatic antioxidant level than that of other lower dose (100mg/kg body weight) extracts treatment group.

CONCLUSION

The result of present study revealed that the ethanolic extract of flower part of *Rhododendron arboreum* significantly reduced the plasma lipid and lipoprotein profile, thus reduced the atherogenic index and cardiac risk ratio. It also significantly reduced the tissues free cholesterol, ester cholesterol, triglycerides, phospholipids and free fatty acid. Then significantly reduced the antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX), glutathione reductase (GRX) and Glutathione-S-transferase (GST) and non-enzymatic antioxidant glutathione levels. This finding provides some biochemical basis for the use of ethanolic extract of flower part of *Rhododendron arboreum* as antihyperlipidemic agent having preventive and curative effect against hyperlipidemia. This study plan to lead further studies is required to again more insight into the possible mechanism of action of this medicinal plant.

REFERENCES

1. Amit Agrawal and Rishikesh Sharma.(20190. A study of antiobesity, hypolipidemic and anti-atherogenic activity of poly herbal formulation against high fat diet-induced obesity in albino rats.*J. Adv. Res. in Pharmaceutical Sciences & Pharmacology Interventions*, Vol. 3(1): pp. 1-5.
2. Amutha Iswarya Devi J and Kottai Muthu A. (2014).*In Vitro* antioxidant activity and total phenolic content of ethanolic extract of whole plant of *Lactuca runcinata* (DC). *International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS)*, Vol. 5(7): pp. 72- 89.
3. Chinwendu M. Chikezie, Okey A. Ojiako, Adamma A. Emejulu and Paul C. Chikezie. (2018).Atherogenicity of diabetic rats administered single and combinatorial herbal extracts.*Bulletin of Faculty of Pharmacy*, Vol. 5(6): pp. 169–174.
4. Dale S Edgerbon, Sylvain Cardin Maya E, Emshwiller and Doss Neal. (2001). Small increases in insulin inhibit hepatic glucose production solely caused by an effect on glycogen metabolism.*Diabetics*, Vol. 50(8): pp. 1807-1882.
5. Fatima Mohamed Hussein Shediwah, Khalid Mohammed Naji, Hussein Saleh Gumaih and Fatima A. Alhadi, Amani Lutf Al-Hammami, Myrene Roselyn D Souza.(2018). Antioxidant and anti-hyperlipidemic activity of *Costus speciosus* against atherogenic diet-induced hyperlipidemia in rabbits.*Journal of Integrative Medicine*, Vol. 6(5): pp. 1-9.
6. Gabriel A, Agborab, Joe A. Vinsonb, Julianne Sortinob and Robert Johnson. (2012). Antioxidant and anti-atherogenic activities of three piper species on atherogenic diet fed hamsters, *Experimental and Toxicologic Pathology*, Vol. 6(4): pp. 387– 391.
7. Gavin Hamilton, Alexandra N Schlein and Michael S. (2017). *In-vivo* triglyceride composition of abdominal adipose tissues measured by H MRS at 3T. *Jouranal of magnetic resonance imaging*. Vol. 45(5): pp. 1455-1463.
8. Geetha Kodali and Ahmadi Banu.(2016). Hypolipidemic activity of ethanolic extract of *Saccharum spontaneum* in atherogenic diet induced rats.*Journal of Pharmacy Research*, Vol. 10(1): pp. 16-20.

9. Helen Yeni Komshian, Marcello Carantoni, Fahim Abbasi and Gerald M Reaven. (2000). Relationship between several surrogate estimate of insulin resistance & quantification of insulin mediated glucose disposal in 490 healthy non diabetic volunteers.*Diabetes Care,* Vol. 23(2): pp. 171-175.
10. Henry Ohem Okpa, Ofem Egbe Enang, Emmanuel Edet Effa, Okon Ekwere Essien and Patrick Ntui Mbu. (2015).Comparative analysis of atherogenic index of plasma and its relationship with cardiovascular risk among patients with diabetes mellitus and concurrent diabetes mellitus with hypertension.*Attending endocrinology clinic in a tertiary hospital south-south Nigeria*, Vol. 14(8): pp. 102-107.
11. Jeyaraman Amutha Iswarya Devi and Arumugam Kottai Muthu.(2015). Hypolipidemic effect of ethanolic extract from whole plant of *Lactuca runcinata* (DC.) in atherogenic diet induced hyperlipidemic rats.*African Journal of Pharmacy and Pharmacology*, Vol. 9 (47): pp. 1080-1087.
12. Jie Jia and Xin Cun Dong. (2009). Evalution of *invivo* antioxidant activities of ganoderma lucidum polysaccharides in STZ diabetic rats. *Food chemistry*, vol. 11(5); pp. 32-36.
13. Jon MC Murdy, Jregory Jay, Selim Suner and Gregory Crauford. (2009). Photonics based *invivo* total haemoglobin monitoring and clinical relevance.*Journal of biophotonics*, Vol. 2(5): pp. 227-278.
14. Jose Castro Perez, Stephen F Previs, David G Mc Laren, Vinit Shah and Gowri Bhat. (2019).*Invivo* DZO labelling to quantity static & dynamic changes in cholesterol & cholesterol esters by high resolution LC/MS.*Journal of lipid research*, Vol. 7(5): pp. 45-56.
15. Jovana Veselinovic, Aleksandar Veselinovic, Andrey Toropov, Alla Toropova, Ivana Damnjanovic and Goran Nikolic.(2014). Monte Carlo method based QSAR modelling of coumarin derivatives as potent HIV-1 integrase inhibitors and molecular docking studies of selected 4-phenyl Hydroxycoumarins. *Scientific Journal of the Faculty of Medicine in Nis.*,Vol. 31(2): pp. 95-103.
16. Kedlaya R and Vasudeval DM. (2004). Inhibitiol of lipid peroxidation by botanical studies.*Life sciences*. Vol. 76(1): pp. 21-28.
17. Klara Valko, Gabriela Invanova Berndt, Paul Beswick and Mark kidney. (2018). Biomimetic HPLC to estimate lipophilicity, protein & phospholipid binding of protential peptide therapeutics.*Admet & D MPK*, Vol. 2(6): pp .162-175.
18. Michael Silveira Coelho, Sabrine De Araujo Aquino and Juliana Machado Latorres. (2019).*Invitro* and *invivo* antioxidant capacity of chia protein hydrolysates and peptides. *Food hydrocolloids*. Vol. 91(5): pp. 19-25.
19. Mooks S and Halkes CJM. Bilesen S and castro cabeza S M. (2004). *In-vivo* regulation of plasma tree fatty acids in insulin resistance.*Metabolism Science direct*.,Vol. 53(9): pp. 76-83.
20. Nada Orsolic, Irena Landeka Jur¡cevic, Domagojikic, Dunja Rogic, Dyana Odeh, Vedran Balta, Eleonora Perak Junakovic, Svjetlana Terzic and David Jutri.(2019). Effect of propolis on diet-induced hyperlipidemia and atherogenic indices in mice *Antioxidants*, Vol. 8(5): pp. 9-22.
21. Ntchapda fidele, Djedouboum Abakar, Talla Emmanuel, Sokeng Dongmo Selestin, Nana paulin, Adjia Hamadjida, Nguimbou Richard Marcel, Bonabe Christian, Gaimatakon Samuel, Ntintang yanou Nicolas and Dimo Theophile.(2014). Hypolipidemic and anti-atherogenic effect of aqueous leaves extract of *Ficus glumosa* (Moraceae) in rats.*Exp. Gerontol*, Vol. 6(3): pp. 31-38
22. Patrick Poulin and Frank Peter Thiel. (2000). A priori prediction of tissue plasma partition coefficient of drug to facilitate the use of physiologically based pharmacokinetic models in drug discovery.*Journal of pharmaceutical science*, Vol. 89(1): pp .16-35.
23. Piaoping Zhou, Mengqiang Wang, Fegjun Xie, Dong Fang Deng and Qicum Zhou. (2016). Effect of dietary carbohydrate to lipid ratios on growth performance, digestive enzyme & hepatic carbohydrate metabolic enzyme activities of large yellow croaker.*Aquaculture*, Vol. 45(2): pp. 45-51.
24. Rihua Xu, Nan Shang and Pinglan Li. (2011).*Invitro* and *invivo* antioxidant activity of exopolysaccharide fraction from bifidobacterium animals RH. *Anaerobe*. Vol. 17(5): pp. 226-231.
25. Saravanan Subramaniam, Ramachandran Subramaniam, Suja Rajapandian, Subasini Uthrapathi, Victor Rajamanickam Gnanamanickam and Govinda Prasad Dubey.(2011). Anti-atherogenic activity of ethanolic fraction of *Terminalia arjuna* bark on hypercholesterolemic rabbits.*Evidence-Based Complementary and Alternative Medicine*, Vol. 7(4). pp. 342- 357.

26. Suresh N Thennadil, Jessica L Rennert, Brian J Wenzel and Kevin H Hazel. (2001). Comparison of glucose concentration in interstitial fluid and capillary and venous blood during rapid changes in blood glucose levels.*Diabetes Technology and Therapeutics*, Vol. 3(3): pp. 357-365.
27. Vijay M Khedkar, Nikhilesh Arya, Evans C Coutinho, Chamanlal J Shishoo and Kishor S Jain. (2014).Docking study of novel antihyperlipidemic thieno[2,3-*d*]pyrimidine; LM-1554, with some molecular targets related to hyperlipidemia - An investigation into its mechanism of action. *Link. Springer*, Vol. 3(5): pp. 67-75.
28. Zhang Q, N Li, Gzhou and Z Li. (2003).*Invivo* antioxidant activity of polysaccharide fraction from *Porphyra haitanesis* in aging mice. *Elsevier*, Vol. 48(2): pp.151-155.

Table 1: Average body weight changes in rats

Values are expressed as mean ± SEM (n=6 rats)

Groups	Initial Weight (g)	Final Weight (g)	Average Body weight gain (g)
Group I	185.10 ± 0.15^{bNS}	240.08 ± 0.10 ^{b**}	54.98 ± 0.54^{b**}
Group II	154.62 ± 0.84^{aNS}	235.75 ± 0.68^{a**}	81.13 ± 0.76^{a**}
Group III	160.44 ± 0.12^{aNS,bNS}	231.49 ± 0.15^{aNS,bNS}	71.05 ± 0.90^{aNS,bNS}
Group IV	142.17± 0.12^{aNS,bNS}	222.62 ± 2.20^{aNS,bNS}	80.45 ± 2.32^{aNS,bNS}
Group V	149.55 ±0.53^{aNS,bNS}	226.64 ± 2.30^{aNS,bNS}	77.09± 1.76^{aNS,bNS}
Group VI	151.34 ± 0.41^{aNS,bNS}	231.56 ± 1.33^{aNS,bNS}	80.22± 1.72^{aNS,bNS}
Group VII	152.75± 0.80^{aNS,bNS}	225.15± 1.42^{aNS,bNS}	72.40± 2.22^{aNS,bNS}

P values : *<0.001, **<0.05

NS : Non significant

a → group I compared with groups II, III, IV, V, VI & VII.

b → group II compared with groups I, III, IV, V, VI & VII.

Table 2: Plasma lipid profile in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Total cholesterol (mg/dl)	Free cholesterol (mg/dl)	Ester cholesterol (mg/dl)	Free fatty acid (mg/dl)	Phospho lipid (mg/dl)	Tri glyceride (mg/dl)
Group I	114.10± 1.33^{b*}	28.80 ±1.08^{b*}	85.29 ±0.90^{b*}	44.84±0.32^{b*}	98.46±0.44^{b*}	84.34±1.32^{b*}
Group II	168.67±1.45^{a*}	40.45±0.80^{a*}	128.22±1.24^{a*}	60.80±0.22^{a*}	148.80±0.50^{a*}	155.33±1.26^{a*}
Group III	112.74±0.48^{a*,b*}	29.07±0.62^{a*,b*}	83.67±0.56^{a*,b*}	39.65±0.62^{a*,b*}	104.28±0.33^{a*,b*}	82.46±0.76^{a*,b*}
Group IV	123.69±0.56^{a**,b*}	35.43±0.68^{a*,b**}	88.26±0.43^{a**,b*}	48.32±0.48^{a*,b}	160.33±0.42^{a*,b**}	89.30± 0.24^{a*,b*}
Group V	115.93± 0.59^{a**,b*}	31.87±0.33^{a*,b**}	84.06±0.26^{a**,b*}	45.04±0.38^{a*,b**}	155.36±0.32^{a**b*}	85.47± 0.22^{a*,b*}
Group VI	112.31±0.52^{a*,b}	24.50±0.37^{a*,b}	87.81±0.50^{a*,b*}	44.84±0.32^{a*,b*}	162.87±0.24^{a*,b*}	82.29± 0.55^{a*,b*}
Group VII	102.50±0.74^{a*,b*}	29.21±0.28^{a*,b*}	73.29±0.56^{a*,b*}	40.98±0.33^{a*,b*}	152.90±0.23^{a*,b*}	82.08±0.30^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 3: Plasma lipid profile in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Atherogenic index	Cardiac risk ratio	Atherogenic Coefficient
Group I	1.29± 0.02^{b*}	1.75± 0.02^{b*}	0.75±0.01^{b*}
Group II	3.65± 0.02^{a*}	3.96±0.38^{a*}	2.96± 0.04^{a*}
Group III	1.40± 0.04^{a*,b*}	1.91±0.02^{a*,b*}	0.91±0.03^{a*,b*}
Group IV	1.95± 0.05^{a*,b*}	2.70±0.03^{a*,b*}	1.70±0.07^{a*,b*}
Group V	1.72± 0.05^{a*,b*}	2.34±0.02^{a*,b*}	1.34±0.06^{a*,b*}
Group VI	1.75± 0.08^{a*,b*}	2.39±0.06^{a*,b**}	1.39±0.04^{a*,b*}
Group VII	1.51± 0.04^{a*,b*}	1.89±0.05^{a*,b**}	0.89±0.02^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 4: Plasma lipoprotein in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	HDL cholesterol (mg/dl)	LDL cholesterol (mg/dl)	VLDL cholesterol (mg/dl)	LDL- c/HDL-c ratio	HDL-c/ TC ratio
Group I	65.06 ± 0.75^{b*}	40.12 ± 1.02^{b*}	18.56 ± 0.48^{b*}	0.61 ± 0.05^{b*}	0.57± 0.006^{b*}
Group II	42.58 ± 2.28^{a*}	100.42 ± 1.56^{a*}	32.30 ± 0.40^{a*}	2.35 ± 0.26^{a*}	0.25 ± 0.016^{a*}
Group III	58.96 ± 0.45^{a*,b*}	30.85 ± 0.58^{a*,b*}	15.04 ± 0.30^{a*,b*}	0.52 ± 0.02^{a*,b*}	0.52 ± 0.003^{a*,b*}
Group IV	45.72±0.28^{a*,b*}	61.85±0.18^{a*,b**}	15.80± 0.15^{a*,b*}	1.35±0.22^{a*,b*}	0.36±0.20^{a*,b**}
Group V	49.50±0.22^{a*,b*}	32.73±0.10^{a**,b*}	16.90±0.17^{a*,b*}	0.66±0.12^{a*,b*}	0.43±0.10^{a**,b*}
Group VI	47.04±0.14^{a*,b*}	37.25±0.17^{a**,b*}	17.36±0.18^{a*,b*}	0.79±0.20^{a*,b*}	0.42±0.14^{a*,b**}
Group VII	54.15±0.20^{a*,b*}	32.64±0.18^{a*,b*}	15.80±0.15^{a*,b*}	0.60±0.21^{a*,b*}	0.53±0.10^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 6: Tissue lipid content of free cholesterol level in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	Free cholesterol (mg/g tissue)		
	Aorta	Heart	Liver
Group I	0.57 ± 0.05^{b*}	0.79 ± 0.07^{b*}	0.85 ± 0.04^{b*}
Group II	2.62 ± 0.012^{a*}	1.16 ± 0.08^{a*}	1.50 ± 0.06^{a*}
Group III	0.68 ± 0.04^{a*,b*}	0.78 ± 0.06^{a*,b*}	0.87 ± 0.05^{a*,b*}
Group IV	0.96 ± 0.06^{a*,b*}	1.02 ± 0.08^{a*,b*}	1.10 ± 0.07^{a*,b**}
Group V	0.88 ± 0.07^{a*,b*}	0.94 ± 0.04^{a*,b*}	0.98 ± 0.03^{a*,b*}
Group VI	0.82 ± 0.04^{a*,b**}	0.95 ± 0.07^{a*,b**}	0.96 ± 0.04^{a*,b**}
Group VII	0.70 ± 0.08^{a*,b*}	0.80 ± 0.05^{a*,b*}	0.89 ± 0.06^{a*,b*}

p values : *< 0.001, **< 0.05
a →group I compared with groups II, III, IV, V,VI & VII.
b →group II compared with groups I, III, IV, V, VI & VII.

Table 7: Tissue lipid content of ester cholesterol level in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	Ester cholesterol (mg/g tissue)		
	Aorta	Heart	Liver
Group I	2.46 ± 0.33^{b*}	2.95 ± 0.24^{b*}	2.10 ± 0.30^{b*}
Group II	7.02 ± 0.38^{a*,b**}	7.20 ± 0.32^{a*}	3.56 ± 0.24^{a*}
Group III	2.82 ± 0.16^{a*,b*}	3.08 ± 0.18^{a*,b*}	2.12± 0.15^{a*,b*}
Group IV	3.22 ± 0.26^{a*,b**}	3.90 ± 0.30^{a*,b**}	2.88 ± 0.08^{a*,b*}
Group V	2.98 ± 0.20^{a*,b*}	3.56 ± 0.26^{a*,b*}	2.54 ± 0.12^{a*,b*}
Group VI	3.02 ± 0.30^{a*,b**}	3.75 ± 0.22^{a*,b**}	2.63 ± 0.09^{a*,b**}
Group VII	2.90 ± 0.10^{a*,b*}	3.14 ± 0.16^{a*,b*}	2.18 ± 0.10^{a*,b*}

p values :*< 0.001, **< 0.05
a →group I compared with groups II, III, IV, V,VI & VII.
b →group II compared with groups I, III, IV, V, VI & VII.

Table 8: Tissue lipid content of triglyceride content in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	Triglyceride (mg/g tissue)		
	Aorta	Heart	Liver
Group I	11.82 ± 0.22^{b*}	12.54 ± 0.18^{b*}	9.87 ± 0.21^{b*}
Group II	25.28 ± 0.18^{a*b**}	49.70 ± 0.24^{a*}	30.14 ± 0.14^{a*}
Group III	14.18 ± 0.10^{a*,b**}	23.10 ± 0.10^{a**,b*}	13.56 ± 0.24^{a*,b*}
Group IV	20.42 ± 0.30^{a*,b**}	31.62 ± 0.32^{a*,b**}	21.25 ± 0.26^{a**,b*}
Group V	17.36 ± 0.24^{a*,b**}	27.34 ± 0.22^{a*,b*}	18.43± 0.18^{a*,b*}
Group VI	16.84 ± 0.12^{a*,b*}	26.45 ± 0.26^{a**,b*}	17.32 ± 0.24^{a*,b*}
Group VII	14.34 ± 0.20^{a*,b*}	23.78 ± 0.14^{a*,b*}	14.08 ± 0.18^{a*,b*}

p values : *< 0.001, **< 0.05
a →group I compared with groups II, III, IV, V,VI & VII.
b →group II compared with groups I, III, IV, V, VI & VII

Table 9: Tissue lipid content of Phospholipid content in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	Phospholipids (mg/g tissue)		
	Aorta	Heart	Liver
Group I	9.56± 0.16^{b*}	25.52 ± 0.22^{b*}	19.20 ± 0.24^{b*}
Group II	18.45 ± 0.20^{a*}	38.86 ± 0.35^{a*}	27.78 ± 0.30^{a*}
Group III	11.76 ± 0.12^{a*,b*}	28.44 ± 0.24^{a*,b*}	20.10 ± 0.28^{a**,b*}
Group IV	13.10 ± 0.18^{a*,b*}	32.76± 0.33^{a*,b**}	23.78± 0.18^{a*,b**}
Group V	12.88 ± 0.22^{a*,b*}	31.10± 0.20^{a*,b*}	22.46± 0.24^{a**,b*}
Group VI	12.76 ± 0.10^{a*,b**}	30.94± 0.12^{a*,b**}	22.00± 0.16^{a*,b**}
Group VII	11.94 ± 0.14^{a*,b*}	29.08 ± 0.18^{a*,b*}	20.00± 0.20^{a*,b*}

p values : *< 0.001, **< 0.05
a →group I compared with groups II, III, IV, V,VI & VII.
b →group II compared with groups I, III, IV, V, VI & VII.

Table 10: Tissue lipid content of free fatty acids in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	Free fatty acids (mg/g tissue)		
	Aorta	Heart	Liver
Group I	12.82 ± 0.30^{b*}	15.12 ± 0.26^{b*}	11.70± 0.20^{b*}
Group II	27.68 ± 0.28^{a*}	49.20 ± 0.24^{a*}	30.64 ± 0.18^{a*}
Group III	14.24 ± 0.16^{a*,b*}	18.21 ± 0.26^{a*,b*}	11.22 ± 0.24^{a*,b*}
Group IV	18.66± 0.22^{aNS,b*}	25.56± 0.18^{a*,b**}	15.64± 0.30^{a*,b**}
Group V	16.24± 0.14^{aNS,b*}	20.30± 0.10^{a*,b*}	13.30± 0.12^{a*,b*}
Group VI	17.04± 0.26^{aNS,b*}	21.68± 0.22^{a*,b**}	12.88± 0.18^{a*,b**}
Group VII	14.88± 0.12^{a*,b*}	19.10± 0.14^{a*,b*}	11.45± 0.10^{a*,b*}

p values : *< 0.001, **< 0.05
a →group I compared with groups II, III, IV, V,VI & VII.
b →group II compared with groups I, III, IV, V, VI & VII.

Table 11: Tissue lipid peroxidation of TBARS in high fat diet rats
[Values are mean ± SEM of 6 rats]

Groups	TBARS (n mol of MDA formed/g tissue)		
	Aorta	Heart	Liver
Group I	19.76 ± 2.92^{b*}	45.40 ± 2.80^{b*}	26.53 ±2.65^{b*}
Group II	70.12 ± 4.20^{a*}	89.12 ± 4.26^{a*}	81.60 ±4.25^{a*}
Group III	19.96± 1.26^{a*,b*}	45.86 ± 2.10^{a*,b*}	25.25± 2.10^{a*,b*}
Group IV	26.32±2.24^{a**,b**}	62.74±2.35^{a**,b**}	33.54±3.12^{a**,b*}

Group V	24.84±3.12^{a**,b*}	58.45±3.08^{a**,b*}	29.30±2.28^{a*,b**}
Group VI	23.66±2.04^{a*,b*}	54.58±3.10^{a*,b*}	30.44±42.14^{a*,b*}
Group VII	20.90±1.90^{a*,b*}	46.23±1.55^{a*,b*}	26.32±1.60^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 12: Tissue lipid peroxidation of conjugated diene in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	Conjugated diene (μ moles/g tissue)		
	Aorta	Heart	Liver
Group I	178.22± 0.72^{b*}	170.48. ± 0.36^{b*}	185.12± 0.48^{b*}
Group II	757.45 ± 3.10^{a*}	273.50 ± 0.52^{a*}	286.24± 0.84^{a*}
Group III	476.62 ± 0.78 ^{a**,b*}	178.23± 0.62 ^{a*,b*}	197.23± 0.20^{a*,b*}
Group IV	610.38±0.85^{a**,b**}	223.14±0.74^{a**,b**}	258.36±0.56^{a**,b**}
Group V	542.54±0.64^{a**,b**}	210.35±0.80^{a**,b*}	234.58±0.40^{a**,b*}
Group VI	561.75±0.88^{a*,b**}	206.68±0.84^{a**,b*}	221.12±0.62^{a**,b*}
Group VII	496.16±0.76^{a*,b*}	181.46±0.70^{a*,b*}	199.90±0.34^{a*,b*}

p values :*<0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 13: Tissue enzymatic antioxidant of superoxide dismutase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	SOD (unit min/mg protein)		
	Aorta	Heart	Liver
Group I	3.10 ± 0.22^{b*}	1.94 ± 0.07^{b*}	3.92 ± 0.20^{b*}
Group II	1.60 ± 0.14^{a*}	0.79± 0.05^{a*}	1.68 ± 0.18^{a*}
Group III	3.02 ± 0.10^{a*,b*}	1.82 ± 0.08^{a*,b*}	3.76± 0.24^{a*,b*}
Group IV	2.20± 0.21^{a**,b**}	0.95± 0.10^{a**,b**}	2.85± 0.22^{a**,b**}
Group V	2.34± 0.26^{a**,b*}	1.55± 0.18^{a*,b**}	2.52± 0.18^{a**,b*}
Group VI	2.56± 0.33 ^{a**,b*}	1.46± 0.14 ^{a**,b**}	2.98± 0.20 ^{a**,b**}
Group VII	2.88± 0.24^{a*,b*}	1.78± 0.20^{a*,b*}	2.60± 0.16^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 14: Tissue enzymatic antioxidant of catalase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	CAT (μ moles of H_2O_2, consumed min/mg protein)		
	Aorta	Heart	Liver
Group I	32.45 ± 2.33^{b*}	48.22 ± 3.74^{b*}	29.34 ± 1.24^{b*}
Group II	22.14 ± 2.45^{a*}	31.68 ± 1.82^{a*}	16.80 ±1.10^{a*}
Group III	31.78 ± 2.78^{a*,b*}	48.76 ± 3.16^{a*,b*}	29.44 ± 1.58^{a*,b*}
Group IV	24.23± 2.26^{a**,b**}	36.43± 3.50^{a**,b**}	21.52± 2.40^{a**,b**}
Group V	25.66± 2.52^{a**,b*}	39.85± 2.78^{a**,b*}	23.40± 2.52^{a**,b**}
Group VI	28.72± 2.81^{a**,b*}	40.91± 2.96^{a**,b*}	24.65±1.63^{a**,b**}
Group VII	30.84± 1.55^{a*,b*}	46.54± 2.34^{a*,b*}	28.78± 1.45^{a*,b*}

p values :*< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 15: Tissue enzymatic antioxidant of glutathione peroxidase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	GPx (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	13.74 ± 1.42^{b*}	15.88 ± 1.18^{b*}	8.78 ± 0.40^{b*}
Group II	6.86± 0.09^{a*}	7.26 ± 0.48^{a*}	5.34 ± 0.42^{a*}
Group III	16.35 ± 1.15^{a*,b*}	15.34 ± 0.44^{a*,b*}	8.742 ± 0.50^{a*,b*}
Group IV	10.48 ± 0.16^{a**,b**}	10.08 ± 0.28^{a**,b**}	6.60 ±0.34^{a**,b**}
Group V	12.02 ± 3.42^{a**,b*}	12.41 ± 0.32^{a**,b*}	7.33 ±0.46^{a**,b*}
Group VI	12.24 ± 0.10^{a*,b*}	12.52 ± 0.20^{a**,b*}	7.56 ±0.38^{a**,b*}
Group VII	15.60 ± 0.12^{a*,b*}	15.10 ± 0.24^{a*,b*}	8.21 ±0.26^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 16: Tissue enzymatic antioxidant of glutathione reductase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	GR (mg of GSH consumed/min/mg protein)		
	Aorta	Heart	Liver
Group I	1.84 ± 0.12^{b*}	2.90 ± 0.21^{b*}	1.58 ± 0.08^{b*}
Group II	0.78 ± 0.22^{a*}	1.29 ± 0.03^{a*}	0.66± 0.04^{a*}
Group III	1.74± 0.14^{a*,b*}	2.87± 0.11^{a*,b*}	1.56 ± 0.12^{a*,b*}

Group IV	1.15±0.10^{a**,b**}	1.96±0.07^{a**,b**}	0.84±0.07^{a**,b**}
Group V	1.46±0.26^{a**,b*}	2.35±0.10^{a**,b*}	1.12±0.10^{a**,b*}
Group VI	1.40±0.11^{a**,b*}	2.42±0.09^{a*,b**}	1.33±0.05^{a**,b*}
Group VII	1.72±0.18^{a*,b*}	2.84±0.05^{a*,b*}	1.51±0.08^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 17: Tissues enzymatic antioxidant of glutathione-S-transferase in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	**Glutathione-S-transferase(GST) (µ mole of CDNB-GSH-conjugate/min/mg protein)**		
	Aorta	**Heart**	**Liver**
Group I	15.76 ± 0.28^{b*}	18.12 ± 0.36^{b*}	22.48 ± 0.30^{b*}
Group II	7.34 ± 0.25^{a*}	9.32 ± 0.22^{a*}	10.56 ± 0.34^{a*}
Group III	13.10 ± 0.18^{a*,b*}	16.48 ± 0.36^{a*,b*}	20.33 ± 0.20^{a*,b*}
Group IV	9.25±0.12^{a*,b*}	11.81±0.24^{a*,b*}	14.36±0.18 ^{a*,b*}
Group V	10.43±0.30^{a*,b*}	13.34±0.30^{a*,b*}	17.18±0.24^{a*,b*}
Group VI	10.62±0.24^{a*,b*}	13.58±0.27^{a*,b*}	18.74±0.36^{a*,b*}
Group VII	12.56±0.10^{a*,b*}	16.64±0.18^{a*,b*}	20.05±0.12^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Table 18: Tissue non enzymatic antioxidant of glutathione in high fat diet rats

[Values are mean ± SEM of 6 rats]

Groups	**Glutathione (mg/g tissue)**		
	Aorta	**Heart**	**Liver**
Group I	5.89 ± 0.28^{b*}	7.72 ± 0.32^{b*}	4.35 ± 0.30^{b*}
Group II	3.08 ± 0.33^{a*}	5.10 ± 0.36^{a*}	2.14 ± 0.35^{a*}
Group III	5.76 ± 0.326^{a*,b*}	7.54 ± 0.28^{a*,b*}	4.28 ± 0.20^{a*,b*}
Group IV	4.29±0.14^{a**,b**}	6.32±0.40^{a**,b**}	3.10±0.18^{a**,b**}
Group V	4.78±0.16^{a**,b**}	6.86±0.33^{a**,b*}	3.55±0.26^{a**,b*}
Group VI	4.12±0.28^{a**,b*}	6.44±0.28^{a**,b*}	3.76±0.34^{a**,b**}
Group VII	5.58±0.10^{a*,b*}	7.46±0.12^{a*,b*}	4.16±0.22^{a*,b*}

p values : *< 0.001, **< 0.05

a →group I compared with groups II, III, IV, V,VI & VII.

b →group II compared with groups I, III, IV, V, VI & VII.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Study and Interpretation of the Physico Chemical Characteristics of Ground Water

T. S. R. Umamageswari*

Associate Professor, Department of Chemistry, PSNA College of Engineering and Technology, Dindigul, Tamil Nadu, India.

Received: 20 Nov 2020 Revised: 06 Dec 2020 Accepted: 07 Jan 2021

***Address for Correspondence**
T. S. R. Umamageswari
Associate Professor,
Department of Chemistry,
PSNA College of Engineering and Technology,
Dindigul, Tamil Nadu, India.
Email: umamageswaritsr1980@psnacet.edu.in

ABSTRACT

The hydro chemical study was carried out in Theni District, Tamilnadu, India, with an objective of understanding the suitability of local groundwater quality for domestic and drinking purposes. This paper deals with the study on the influence of environmental parameters on the water quality. Ground water samples have been collected from five taluks of Theni District. The samples have been analyzed for 10 physico chemical parameters as per standard procedures. The analyzed parameters are such as pH, total dissolved solids, total hardness, total alkalinity, calcium, magnesium, chloride, iron, sodium and potassium. Based on the parameters, water quality index calculated. Results indicate that study area is found to lie between bad and medium water quality. This analysis also reveals that the ground water of this area needs some degree of treatment before consumption and it also need to be protected from further contamination.

Keywords: Physico chemical parameters, WHO, Ground water, water quality index and Theni District

INTRODUCTION

Groundwater is the principal source of drinking water in our country and indispensible source of our life. "No life without water" is a common saying depending upon the fact that water is the one of the naturally occurring essential requirement of all life supporting activities [1]. Groundwater has unique features which rendered them suitable for public water supply. They have excellent natural quality usually free from pathogens; colour and turbidity can be consumed directly without treatment. The healthy aquatic ecosystem is depended on physical, chemical and biological characteristics [2]. The quality of water in any ecosystem provides significant information about the

available resources for supporting life in that ecosystem. Good quality of water resources depends on a large number of physical, chemical and biological characteristics. The water supply for human consumption is often directly sourced from groundwater without biochemical treatment and the level of pollution has become a cause for major concern. The water used for drinking purpose should be free from toxic elements, living and non living organisms and excessive amount of minerals that may be harmful to health. Keeping this in focus, the quality aspects of ground water in Theni district were analyzed for general water quality, pollution due to industrial discharges and municipal sewage. So the present study is analyzed the suitability of ground water quality for drinking and domestic purposes by calculating the water quality index

MATERIALS AND METHODS

Study area

Theni district lies between nine degree thirty minutes and ten degree thirty minutes on the North latitude and between seventy seven degree and seventy eight degree thirty minutes to the East longitude. The total area covered by this district is 3242.3 square kilometers. The maximum temperature experienced in this district is 40.5 degree Celsius and the minimum temperature over here is 16 degree Celsius. The economy of the study area is mostly dependent on agriculture. The total area under cultivation in the district of Theni is 126148 hectares. The district of Theni is having two revenues, five revenue taluks, seventeen revenue firkas and one hundred and thirteen revenue villages. The map of the study area given in Fig.1. In order to determine the water quality of study area, the ground water samples collected from five taluks namely Periyakulam (S_1), Bodinayakanur (S_2), Theni (S_3), Aundipatti (S_4) and Uthamapalayam (S_5) in triplicates. Samples were collected in polythene bottles and analyzed for various water quality parameters as per standard procedures APHA, AWWA, WPCF (1985) [3] given in Table 1.The experimental values were compared with standard values recommended by Indian standards [4] and World Health Organization [5] for drinking purposes.

Water Quality Index (WQI)

WQI may be defined as a 'rating that reveals the composite influence of a number of water quality parameters on the overall water quality'[6] .Water quality index provide information on a rating scale from 0 to 100.The water quality index has been considered to give a criteria for water classification based on the use of standard parameters for water characterization[7-10].This index is a mathematical instrument used to transform large quantities of water characterization data in to a single number, which represents the water quality level.WQI is calculated from the point of view of the suitability of ground water for human consumption.

RESULTS AND DISCUSSION

pH

pH of water is generally influenced by geology of catchments area and buffering capacity of water and also it is a measure of intensity of acidity or alkalinity .The pH of all sampling sites showed within the limit of 6.5-8.5 suggested by BIS and WHO. Dissolved gases such as carbon dioxide, hydrogen sulphide and ammonia also affect the pH of water. pH lower than 4 will produce sour taste and higher values above 8.5 bitter tastes. Higher value of pH has the scale formation in water heating apparatus and reduces the germicidal potential of chlorine. pH below 6.5 starts corrosion in pipes, there by releasing toxic metals such as Zn, Pb, Cd and Cu. The pH values of all sampling sites above 7 indicates slightly alkaline nature is due to the presence of fine aquifer sediments mixed with clay and mud which are unable to flush off the salts during monsoon rain and hence retained longer on other seasons. The value of pH greater than 11 causes eye irritation and other skin disorders. The water having pH range10 to 12.5 can cause hair to swell and gastrointestinal irritation may occur. No such problems were found in ground water samples collected from the study area. Since, their pH of the sampling sites within the range of 6.5-8.5.

Total Dissolved Solids

The fluctuations in electrical conductivity correlated positively with the total dissolved solids which are the common indicators of polluted waters. In natural waters, dissolved solids consists mainly of inorganic salts such as of calcium, magnesium, sodium, potassium, bicarbonate, chloride and sulphate ions and small amount of organic matter and dissolved gases. Water containing more than 500mg/l of TDS is not considered desirable for drinking water supplies, though more highly mineralized water is also user where better water is not available. For this reason, 500 mg/l as the desirable limit and 2000mg/l as the maximum permissible limit has been suggested for drinking water. All the samples were found above the desirable limit but well within the maximum permissible limit of 2000mg/l by Indian standards. High concentration of TDS was observed for all sampling sites due to long residence time factors of litho logy of water bodies. High TDS concentration cause gastrointestinal irritation in human and also laxative effect particularly upon transits.

Total hardness

Total hardness is due to the presence of divalent cations of Calcium and Magnesium in the ground water. Hardness is the property of water which prevents lather formation with soap and increases the boiling point of water. The low and high value of hardness has advantages and disadvantages. Absolutely soft water is tasteless. Moderately hard water is preferred to soft water for irrigation purposes. Absolutely soft water is corrosive and dissolves the metals. More cases of cardiovascular diseases are reported in soft water areas. The hardness of the sampling sites are S_1 (220) falls within the limit defined by BIS. Other sampling sites such as S_2 (343) S_3(540) S_4 (376)and S_5 (450) was found to exceed the limit The high level of hardness in above mentioned sampling sites indicates the discharging of sewage effluents in to the ground water. The hardness of water is not a pollution parameter but indicates water quality.

Total Alkalinity

The alkalinity of water is caused mainly due to hydroxides, carbonate and bicarbonate ions. The value of alkalinity in water provides an idea about the natural salts present in water. Alkalinity of water is its quantitative capacity to react with a strong acid to a designated pH. The desirable limit of alkalinity in drinking water is 200mg/l as per Indian standards. In present investigation, the alkalinity of all sampling sites is observed as high than the desirable limit prescribed by BIS. This can be explained as a result of increased effluent discharges and also due to the presence of country rocks. Excess alkalinity in water is harmful for irrigation which leads to soil damage and crop yields. The high alkalinity value at the sampling sites is also due to the action of carbonates upon the basic materials in the soil.

Calcium and Magnesium

The desirable limit of calcium is 75mg/l and magnesium is 30mg/l as per Indian standards. In ground water of the study area, the values of calcium at the sampling site S_2 (65) and the value of magnesium at S_1(21) were observed as below the desirable limit. This is due to the dilution effect on rain water. While other sampling sites, the concentration of calcium and magnesium was observed as high than the desirable limit. High concentration of calcium and magnesium in above mentioned sampling sites was also reflected the concentration of total hardness in the same sampling sites. In ground water, the calcium content generally exceeds the magnesium content in accordance with their relative abundance in rocks. No sample of the study area exceeds the maximum permissible limit prescribed for Ca and Mg.

Chloride

The most important source of chlorides in the water is due to the discharge of domestic sewage. Chloride is not utilized directly and indirectly for aquatic plant growth and hence its existence in the aquatic ecosystems is regarded as pollution. BIS and WHO have prescribed 250mg/l as the maximum desirable limit for chloride. In the study area, the concentration of chloride for all sampling sites is found to exceed the limit of 250mg/l. This can be attributed to the mixing of industrial effluents in sewage which build up high amounts of organic and inorganic ions like chloride.

High concentration of chloride imparts a salty taste to water and people who are not accustomed to high chlorides are subjected to laxative effects, gastrointestinal problems and dehydration. .

Iron

Iron occurs naturally in ground water. The Bureau of Indian standards and WHO has recommended is 0.3mg/l as the desirable limit in drinking water. It is evident from the collected data all sampling sites found to exceed the desirable limit. High concentration of iron generally cause inky flavor, bitter and astringent taste. It can also discolour clothes, plumbing fixtures and cause scaling which encrusts pipes. Excessive concentration may promote bacterial activities in pipe and service mains causing objectionable odours and 'red rot 'disease in water High concentration of iron in water is not suitable for processing of food, beverages, ice, dyeing and bleaching. Water with high concentration of iron when used in the preparation of tea, coffee, interacts with tannin giving a black inky appearance with a metallic state.. Iron is an essential trace element for the human body. The higher concentration of iron at all the sampling sites causes cell damage, mutation of genes and also increase the hazard of pathogenic organisms. The concentration of iron in natural water is controlled by both physic chemical and microbiological factors.

Sodium

Sodium in drinking water is not a health concern for most of the people, but may be an issue for someone with heart diseases, hyper tension and kidney disease. Studies have shown that reducing salt intake will lower blood pressure in people with hyper tension, but cannot be conclusively inferred that increased sodium intake will cause hyper tension. The concentration of sodium in drinking water is 200mg/l as per WHO standard. The lower concentration of sodium at the sampling sites S_2(110) and S_5 (189) can be explained on the basis of lower microbial activity. The high concentration at the remaining sampling sites is due to high rate of mineralization in the sediments.

Potassium

Potassium is an essential element for human beings, plants and animals. It is derived in food chain mainly from vegetation and soil. The main sources of potassium in ground water include rain water, weathering of potash silicate minerals, use of potash fertilizers and use of surface water for irrigation. It is more abundant in sedimentary rocks and commonly present in feldspar, mica and other clay minerals. WHO has prescribed the guideline level of potassium is 200mg/l for drinking water. The concentration of potassium at all the sampling sites showed within the desirable limit. Excessive quantity of potassium in drinking water may cause dehydration.

Calculation of Water quality index (WQI)

The WQI was calculated considering ten physico chemical parameters such as pH, total dissolved solids, total hardness, total alkalinity, magnesium, calcium, chloride, iron, sodium and potassium using WHO standards. In this method the quality rating scale has been assigned to the parameters, which is also weighted according to its relative importance in the overall water quality. The maximum weight of 4 has been assigned to the parameters like pH and TDS due to their major importance in water quality assessment. Other parameters like sodium, potassium and iron are assigned the minimum weight of 1 as they play minimum roles in the water quality assessment. The following formula is used for this calculation.

$$WQI = \sum q_i W_i \quad \text{as } W_i = 1 \text{----------(1)}$$

For calculating WQI the sub index (SI) is first calculated for each parameters, which is

$$(SI)_i = q_i W_i \text{----------(1a)}$$

and thus the formula is

$$WQI = \frac{(SI)_i}{(w_i)} \text{----------(1b)}$$

The unit weight of each parameter is calculated by the formula

$$W_i = \frac{w_i}{\sum_{i=1}^{n} w_i} \text{----------(1c)}$$

The quality rating scale (q_i) for the parameters have been divided into four stages such as permissible, slight, moderate and severe for which quality rating (q_i) ranges from 0 to 100. The computed WQI can be categorized into five types from excellent water to very bad water [11]. It is found that none of the sampling sites showed excellent water and very bad water category throughout the study period. The sampling site S_1(71.23) classified as 'Good', the sampling sites such as S_2 (50.12) and S_4 (54.34) classified as 'Medium' and rest of the sampling sites S_3 (45.67) and S_5 (41.43) classified as 'Bad' water category.

CONCLUSION

This study has thrown light on quality and suitability of ground water in Theni district. In respect of all evaluating criteria, the sampling sites in ground water not potable and need to be treated before consumption. For effective ground water management and sustainability, this assessment should be adopted on regular basis to monitor the ground water system of the area apart from health education hygiene related issues to reduce indiscriminate waste dump in the study area.

REFERENCES

1. APHA, AWWA and WPCF, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington D.C., 1995; 14 .
2. Ayibatatele NB, First Seosun Environmerita Baseline Survey, In Proc. of internal .conf. on water and environ 1992; 1: 4-26.
3. Venkatesharaju K, Ravikumar P, Somashekar RK, Prakash KL, Physico chemical and Bacteriological Investigation on the River Cauvery of Kollegal Stretch in Karnataka. Journal of Science Engineering and Technology 2010 ; 6(1) :50-59.
4. WHO (World Health Organization), (2004).Guideline for Drinking water quality recommendations. World Health Organization, Geneva, Switzerland.
5. BIS Indian Standards Specification for Drinking Water quality. IS 10500 New Delhi, Bureau of Indian Standards (1999).
6. Shankar SB, Sanjeev L Assessment of water quality index for the ground waters of an industrial area in Bangalore, India. Environmental Engineering Science 2008; 25(6):911-915
7. Pesce SF, Wunderlin DA, Use of water quality indices to verify the impact of Cordoba city (Argentina) on Suquiya River. Water Research, 2000; 34(11):2915-2926
8. Bordalo A.A, Nilsumranchit W, Chalrmwat K. Water quality uses of the Bangpakong River (Eastern Thailand).Water Research, 2001: 35(15) : 3635-3642.
9. Cude C. Oregon water quality index: a tool for evaluating water quality management effectiveness. American Water Resources Association, 2001; 37(1):125-137.
10. Hector Hernandez-Romero AH, Tovilla- Hernandez,C, Malo, EA ,Bello-Mendoza, R. Water quality and presence of pesticides in a tropical coastal wetland in southern Mexico. Marine Pollution Bulletin. 2004; 48: 1130-1141.
11. Tiwari TN, Mishra.MA, A preliminary assignment of water quality index of major Indian rivers. Indian J. Environ Proc. 1985; 5: 276-279

Table 1. Water quality parameters units and analytical methods used

S.No	Parameters	Abbreviation	Units	Analytical Methods	Instruments
1.	Total Dissolved Solids	TDS	mg/L	Filtration and Gravimetric method	Temperature controlled oven
2.	pH	pH	pH unit	Instrumental	pH meter
3.	Total Hardness	Hardness	mg/L	Digital Titrimetric	EDTA Titration
4.	Total Alkalinity	Alkalinity	mg/L	Digital Titrimetric-	Neutralising With standard HCl
5.	Calcium	Ca	mg/L	Digital Titrimetric	EDTA Titration
6.	Magnesium	Mg	mg/L	Digital Titrimetric	EDTA Titration
7.	Chloride	Cl	mg/L	Digital Titrimetric	Argentometric Titrimetric method
8.	Sodium	Na	mg/L	Flame photometric method	Flame Photometer
9.	Potassium	K	mg/L	Flame photometric method	Flame Photometer
10.	Iron	Fe	mg/L	Colorimetric method	UV – VIS Spectrophotometer

Fig.1 Map of the study area

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

All About Panchagavya for Human usage – A Review

S. Parkavi[1], P. Ganesh[2]* and M. Kokila[1]

[1]Ph.d., Research Scholar, Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.
[2]Assistant Professor, Department of Microbiology, Faculty of Science, Annamalai University, Annamalai Nagar, Chidambaram, Tamil Nadu, India.

Received: 27 Nov 2020 Revised: 28 Dec 2020 Accepted: 18 Jan 2021

***Address for Correspondence**
P. Ganesh
Assistant Professor,
Department of Microbiology,
Faculty of Science, Annamalai University,
Annamalai Nagar, Chidambaram, Tamil Nadu, India.
Email: drpg1974@gmail.com

ABSTRACT

Panchagavya is an ancient traditional medicine in India. In ancient India, people are most beloved in treating various diseases by natural and traditional manner. By using natural or traditional medicine they are living healthily. Panchagavya is the combined form of five cow products such as milk, urine, dung, curd and ghee (clarified butter). Many researchers and scientists worked on Panchagavya as a single or with multiple herbs or combined with herbal drugs. This review is about the human usage of Panchagavya. The treatment by using Panchagavya is treated as 'Panchagavya chikitsa' or 'Cowpathy' in India. Panchagavya therapy or Cowpathy utilizes five products as these possess medicinal properties and are used as single or in combination with some other drugs of herbs, animal or mineral origin for the treatment of several disorders and diseases. Panchagavya products are rich in nutrition, amino acids, proteins, vitamins, minerals, and hormones. These products are known to cure several human diseases and enhance immune power and also good for animals, which provides rich nutrition and helps to boost the immune system. Nowadays, humans are frustrated by an antibiotic that is resistant to numerous organisms. So, a natural product leads to no side effects and also cures diseases. However, scientific validation is much more important for world acceptance on the natural products for human uses. This review mainly based on the research findings of Panchagavya and the five products.

Keywords: Panchagavya, ghrita, antioxidants, antibacterial, anticancer.

INTRODUCTION

Panchagavya elements such as Cow Urine, Cow Milk, Cow Dung, Cow Curd and Cow Ghee are mentioned in traditional folk medicine in ancient India to treat different human diseases and are also used as good organic manure for soil and also for the treatment of various plant diseases. Panchagavya is a Sanskrit word, meaning the five key elements derived from the cow (milk, urine, dung, curd and ghee). These five items possess medicinal properties that are used alone or combined with other herbs against many human and animal diseases. In India, the treatment using Panchagavya is called "Panchagavya therapy" or "cowpathy." The formulation of Panchagavya is the ghrita form of Ayurveda. In India, Cow is celebrating as a divine mother and also known as Kamadhenu and Go-matha or Gaumata. The ancient times of India, traditional therapies based on Panchabhootas (Five elements of nature – Earth, Water, Fire, Air and Space) and the health is affected by Tridoshas *viz.,* Vadha (air), Pitha (fire) and Kapha (Phlegm). Any disturbance in the harmony of the natural ratio of the five elements may cause the disease. Based on these fundamental principles of life, different remedial systems were developed *i.e.,* Vrikshayurveda for plants, Mrigayurveda for animals and Ayurveda for human beings (Charaka - Samhita, 1981). In Vedas, uses of cow derived products have been mentioned for treating tridoshas. Various Scientists and researchers have found them to be a rich source of essential elements as well as minerals and hormones in the cow products.

Panchagavya therapy or Cowpathy utilizes five products as these possess medicinal properties and are used as single or in combination with some other drugs of herbs, animal or mineral origin for treatment of several disorders and diseases like flu, allergies, colds, cough, arthritis, rheumatoid arthritis, leucoderma, leucorrhoea, alopecia, asthma, hyperlipidemia, renal disorders, dietary and gastrointestinal tract disorders, acidity, ulcer, wound healing, heart disease, skin infections/diseases, tuberculosis, chickenpox, hepatitis, leprosy and other bacterial/viral infections, aging, chemical intoxication, worm infestations, obesity, etc. Panchagavya has disinfectant and antiseptic properties. Panchagavya is used as fermented product in agriculture along with vermicompost, biopesticides and biofertilizers. Each Panchagavya element has distinct qualities and uses in health, agriculture and other fields. Scientists and Physicians are facing problems in modern allopathic treatment due to the multiple drug resistance in microorganisms, the presence of antibiotic residues in the food chain and/or associated allergies and autoimmune disorders in humans and animals. Immunity is reducing drastically as a result of the environmental pollution, use of agrochemicals in agriculture and the presence of pesticides, heavy metals, fungal toxins, etc., in the food chain. Deficient functioning of macrophages leads to the inefficacy of antibiotic drugs, the development of resistance in bacteria, recurrent infections, and or decreased immune status of an individual.

Panchagavya is an organic product derived from five products evolving from cow and it has been used in Indian traditional medicine since time immemorial. But the Panchagavya process is fermented. “I have modified this Panchagavya by adding a few more ingredients and the modified version has a lot of beneficial effects on a variety of crops and livestock", said Dr. K. Natarajan, President of the Rural Community Action Centre (RCAC), a non-governmental organization, actively engaged in promoting the concepts of organic farming and biodiesel in the rural areas of Tamil Nadu."The present form of Panchagavya is a single organic input, which can act as a growth promoter and immunity booster. I am essentially preparing a product containing 4 kg gobar gas slurry, 1 kg fresh cow dung, 3 liters of cow urine, 2 liters of cow's milk, 2 liters of cow's curd, 1 kg cow's ghee, 3 liters of sugarcane juice, 12 ripe bananas, 3 liters of tender coconut water, and 2 liters of toddy (if available). This will make about 20 liters of Panchagavya. The concoction is stored in a wide-mouthed earthen pot or concrete tank in open. Sufficient shade should be provided, and the contents should be stirred twice a day, both in the morning and in the evening. In seven days, the modified Panchagavya will be ready, and it can be diluted before use on plants and animals," says Dr. K. Natarajan. As per WHO (world health organization), the 20th-century wonder drugs ‘antibiotics’ will not remain useful and become almost ineffective by the year 2020. In these circumstances, one has to think over the alternative therapeutic approaches to control the infections (Dhama *et al*., 2012; Chauhan, 2005; Garg and Chauhan, 2003a). Now-a-days peoples are frustrated by the heavy medicines of allopathy and are using Cowpathy and being

benefited by the Panchagavya products. However, scientific validation of Panchagavya products is required for its worldwide acceptance and popularity in the terms of agricultural, energy resources, nutrition and medicinal properties to exploit the optimal Power of Panchagavya for the service of mankind, regardless of scientific validation, people are using and getting benefits of it (Dhama *et al*., 2012). This review elaborates on the versatility of the Panchagavya therapy/chikitsa (Cowpathy) for human's health.

Panchagavya Properties

In ancient literature of Ayurveda for preparing Panchagavya by using various formulae, used as single or combined with multiple herbal drugs. According to the ancient literature such as Chakara Samhita, Chaukambha Sanskrit Pratistana, *etc*., revealed the various formulations of Panchagavya which is used alone or combined with multiple herbal drugs. The different formulations of Panchagavya are as Swapla – Panchagavya ghrita, Panchagavya ghrita (means ghee formulations) and Mahapanchagavya gritha (means the addition of 18 or 24 herbs with Panchagavya to prepare mahapanchagavya ghrita). These different formulations are used to treat various human diseases such as skin diseases, vitiligo, cough, cold, chronic illness, *etc*. The treatment for human diseases by using Panchagavya is popularly known as Cowpathy or Panchagavya chikitsa or therapy. Panchagavya therapy has been proposed as an alternative and useful prophylactic and therapeutic approach for livestock, poultry and human health (Dhama *et al*., 2012; Mathivanan *et al*., 2008; Dhama *et al*., 2005a). Panchagavya constitutes five substances obtained from cow *viz*. urine, milk, ghee, curd and dung. All these products possess medicinal properties and are used singly or combined with other herbs for therapeutic purposes. Panchagavya elements possess high nutritional value such as cow milk, curd and ghee, cow urine and dung can act as an alternate and cheaper source of energy, biogas, fuel and electricity (Chauhan, 2004; Dhama *et al*., 2005; Alves, 2008; Dhama *et al*., 2013). Panchagavya formulated from native breeds of cows which is beneficial for human uses. The five products in Panchagavya are cow urine, cow dung, cow milk, cow curd and cow ghee. In the literature of Ayurveda, formulation of Panchagavya in the ratio of 2:1:6:12:2 (urine, dung, curd, milk and ghee). Panchagavya products are rich in nitrogen, sulfur, phosphate, sodium, manganese, chloride, magnesium and calcium salts, acids like carbolic, succinic and citric, vitamins such as A, B, C, D & E, minerals and hormones. These products are known to cure several human ailments and enhance immunity by inducing immune modulation through enhancement of both cellular and humoral immune responses, up - regulating the lymphocyte proliferation activity, reducing apoptosis in lymphocytes. They act as anti-aging agents by preventing the free radicals formation and efficiently repairing the damaged DNA (Dhama *et al*., 2012 & 2005a). The individual properties of five elements of Panchagavya and combined Panchagavya with the herbal drug studies such as antibacterial, antifungal, anticancer properties, immunological aspects, pharmacological aspects, *etc*. by various researchers.

Immunological aspects

Gajbhiye *et al*., (2014) studied the Immunostimulant activity of a medical preparation of Panchagavya and studied by using combined herbs such as pomegranate (*Pumica granata*), and fed to mice orally. The oral uptake of Panchagavya with the pomegranate extract stimulating the immunity of mice was recorded in their study.

Antimicrobial and Antioxidant activity

Edwin *et al*., (2008) studied the antimicrobial and antioxidant activities of cow urine (CU) and cow urine distillate (CUD). In this comparative study freshly collected cow urine showed better results than distillated cow urine. Sathasivam *et al*., (2010) evaluated the activity of cow urine against *Aspergillus flavus*. *In–vitro* analyses of antifungal activity of cow urine compared with two fungal species of *Aspergillus*. In their comparative analysis, maximum growth suppression was observed in *Aspergillus niger* than *Aspergillus flavus*. Athavale *et al*., (2012) evaluated the antioxidant activity of traditional ayurvedic preparation of Panchagavya and concluded that Panchagavya has high antioxidant potential and indicated that, this forms the basis of treating cancer by using Panchagavya. Raad *et al*., (2013) studied the antibacterial activity of cow urine against some pathogenic and non – pathogenic bacteria.

They tested different cow urine samples against 14 pathogenic and non – pathogenic bacteria cultures. They concluded that all the different samples of cow urine showed clear evidence of bioactive compounds and also the presence of antibacterial activity. Waziri and Suleiman (2013) analyzed the antimicrobial activities of evaporated cow dung extracts against some pathogens and also analysed some elements in evaporated cow dung extracts.

Kumar *et al*., (2013) studied the antioxidant activities of cow milk caseinates hydrolyzed with different proteases. The analytical study revealed the highest antioxidant activities of a peptic hydrolysate of cow milk casein. Jirankalgikar Nikhil *et al*., (2014) studied about *In – Vitro* antioxidant activity and High – Performance Thin Layer Chromatography (HPTLC) profile of cow dung, and revealed the cow dung natural antioxidants by their studies. Deepika *et al*., (2016) studied about the determination of the antimicrobial activity of Panchagavya against urinary tract infections. They studied natural products such as Panchagavya (cow dung, milk, curd, ghee, and urine), buttermilk and honey. Hoh and Dhanashree (2017) studied the effect of cow urine's antifungal activity on Candida species. They described that the distillated cow's urine acted as alternative drug for antifungal diseases.

Karami *et al*., (2017) evaluated the antagonistic activities of cow milk against some pathogens. They isolated and identified probiotic *Lactobacillus* bacteria from the dairy product. And found the isolated *Lactobacillus* species are more potential against some pathogens. Dave *et al*., (2018) studied an *In – Vitro* comparative analysis of the antimicrobial effect of cow urine and goat urine against certain dental caries causing pathogens. Both the urine samples showed more effective medicinal potent against dental caries pathogens. Based on this comparative study, goat urine was found more potent than cow urine. Rachana and Sreepada (2019) evaluated the cow urine antioxidant activity and also studied anti-inflammatory activities by using raw and distilled cow urine. They concluded that raw cow urine has more antioxidant and anti-inflammatory activities, which are compared to distillated cow urine. Joshi *et al*., (2019) studied *in-vitro* activities of antibacterial and antioxidant potential of cow urine obtained from various altitudinal and climate region of Nepal. They concluded that a cow urine sample from Nepal shows active antibacterial effects and natural antioxidants.

Anticancer activity

Research works carried out by Go - Vigyan Anusandhan Kendra (Cow Science Research Center) at Nagpur revealed the beneficial properties of cow urine in the treatment of cancers. Further extensive research on cow urine therapy against fighting cancer carried out by Scientists of Central Institute of Medicinal and Aromatic Plants (CIMAP), CSIR Center at Lucknow, along with collaboration with Go - Vigyan Anusandhan Kendra, Nagpur and confirmed this milestone achievement. Studies highlight the role of cow urine in curing cancers and that cow urine enhances the efficacy and potency of anti-cancer drugs. They patented this work and granted U.S. Patent (No. 6896907) in the field of treatment of cancers (Amar Ujala, July 19, 2005). Jain *et al*., (2010) discussed anticancer activities of cow urine therapy on different cancer patients in Madsaur district. They concluded that cow urine therapy controls the cells in cancer patients. Praveesh *et al*., (2011) analyzed the anti-cancer and also studied the anti-hypertensive effect of cow milk. They fermented cow milk by two lactic acid bacteria *Lactobacillus plantarum* and *Lactobacillus casei.* They concluded that the peptides derived and isolated from cow milk are used as medicines. Rita and Kansal (2011), studied comparative analyses of cow ghee *verses* soyabean oil against mammary carcinogenesis. Experimental study conducted on 7, 12 – dimethylbenz (a) – anthracene induced mammary carcinogenesis and expression of cyclooxygenase – 2 and peroxisome proliferators activated receptor in animal rats. They concluded that cow ghee can protect us against mammary carcinogenesis.

Other studies

Achalia *et al*., (2003) studied hepatoprotective activities of panchagavya ghrita (PGG) against Carbontetrachloride (CCl_4) induced hepatoxicity in rats, PGG showed significant reduction in CCl_4 induced hepatoxicity in rats by given oral route. Charde *et al*., (2006) formulated cow ghee with honey equally as Madhu ghrita and studied anti-inflammatory activity by screening carrageenan – induced rat paw odema method and also studied wound healing potential of madhu ghrita and concluded that ghrita is effective in wound healing.

Dhurvey *et al*., (2012) evaluated the cow ghee physico – chemical properties, which are before and after hydrogenation. They analysed cow ghee and hydrogenated cow ghee and used for formulating medicine as well as nutritional purpose and concluded ghee could be safe and can be routinely up taken in regular diet.

Nariya *et al*., (2012) analyzed the traditional ayurvedic preparation of Panchagavya using various physico – chemical properties and High Performance Thin Layer Chromatography (HPTLC) methods. Establishment of data and profile of Panchagavya is very useful for quality control and controlling batch to batch variations. Amit Kumar *et al*., (2013 & 2014) studied Novel and synergistic antinociceptive activity of different compositions of Panchagavya, which is combined with *Aloe barbedansis* Mill by using tail immersion model method of mice. And also studied about synergistic anthelmintic activity of Panchagavya and *Aloe barbedansis* Mill. Sachdev *et al.,* (2012) studied the effect of Gomutra ark on alloxan-induced diabetes rats and found that cow urine have high therapeutic index and significant anti-diabetic effect due to its antioxidant activity. Patel *et al*., (2018) reported the first *in vivo* prophylactic (anti-infective) potential of a Panchagavya mixture against certain pathogenic bacteria and concluded that Panchagavya has immunomodulatory potential leading to protection against bacterial infections.

COVID – 19 treatments

COVID – 19 (Corona virus) is one of the recent pandemic diseases in world wide. In India, various treatment using natural methods and ayurveda are ongoing to defense and protect the people from corona viruses. A recent research based on Panchagavya to treat against corona virus is under progress. Tijare *et al*., (2020) evaluated and proved the preliminary findings of Panchagavya medicine in COVID – 19 patients. The patients affected by corona virus are treated with Panchagavya and Gomutra arka and concluded patients suffered from corona virus get recovery from corona virus. They concluded that Panchagavya and Gomutra arka treatment is safe and effective therapy.

CONCLUSION

This review mainly focused on the various research findings on five products of cow and Panchagavya for human use. Panchagavya is a safe for human use, as it is being used for ten decades in India. But the formulations of Panchagavya with cow dung and cow urine is not satisfied by many peoples. Panchagavya or Cowpathy is a new approach from ancient literature medicine, and surely a promising drug formulation in further years. Many researchers studied about anti-epileptic, nootropic, not only for humans, studies on animal's diseases and plant diseases too on using Panchagavya. A research reveals the many potential properties of five products of cow, formulated Panchagavya and combined form with various herbs. Further findings should be done in large population studies, new drug development approach and the various possibilities to eradicate diseases.

REFERENCES

1. Achalia G.S., Kotagle N.R., Wadodkar S.G., Dorle A.K. Hepatoprotective activity of Panchagavya Ghrita against Carbontetrachloride induced Hepatotoxicity in rats, Indian Journal of Pharmacology, 2003, 35: p. 308- 311.
2. Achliya, Girish S., Sudhir G, Wadodkar and Avinash K Dorle (2004). Neuropharmacological actions of Panchagavya formulation containing *Emblica officinalis Gaerth* and *Glycyrrhiza glabra Linn* in mice. Indian Journal of Experimental Biology Vol. 42, pp. 499-503.
3. Alves RRN (2008). Animal-based remedies as complementary medicine in Brazil. Forschende Komplementarmedizin., 15(4): 226-227.
4. Athavale, A., Jirankalgikar, N., Nariya, P., & Des, S. (2012). Evaluation of in-vitro antioxidant activity of panchagavya: a traditional ayurvedic preparation. *Int J Pharm Sci Res*, *3*, 2543-9.
5. Caraka-Samhita, Editor-Translator P. Sharma (1981), Chaukhambha Orientalia, Varanasi, India, Volume 1, p. 213

6. Charde MS, Fulzele SV, Satturwar PM et al (2006). Wound healing and anti-inflammatory potential of madhu-ghrita. Indian J Pharm Sci 68:26–31.
7. Chauhan R.S. (2000). Panchgavya dwara prakritik chikitsa. Kheti sansar, **3:** 21-23.
8. Chauhan R.S. (2002a). Panchgavya: desi gay se prapta ek adbhut aushadhi pashudhan, **17 (3):** 5-7.
9. Chauhan R.S. and Sharma, R. (2002). Desi gayo ki upyogita thatha unki sankar evam videshi nasl ki gayon se tulna. Pashupalan, May 2002, 31-33.
10. Chauhan R.S. and Singh, B.P. (2001). Panchgavya dwara prakritik chikitsa. Asian kisan sansar, **2 (3)**: 29-31.
11. Chauhan, R.S. (2002b). Medical importance of Panchagavya (Cow therapy). In: National Symposium on Historical Overview on Veterinary Sciences and Animal Husbandry in Ancient India, Vedic and Ashokan Period). IVRI, Izatnagar, April 16-17, 2002.
12. Chauhan, R.S. (2003a). Gomutra se sarir ki rog pratirodhi chamta me vridhi. National Seminar on Veterinary Sciences–Research Directions in the Next Decade by Commission for Scientific & Technical Terminology, Ministry of HRD, Delhi; IVRI, Izatnagar & Dr. C.M. Singh Endowment Trust, Bareilly, Feb. 27-28, 2003, at IVRI, Izatnagar (U.P.).
13. Chauhan, R.S. (2003b). Panchgavya se rogpratirodhi chamta me vridhi. Prakriti smarika **pp** 9.
14. Chauhan, R.S. (2003c). Neoplasms, in Illustrated Special Veterinary Pathology, RS Chauhan (Editor), IBDCO Publishing Division, Lucknow, India. Chapter 1, **Pp:** 1-67.
15. Chauhan, R.S. (2004a). Govansh Evam Manav Shwashthya. Cowtherapy Society, Pantnagar.
16. Chauhan, R.S. (2004b). Panchgavya therapy (Cowpathy): Current status and future directions. *The Indian Cow. July-September Issue* **1:** 3-7
17. Chauhan, R.S. (2005). Cowpathy: A new version of Ancient Science. Employment News, Vol. xxx, No.15, 9-15 July, 2005, pp 1-2.
18. Chauhan, R.S., Singh D.D., Singhal, L.K. and Kumar, R. (2004): Effect of cow urine on the interlukin-1 and 2. *Journal of Immunology and Immunopathology*, **6** (1):38-39.
19. Chauhan, R.S., Singh, B.P. and Singhal, L.K. (2001) Immunomodulation with Kamdhenu Ark in mice. *Journal of Immunology and Immunopathology*, **3:** 74-77.
20. Chauhan, R.S., Singh, B.P., Singhal, L.K., Agrawal, D.K. and Singh, A.K. (2001). Enhancement of phagocytic activity of leucocytes in mice with Kamdhenu ark. In: XVI Annual Convention of IAVA and National Symposium on Animal Structural Dynamics to Improve Health and Production, Pantnagar. November 8-10, 2001.
21. Chauhan,. R. S. (2003). Immunomodulation: pesticides and panchgavya. National conference on panchgavya, ayurveda and agricultural system national environmental institute, Nagpur April, 28-29, 2003.
22. Dave, P., Gill, V., Ajitkumar, P. A., Patel, P., Raghu, A. A., & Tulluri, S. L. S. T. (2018). Antimicrobial Effect of Cow Urine and Goat Urine against common Dental Caries Pathogens: An in vitro Comparative Study. *Journal of Advanced Medical and Dental Sciences Research*, *6*(8), 85-89.
23. Deepika M, Nashima K, Rajeswari S. (2016). Antimicrobial activity of panchagavya against urinary tract infection. Internationat Journal of Current Pharmacological Research. 8(3):68-70.
24. Dhama, K., R.S.Chauhan and L.Singhal, (2005). *Int. J. Cow Sci*., 1: 1-25.
25. Dhurvey YR, Kawtikwar PS, and Sakarkar DM.. (2012): Evaluation of physicochemical properties of cow ghee before and after hydrogenation. *Evaluation* 4, no. 1 185-189.
26. Edwin J., Sheej E., Vaibhav T., Rajesh G., Emmanuel T. (2008). Antioxidant and antimicrobial activities of cow urine. Global J. Pharmacol. 2: 20–22.
27. Gajbhiye SP, Padmanabhan U, Kothari S, et al. (2015). Immunostimulant activity of a medical preparation panchagavya. Int J Res Pharm Sci.; 5(3): 1–5.
28. Hoh, J. M., & Dhanashree, B. (2017). Antifungal effect of cow's urine distillate on Candida species. Journal of Ayurveda and integrative medicine, *8*(4), 233-237.
29. Jain NK, Gupta VB, Garg R, Silawat N. (2010). Efficacy of cow urine therapy on various cancer patients in Mandsaur District, India - A survey. Int J Green Pharm; 4: 29-35. Jain NK, Gupta VB.

30. Jirankalgikar, N., Nariya, P., & De, S. (2014). In vitro antioxidant activity evaluation and HPTLC profile of Cow dung. *International Journal of Green Pharmacy (IJGP)*, *8*(3).
31. Joshi, D. R., Aryal, P., Chaudhary, M. K., BK, P. K., Yadav, S. P., Rawal, P.,& Bhandari, R. (2019). Study of in-vitro Antioxidant and Antibacterial Activity of Cow Urine from Different Altitudinal and Climatic Region of Nepal. *Microbiology Research Journal International*, 1-8.
32. Kansal, V. K., Rani, R., & Bhatia, E. (2011). Cow Ghee Protects from Mammary Carcinogenesis: Mechanism. *Chemical Analysis of Value Added Dairy Products and Their Quality Assurance*, 68.
33. Karami, S., Roayaei, M., Hamzavi, H., Bahmani, M., Hassanzad-Azar, H., Leila, M., & Rafieian-Kopaei, M. (2017). Isolation and identification of probiotic Lactobacillus from local dairy and evaluating their antagonistic effect on pathogens. *International Journal of Pharmaceutical Investigation*, *7*(3), 137.
34. Kumar S.,(2013) Antioxidative property of cow milk caseinates hydrolyzed with different proteases, International Journal of Pharmacy and Pharmaceutical Sciences, Vol 5 (2)
35. Kumar, R., Kumar, A., Kumar, K., Gupta, V., Shrivas, T., & Tripathi, K. (2014). Synergistic anthelmintic activity of different compositions of panchagavya and Bauhinia variegate Linn. *Int J Phytopharmacol*, *5*, 120-2.
36. Nariya P., (2012). Analytical study and HPTLC profile of Panchagavya- a traditional ayurvedic preparation, Asian journal of biochemical and pharmaceutical research, 2 (2).
37. Patel P, Joshi C, Funde S et al. Prophylactic potential of a Panchagavya formulation against certain pathogenic bacteria [version1: referees: 3approved] F1000 Research 2018, 7:1612 (https://doi.org/10.12688/f1000research.16485.1)
38. Praveesh, B. V., Angayarkanni, J., & Palaniswamy, M. (2013). Therapeutical properties of cow milk fermented with Lactobacillus plantarum and Lactococcus casei. *African Journal of Biotechnology*, *12*(21), 3296-3301.
39. Raad, S., Deshmukh, D. V., Harke, S. N., & Kachole, M. S. (2013). Antibacterial activity of cow urine against some pathogenic and non-pathogenic bacteria. *International Journal of Pharmaceutical Sciences and Research*, *4*(4), 1534.
40. Rachana B and Sreepada KS (2019): Antioxidant and anti-inflammatory activities of cow urine from Malnad gidda - an indigenous breed. Int J Pharm Sci & Res; 10(2): 612-18. doi: 10.13040/IJPSR.0975-8232.10(2).612-18.
41. Sachdev, D. O., Gosavi, D. D., and Salwe, K. J. (2012). Evaluation of antidiabetic, antioxidant effect and safety profile of gomutra ark in Wistar albino rats. Ancient Science of Life. 31(3): 84–89. http://doi.org/10.4103/0257-7941.103180.
42. Sathasivam A., Muthuselvam M., Rajasekran R. (2010). Antimicrobial activities of cow urine distillate against some clinical pathogens. Global J. Pharmacol. 4: 41–44.
43. Tijare P, Ambatkar N, Tiwari V. Clinical Improvement In COVID19 Patients With Timely Intervention Of Panchagavya Medicine: A Preliminary Finding. Int J of Pharmc Res [Internet]. 2020 Jun. 28 [cited 2020 Sep. 20]; 10 (6): e5449. Available from: https://www.ssjournals.com/index.php/ijpr/article/view/5449
44. Waziri, M., & Suleiman, J. S. (2013). Physicochemical properties and antimicrobial activity of evaporated extract of cow dung against some pathogens. *Journal of Scientific Research*, *5*(1), 135-141.

Table 1. The properties of Panchagavya for human health are

Five elements of Panchagavya	Other names	Quantity of Panchagavya preparation for human use (app.)	General Uses and Therapeutic uses
Cow urine	Gomutra/Gomootra	60 ml	➢ Used as Cow Urine therapy – Gomutra ark (fresh cow urine or steam distillated cow urine). ➢ Cow urine tablets (Gomutra pills).

			➢ It is effective medicine and good antiseptic. ➢ Research reveals that cow urine contains urea, nitrogen, sulphur, phosphate, sodium, vitamins A, B, C, D, and E, minerals, calcium salts, irons, gold, hormones, etc. ➢ Cow urine therapy cures various disease ailments as chronic like cancer, AIDS, psoriasis and other skin disorders. ➢ It helps in face wrinkles and anti-aging.
Cow dung	Gomaya/Gos'akr't/Gomaeya	5 g (very little amount – half thumb size app.)	➢ Used as small amount or cow dung juice by filtered seven times with muslin cloth. ➢ Effective for killing microorganisms, antiseptic and good disinfectant. ➢ Fresh cow dung contains highest amount of vitamin B_{12}. ➢ Cow dung in cosmetics used to treat various skin diseases like ringworm, itching, psoriasis. In shampoo it helps to rid dandruff and dryness of hair. ➢ By the combination with dung and urine and also with other herbs to treat various diseases. ➢ Using as cow dung tooth powder removes cavity problems, bad breath, pyorrhea, gum bleed, etc.
Cow milk	Godugdha/Goksheera	400 ml	➢ Cow milk is important, easily digestible and healthy food for infants (next to breast milk) and all ages of peoples. ➢ It contains low calories, cholesterol, and numerous micro nutrients, protein, vitamins A, B complex groups and C, and calcium. ➢ Treating numerous properties as peptic ulcer, skin cancer, skin diseases and other diseases. ➢ Cow milk contains numerous good lactic acid bacteria.
Cow curd	Godadhi	180 ml	➢ Cow curd used as probiotic it produces lot of lactic acid producing bacteria. ➢ It produces antifungal metabolites such as phenyl lactic acid,

			proteinaceous compounds, etc. ➢ Good for antifungal effects on hair as dandruff, dry scalp, prevents hair damaging. ➢ Used to treat various diseases as gastrointestinal tract problems – ulcers, irritable bowel syndrome, colon cancer. ➢ Myth - it increases concentration of brain.
Cow ghee	Goghrit/Goghr'ta (Goghrita)	60 ml	➢ Ghrita means ghee, formulating medicines as ghee. Used as various Ghrita in Ayurveda. ➢ Traditionally believed to improve memory, intelligence and resistance to various infections. ➢ Researches reveals cow ghee has anti – aging, immunostimulant, antifungal activity, purifies blood and promotes wound healing properties. ➢ It is used with various herbs to cures certain skin diseases. ➢ It exhibits anti chollestric and it safe for use.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Estimation of Cell Suspension Culture with Enhanced Aporphine of *Annona squamosa* L.

Mamta Choudhary and Khushbu Verma*

Faculty of Agriculture and Veterinary Science, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.

Received: 01 Nov 2020 Revised: 22 Nov 2020 Accepted: 24 Dec 2020

***Address for Correspondence**
Khushbu Verma
Faculty of Agriculture and Veterinary Science,
Jayoti Vidyapeeth Women's University,
Jaipur, Rajasthan, India.
Email: vermakhushbu25@gmail.com/fbtdepartment@jvwu.ac.in

ABSTRACT

Annona squamosa L. is an economically important species. *Annona squamosa* L. leafs are significant as herbal substitute for diverse diseases. Present study's main objective to evaluate the enhancement of Aprophine- Alkaloid in Cell suspension culture of *Annona squamosa* L. Suspension culture with treatment of Salicylic Acid (SA) was experienced on modified Murashige and Skoog (MS) strains with specific combination of Growth Regulators (3.0 mg L-1 BAP and 4.0 mg L-1 NAA). Cell cultures were treated with different concentration of Salicylic acid (SA) (25, 50, 75 and 100 mg/L) separately. Thin Layer Chromatography (TLC) and High pressure liquid chromatography (HPLC) was performed for the quantitative analysis of isolated secondary metabolites-Aprophine in isolated control and Salicylic acid treated Cells. Retention time for the recognition of Aprophine, was reported at 5.75 min and 6.5 min respectively at 254 nm. Highest accumulation was observed in 100 µM Salicylic acid treated cell culture in comparison to control. The results of this study revealed enhanced accumulation in Cell suspension culture of *Annona squamosa* L. by using Salicylic acid.

Keywords: Aprophine, *Annona squamosa* L., Growth Regulators, Suspension culture, Salicylic acid.

INTRODUCTION

The relation of herbal medicine inaugurates with human progression. The term medicinal plants cover different kinds of plants and a number of these plants have medicinal accomplishment. The name of the genus, "Annona", came from the Latin word "anon", which means "annual product", that submits to the yielding of fruits of the varied species of this genus of *Annona squamosa* L. (Morton J. 1987) (Sandeep and Abhilasha, 2017). *Annona squamosa* L. plant is accredited through the medicinal potentials which comprise anti-fertility and antitumour potential. Leaves of

Annona squamosa has been reported that its is having an anti-diabetic activity, anti-lipidimic, insecticidal, anti-tumor, antioxidant and anti-inflammatory activity (Gajalakshmi *et al*., 2011). This is a very noticeable plant in the Ayurveda medicine for curing diverse diseases. This plant is predictably used to treat dysentery, heart problems, worm infestation, constipation, antibacterial infections, dysuria, fever and ulcers. It is originate rising extrovertly and extensively in the hilly territory, waste domain and has turned into entirely naturalized in numerous districts of Rajasthan, Punjab, and Andhra Pradesh (Pathak and Zaman 2013). Aprophine is an alkaloid which is prominently present in *Annona squamosa* L. it is highly present in leaves and tender stem (Morita et al. 2000). Aprophine has various medical applications which can apply in disease curing such from Antineoplastic Agents to Motor Dysfunction Diseases (Nabavi et al. 2017). The aim of the present study to elevate the concentration of aprophine in Cell suspension culture of *Annona squamosa* L. by using chemical elicitors to popularize its commercial use for medical purpose.

MATERIAL AND METHODS

Preparation of Explant and Callus Induction of *Annona squamosa* L.

Young fresh leaves of *Annona squamosa* L. were collected from the Herbal Garden of Jayoti Vidyapeeth Women's Uniersity, Jaipur, Rajasthan. Explants were prepared according to the method described. Young leaves were incubated in the dark at 25 ± 2ºC for three weeks for sprouting buds. Then leaf tips (about 0.5 cm) from the used as an explant for callus induction. *Annona squamosa* L. callus was induced and proliferated according to the method described by (Ali et al. 2016). Sterilized leaf explants were planted in MS (Murashige and Skoog) medium supplemented with auxin and cytokinen hormones combinations 3.0mg/l BAP (6-benzylaminopurine)+4.0 mg/l NAA (Naphthalene acetic acid) as plant growth regulator and were incubated at 25 ± 2º C and photo-period of 16/8h light/dark. Fresh Callus samples were further taken out for the preparation of Cell suspension culture.

Cell suspension culture maintenance

Green color Callus culture with 15 days old age, were chosen to create cell suspension culture. The preliminary inoculums were prepared in an Erlenmeyer flask (150 ml) with 50 mL of modified liquid MS medium having plant growth hormones (3.0mg/l BAP+4.0 mg/l NAA). Two successive subcultures had completed by changing 40 ml of used media with the similar quantity of fresh media with 7 days intervals. Bulky clumps of callus were removed after each subculture by using a 500 micron pore nylon sieve. Following two subcultures, 10 ml of thin cell media (0.1 mL / 5 mL PCV culture) was shifted to new 40 ml media.

Salicylic acid treatment

Well developed cell cultures were transferred to the Media treated with different concentration of Salicylic acid (SA) (25, 50, 75 and 100 mg/L) separately. Elicitor treatment was maintained at 25° ± 2°C, photoperiod of 16/8h light/dark for three weeks in Incubator – Shaker. Triplicates were considered for every treatment to get accuracy of the experimentation.

Preparation of plant extracts

For TLC analysis, extraction of 1 g was done to each sample (a powdered form of cells) with 50 ml of solvent (ethanol) in a beaker during 24 hours at room temperature (Zarzycki 2014). For HPLC analysis, 5 gram powder of cells (for control and each treated sample separately) was added in 100 ml distilled water, heat it at 50 ºC for 30 minutes. After cooling process, it was mixed with 12.5 gram Magnesium oxide (Mgo) and heated again at 50 ºC for 25 minutes. Then it was cooled and 25 ml concentrated ethanol was mixed. This solution was filtered through normal filter paper followed by Whatman filter paper. Solutions were dried in petriplates. For the final step, 5 ml methanol was added in dried petriplates to make a final extract. Then extract was collected in bottles for further analysis (Stevigny et al. 2004).

Quantitative analysis of Aprophine - Alkaloid

Estimation of Aprophine through TLC detection method

Ethanolic extract of suspension culture of different concentration (Control, 25, 50, 75 and 100 mg/L Salicylic Acid) had applied as a spot on the plate 2.0-2.5 cm apart from the edge by means of a micropipette. The solvent was allowed to evaporate.

Plate development

Separation had carried out in a TLC chamber with the developing solvent i.e. mobile phase methanol: water (9: 1 v / v) to a depth of about 1.5 cm. This was a permissible to stand for at least 1 hour with a lid covering the top of the tank to make sure that the atmosphere within the tank becomes saturated with vapors of solvent (equilibration). After an equilibration, the lid was detached and the sample loaded. TLC plates were placed vertically in tank in such a manner that the sample spot should not be in contact with the solvent. The plates were taken out of the TLC chamber when the mobile phase covered two-third the length of the TLC plate and dried (Janac et al. 2008).

Analytical detection

After drying, the plates were exposed to iodine vapors by insertion in the chamber that had saturated with iodine vapors. The developed results were observed under visible light. The Rf value of the diverse spots that were recorded was calculated.

Estimation of Aprophine through High-performance liquid chromatography (HPLC)

Aporphine is an alkaloid which formulates the center of a group of quinoline alkaloids. It can subsist in any of two en-antiomeric forms such as (R)-aporphine and (S)-aporphine. Numerous diverse derivatives have been isolated and characterized from plants. Hence, it's noticeable that numerous peaks are analogous to different Aporphine derivatives would be analyzed in HPLC estimation. Consequently, by combining the peaks in connecting a particular retention time (RT) based on the reference, Aprophine molecule we predicted for the presence/induction of alkaloids in our provisions (Sarma et al. 2002).

RESULTS AND DISCUSSION

Annona squamosa L. Cell suspension culture was established by Callus culture shifting on liquid MS medium (3.0 mg L^{-1} BAP and 4.0 mg L^{-1}NAA). Cell Suspension culture was sub cultured by 2 speedy sub culturing procedures. Cell suspension culture was treated after 15th day of successive subculture in MS media having growth hormone (3.0 mg L^{-1} BAP and 4.0 mg L^{-1} NAA) with various concentration of mg L^{-1} Salicylic acid separately (Figure 1). Cell suspension culture is a procedure of maintaining hereditarily similar cells in Liquid MS medium. Suspended cells are perpetually developed and used to enhance varied secondary metabolites through rising progression or by over expression of gene answerable for production of secondary metabolites (Dudareva *et al.* 2013).

Quantitative Estimation through TLC of Cell suspension culture of *Annona squamosa* L.

Secondary metabolites were isolated from suspension culture sample in the form of methanol extracts which were loaded to TLC plates and run in the diverse ratios of methanol and distilled water solvent system were used as mobile phase to establish the elute with finest presentation (methanol / distilled water; 9:1) . Subsequent each separation, the TLC plates were incubated at room temperature. In the current cram, the ratio of methanol and distilled water were found for suspension culture through Rf value (4.56, 0.473, 0.578, 0.7631 and 0.3157) in a appropriate solvent arrangement (methanol: distilled water; 9:1). In previous reports, equivalent conclusion of TLC studies uncovered greatest amount of constituent of the ethanol extract, as it had a highest quantity of well - determined stains. Three chief bands were realistic on UV-spray and iodine long plates. Rf value was measured as distance covered by the solute / Distance traveled by the solvent. The value of Rf for the three bands were

recorded as 0.81, 0.68 and 0.38 correspondingly. The Rf value for quinones and steroids is evident at a value of Rf ~ 0.81, geraniol at 0.68 and amino acids at 0.38 and there was no overlap of compounds. This TLC profile can provide the distinguishing impression of the *Annona squamosa* leaf (Vanitha *et al*. 2012).

Quantitative Estimation through HPLC of Cell suspension culture of *Annona squamosa* L.

High pressure liquid chromatography (HPLC) was further performed for the quantitative analysis of isolated secondary metabolites.

Effect of Salicylic acid as AN elicitor on accumulation of Aprophine Alkaloid

The responsibility of Salicylic acid treated as an elicitor for aprophine accumulation was resolute in *Annona squamosa* suspension cultures had been treated with different concentrations of Salicylic acid. According to HPLC analysis, it has been pragmatic in suspansion cultures where aprophine accumulation in suspension cultures had augmented significantly at 25 µM (677.523 ppm) 50 µM (934.71 ppm), 75 µM (582.92 ppm) and 100 µM (1000 ppm) Salicylic acid concentrations with respect to control (185.595 ppm) untreated suspension culture. Maximum aprophine accumulation in suspension cultures was obtained with 100 µM Salicylic acid concentration in comparison to control. Salicylic acid treated as an elicitor has very important role in elevating the secondary metabolite (aprophine) concentration in the cells of *Annona squamosa* (Table 1) (Figure 3-8).

Similar result were observed in various studies of other workers such as Gupta *et al*.,(2012), and Alam *et al*.,(2011). Throughout their studies, HPLC profiles of *Abelmoschus moschatus* were analyzed and two phenolic compounds gallic acid (2.77 min) and hyperzooid (7.31 min), which had different elution times, were examined. These previous studies strongly supports the analysis of the present study. Present study's results is also being supported with the reports of Kavitha and Mohideen (2017). The bioactive components of *Abelmoschus moschatus* flowers were analyzed through HPLC, UV VIS and FTIR. Phytochemical examination of *Abelmoschus moschatus* flowers explained the presence of flavonoids, terpenoids, tannins, saponins, glycosides, triterpenoids, phenol and anthroquinones. All the findings of the present study revealed that Salicylic acid (SA) showed significant effect on enhancement of Aprophine in Cell suspension culture of *Annona squamosa*. Highest accumulation was observed in 100 µM Salicylic acid treated cell culture to comparison to control. This finding support to the fact of positive regulation of salicylic acid on the enhancement of Secondary metabolite.

CONCLUSION

Annona squamosa L. is an efficiently significant species. *Annona squamosa* L. leafs are useful as herbal alternative medicine for diverse diseases. The chief purpose of this current study to assess the augmentation of Aprophine-Alkaloid in *Annona squamosa* L. Cell suspension culture. Cell Suspension culture was developed in modified Murashige and Skoog (MS) strains with growth hormones (3.0 mg L-1 BAP and 4.0 mg L-1 NAA) with treatment of different concentration of Salicylic acid (SA) (25, 50, 75 and 100 mg/L) separately. Thin Layer Chromatography (TLC) and High pressure liquid chromatography (HPLC) analysis showed that uppermost increase was in 100 µM Salicylic acid treated cell culture to comparison to control cell culture. This study revealed the accumulation of Aprophine in Cell suspension culture of study plant under Salicylic acid treatment. This study also helps to understand the future scope of Cell suspension culture for mass production of Secondary metabolites by using Elicitors.

ACKNOWLEDGEMENT

Authors are thankful to Hon'ble Chairperson JV'n Vidushi Garg and Hon'ble Founder and Advisor JV'n Dr. Panckaj Garg, Jayoti Vidyapeeth Women's University, Jaipur (Rajasthan) for their kind cooperation, encouragement, and providing the facilities of University Innovation Center and other laboratories.

REFERENCES

1. Alam N, Hossain M, Khalil MDI, Moniruzzaman M, Sulaiman SA and Gan S H. High Catechin Concentrations Detected In Withania somnifera (Ashwagandha) By High Performance Liquid Chromatography Analysis. Bmc Complementary and Alternative Medicine. 2011; 11:65.
2. Caroline S, Wautier M-C, Jiwan JLH, Chiap P, Hubert P and Hubert P. Development and Validation of a High Performance Liquid Chromatographic Method for Quantitative Determination of Aporphine Alkaloids from Different Samples of *Cassytha filiformis*.Planta Medica. 2004;70:764-770.
3. Dudareva N, Klempien A, Muhlemann J K and Kaplan I. Biosynthesis, Function and Metabolic Engineering Of Plant Volatile Organic Compounds. New Phytologist. 2013; 198(1):16-32.
4. Gajalakshmi S, Divya R, Deepika V D, Mythili S and Sathiavelu A. Pharmacological activities of Annona squamosa: a review. International Journal of Pharmaceutical Sciences Review and Research. 2011; 10(2):24-29.
5. Gupta RK., Kesari AN, Murthy PS, Chandra R, Tandon V and Watal G. Hypoglycemic and Antidiabetic Effect of Ethanolic Extract of Leaves of *Annona squamosa L.* In Experimental Animals. Journal of Ethanopharmacology 2012; 99(1): 75-51.
6. Janac´kovic P, Teševic V, Marın PD, Milosavljevic S, Duletic -Lauševic S, Janac´kovic S and Veltic M. Brine shrimp lethality bioassay of selected Centaurea L. Species (Asteraceae). Archives of Biological Sciences. 2008; 60 (4): 681-685.
7. Kavitha R and Mohideen AMU. Exploration of Phytocompounds in *Abelmoschus moschatus* Flowers Using Hplc, Uv-Vis and Ftir Techniques. International Journal of Chemical Studies. 2017 5(6); 702-706.
8. Morita H, Sato Y, Chan KL, Choo CY, Itokawa H, Takeya K and Kobayashi J. Samoquasine A, a Benzoquinazoline alkaloid from the seeds of *Annona squamosa*. Journal of Natural Products. 2000; 63: 1707-8.
9. Morton, J. Sugar Apple. In Fruits of warm climates. Edited by Julia F. Morton. Echo Point Books & Media, Miami, FL. 1987; pp. 69–72.
10. Nabavi SM, Uriarte E, Fontenla JA., Rastrelli L and Sobarzo-Sanchez E. Aporphine Alkaloids and their Antioxidant Medical Application: From Antineoplastic Agents to Motor Dysfunction Diseases. Current Organic Chemistry. 2017; 21 (4): DOI**:** 10.2174/1385272820666161017165735 **.**
11. Pathak K and Zaman K. An Overview on Medicinally Important Plant - *Annona reticulata* Linn. International Journal of Pharmacognosy and Phytochemical Research. 2013; 5(4): 299-301
12. Ptak A, El Tahchy A, Skrzypek E, Wojtowicz T and Laurain-Mattar D. Influence of Auxins on Somatic Embryogenesis and Alkaloid Accumulation In *Leucojum aestivum* Callus. Central European Journal of Biology. 2013; 8(6): 591-599.
13. Sandeep and Abhilasha M. Annona squamosa: Seetaphal: A Review. Ip International Journal of Comprehensive and Advanced Pharmacology. 2017; 2(3);72-81.
14. Sarma BK, Singh UP and Singh KP. Variability in Indian isolates of *S. rolfsii*. Mycologia. 2002; 94(6): 1051-1058
15. Vanitha V, Umadevi KJ and Vijayalakshmi K. Determination of Bioactive Components of *Annona Squamosa* L Leaf By Gc- Ms Analysis. International Journal of Pharmaceutical Sciences and Drug Research. 2012; 3(4): 309-312.
16. Zarzycki PK. Staining and Derivatization Techniques for Visualization in Planar Chromatography. In Instrumental Thin-Layer Chromatography. Edited by Poole CF. Elsevier. 2014; 191-237.

Table: 1 Concentration Salicylic acid treated sample in callus and suspension culture samples present Aprophine part per million (ppm).

Concentration Salicylic acid treated sample	Suspension culture aprophine present in samples (ppm)
Control	185.595
25	677.523
50	934.71
75	582.92
100	1000

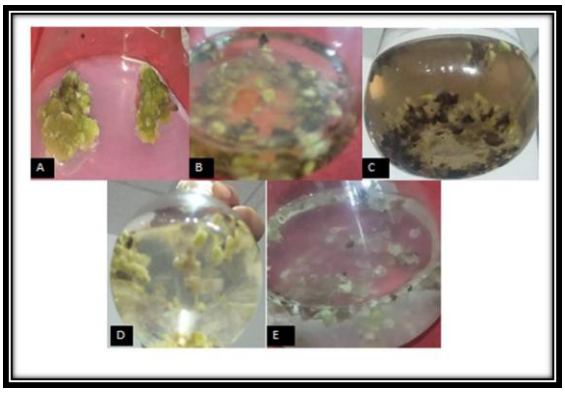


Fig. 1: Cell suspension culture of *Annona squamosa L.* A. *In vitro* developed callus of *Annona squamosa L.* B. Shifting of callus clumps on liquid MS medium (3.0 mg L^{-1} BAP and 4.0 mg L^{-1}NAA).C. Cell Suspension culture after first subculture. D. Cell Suspension culture after second subculture. E. Cell Suspension culture after 15th day of consecutive subculture in liquid MS Medium (having 3.0 mg L^{-1} BAP and 4.0 mg L^{-1} NAA) with various concentration of mg L^{-1} Salicylic acid.

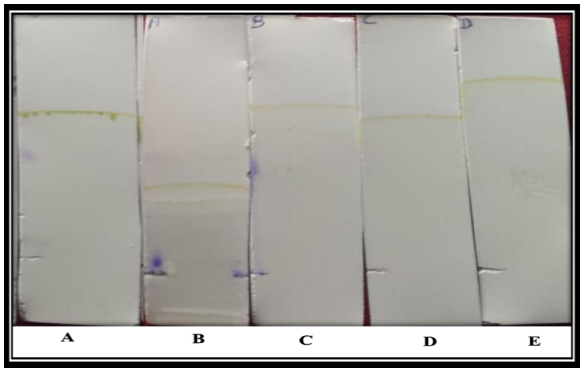


Fig. 2: Methanol extract of Cell suspension culture of *Annona squamosa* L. (A) TLC result without Salicylic Acid (SA) (B) TLC in 25 µM L^{-1} Salicylic Acid (SA) added medium. (C). TLC in 50 µM L^{-1} Salicylic Acid (SA) added medium. (D). TLC in 75 µM L^{-1} Salicylic Acid (SA) added medium. (E). TLC in 100 µM L^{-1} Salicylic Acid (SA) added medium

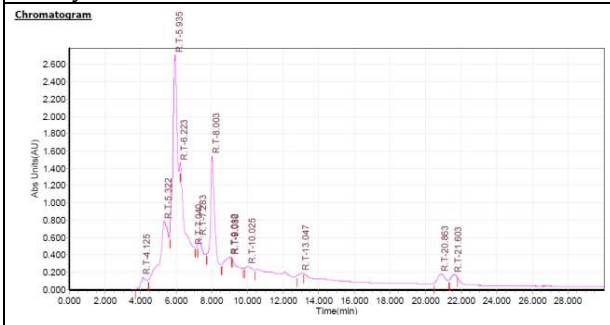


Fig. 3. Quantitative analysis through HPLC for Aprophine standard

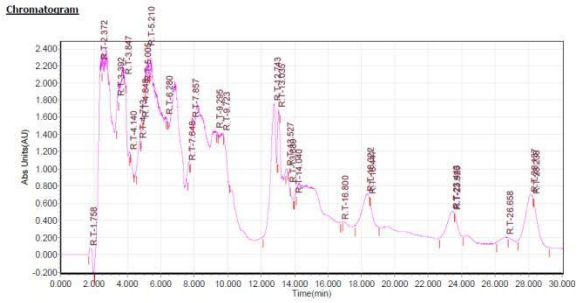


Fig. 4. Quantitative analysis through HPLC for Cell suspension culture of *Annona squamosa* L. (Control- without Salicylic Acid Treatment)

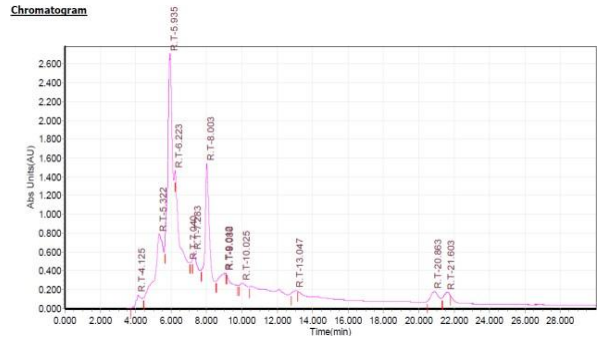


Fig. 5. Quantitative analysis through HPLC for Cell suspension culture of *Annona squamosa* L. (Treated with 25 µM Salicylic Acid)

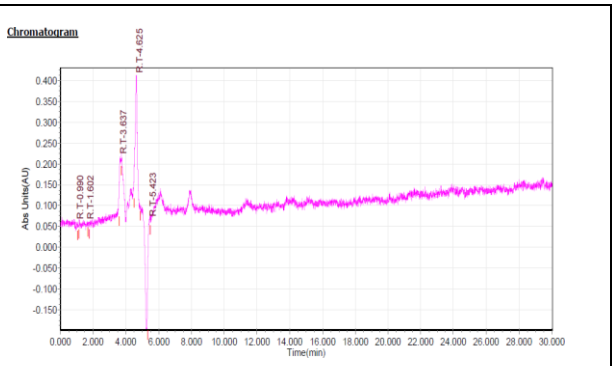


Fig. 6. Quantitative analysis through HPLC for Cell suspension culture of *Annona squamosa* L. (Treated with 50 µM Salicylic Acid)

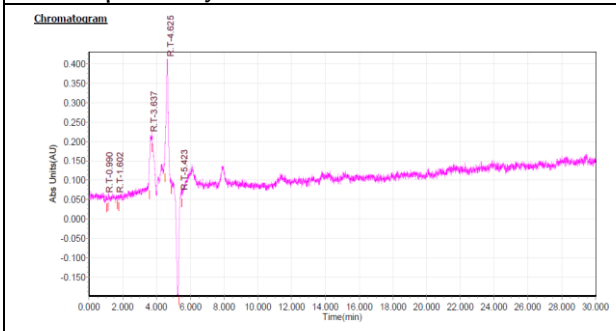


Fig. 7. Quantitative analysis through HPLC for Cell suspension culture of *Annona squamosa* L. (Treated with 75 µM Salicylic Acid)

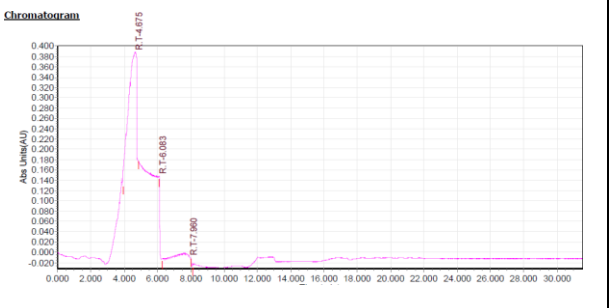


Fig. 8. Quantitative analysis through HPLC for Cell suspension culture of *Annona squamosa* L. (Treated with 100 µM Salicylic Acid)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of *In vitro* Antidiabetic Activity of Ethanolic Extract of *Dracaena terniflora* Roxb. Roots

Kavitha Vasudevan[1]*, R. Sanilkumar[2] and Sabu M.C[1]

[1]Mookambika College of Pharmaceutical Sciences and Research, Muvattupuzha, Kerala, India.
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.

Received: 17 Nov 2020 Revised: 19 Dec 2020 Accepted: 22 Jan 2021

***Address for Correspondence**
Kavitha Vasudevan
Mookambika College of Pharmaceutical Sciences and Research,
Muvattupuzha, Kerala, India.
Email: kavithamanoj28@gmail.com

ABSTRACT

Diabetes is a major metabolic disorder whose prevalence is increasing daily. Medicinal plants have played an important role in the prevention and treatment of type 2 diabetes via prophylactic and therapeutic management. Current treatments possess undesirable side-effects and therefore investigations into alternative remedies are going on which may be cost-effective and devoid of such side-effects. *Dracaena terniflora* Roxb. is used traditionally in the management of diabetes in folklore medicine by various tribal communities in Kerala, India. The study seeks to evaluate the glucose uptake, α-glucosidase and α-amylase inhibitory activities of the roots to support its folkloric use. The crude extract is also evaluated for its phenolic and flavonoid content. The possibility of the extract for cytotoxicity was evaluated using MTT assay in HepG2 cells. The Ethanolic root extract showed a significant 50% α-amylase inhibitory (IC_{50}) activity at a concentration of 336 µg/ml and the 50% alpha glucosidase inhibitory activity (IC_{50}) was found to be 789.184 µg/ml. *In vitro* glucose uptake assay on cultured HepG2 cell lines also showed the occurrence of a dose dependant increase of glucose uptake with increasing concentrations of root extract. The results of the study therefore clearly indicate the potential of this Plant to manage diabetes.

Keywords: *Dracaena terniflora* Roxb. alpha-amylase, alpha-glucosidase, glucose uptake, diabetes, cytotoxicity, Hep G2 cell lines.

INTRODUCTION

Diabetes mellitus is a heterogeneous metabolic disorder characterized by the presence of hyper glycaemia due to impairment of insulin secretion, defective insulin action or both. The chronic hyper glycaemia of diabetes is associated with relatively specific long-term micro vascular complications affecting the eyes, kidneys and nerves, as

well as an increased risk for cardiovascular diseases. The majority of cases of diabetes can be broadly classified into 2 categories: type 1 diabetes and type 2 diabetes. Gestational diabetes (GDM) refers to glucose intolerance with onset or first recognition during pregnancy [1]. Symptoms of marked hyper glycemia include polyuria, polydipsia, weight loss, sometimes with polyphagia, and blurred vision. Impairment of growth and susceptibility to certain infections may also accompany chronic hyper glycemia. Long-term complications of diabetes include retinopathy with potential loss of vision; nephropathy leading to renal failure; peripheral neuropathy with risk of foot ulcers, amputations, and Charcot joints; and autonomic neuropathy causing gastrointestinal, genitourinary, and cardiovascular symptoms and sexual dysfunction. Patients with diabetes have an increased incidence of atherosclerotic cardiovascular, peripheral arterial and cerebrovascular disease [2]. High prevalence, variable pathogenesis, progressive process, and complications of diabetes all highlight the urgent need for effective treatments. Nowadays, different treatments, such as insulin therapy, pharmacotherapy, and diet therapy, are available to control diabetes. There are several types of glucose-lowering drugs that exert anti-diabetic effects through different mechanisms. These mechanisms include stimulation of insulin secretion by sulfonylurea and meglitinides drugs, increasing of peripheral absorption of glucose by biguanides and thiazolidine diones [3], delay in the absorption of carbohydrates from the intestine by alpha-glucosidase, and reduction of hepatic gluconeogenesis by biguanides [4].

Plants are natural antioxidants and effective herbal medicines, in part due to their anti-diabetic compounds, such as flavonoids, tannins, phenolic, alkaloids, glycosides etc improve the performance of pancreatic tissues by increasing the insulin secretion or decreasing the intestinal absorption of glucose [5]. Many plants have been used for the treatment of diabetes mellitus in Indian system of medicine and in other ancient systems of the world. Most of them seem to act directly on pancreas (pancreatic effect) and stimulate insulin level in blood. Some have extra pancreatic effect also by acting directly on tissues like liver, muscle etc. and alter favourably the activities of the regulatory enzymes of glycolysis, gluconeogenesis and other pathways. Since the plant products have fewer side effects, they have the potential as good hypoglycaemic drugs. They may also provide clues for the development of new and better oral drugs for diabetes [6]. *Dracaena terniflora* Roxb. (*Pleomele terniflora* Roxb.) is known as Manjakkanda or Manjakantha (in Malayalam). The synonyms of this plant include Elathaani, dwarf dracaena or wild dracaena of the family Liliaceae. (formerly in Dracaenaceae or Agavaceae). It has wide spread uses in ethno medicine. According to tribal literature available, the herbal preparation containing *Dracaena terniflora* Roxb. is used for treating spermaturia(7). The fresh juice of this plant is used in the treatment of diabetes by the Kurunarippullu tribes of Wayanad(8)(9). roots boiled with rice are taken internally for jaundice and root of this plant is used for the treatment of various liver disorders especially jaundice by the various tribal communities of Kerala (10).Traditionally the root extract is used for piles, Fruits boiled in coconut oil are used against head ache(11). Despite the widespread folklore uses of *Dracaena terniflora* Roxb. there are no results on the scientific validation of its traditional medicinal claim. Thus the aim of the present study was to evaluate proposed claim of antidiabetic activity of the roots of *Dracaena terniflora* Roxb.

MATERIALS AND METHODS

Collection and preparation of extracts

The whole plant of *Dracaena terniflora* Roxb. were collected in March 2017 from the semi forest regions of Wayanad and identified by Dr. Sr. Tessy Joseph, H.O.D department of botany, Nirmala College, Muvattupuzha, kerala, India where a herbarium specimen was deposited (Voucher number NCH/2017/538). Fresh plant materials were washed thoroughly in running tap water to remove adhering impurities, shade dried to constant weight. The roots and aerial parts were separated. Roots were coarsely powdered separately and passed through a 40-mesh sieve. It was stored in a tightly closed container. Fifty grams of the dried root powder was extracted with ethanol by using soxhlet apparatus. To ensure the complete extraction process, exhaustive extraction was applied for 10 hours. After that the extract was recovered from the solvent by evaporation in a rotary evaporator at 60^0C and final drying was done by

keeping the extract in desiccators for 1 hour to yield a reddish brown coloured semisolid sticky mass. The percentage yield of the ethanolic extract was obtained to 6.31%

Preliminary Qualitative phytochemical analysis (12) (13)

The preliminary phytochemical analysis of extract was carried out using standard procedures to identify the various constituents and find out the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, proteins, coumarins and triterpenoids and absence of anthocyanins and glycosides.

Determination of Total phenolic content (TPC) (14) (16)

Folin-Ciocalteau method was used for the determination of the total phenolic content of the root extract using expressed as milligram of gallic acid equivalent (GAE) per gram of the extracts with minor modifications as previously reported and was carried out in triplicates.

Determination of total flavonoid content (TFC) (15) (16)

The total flavonoid content was determined according to the aluminium chloride colorimetric method and expressed as milligram of quercetin equivalent (QE) per gram of extract. The determination of total flavonoid in the extract was also carried out in triplicates.

1) *In vitro* Antidiabetic activity

In vitro Antidiabetic activity of the DTR ethanolic root extract was investigated by studying the inhibitory effects on the level of α-amylase , α-glucosidase and glucose uptake assay from the liver. Acarbose was used as a standard drug to compare the inhibitory effects of alpha amylase and alpha glucosidase. Metformin was used as the standard drug for the assay of glucose uptake from the liver. Glucosidase enzymes catalyze hydrolysis of starch to simple sugars. In humans, these enzymes aid digestion of dietary carbohydrates and starches to produce glucose for intestinal absorption, which in turn, leads to increase in blood glucose levels. Inhibiting the function of these enzymes in patients with type-2 diabetes may reduce hyperglycemia. Glucosidases are named on the basis of their substrates, types of linkages hydrolyzed, and precise mechanism of action. Alpha amylases (e.g., salivary and pancreatic alpha amylases) act on long chain carbohydrates, while alpha glucosidases (e.g., maltase glucoamylase and sucrase isomaltase) act on shorter starch chains and disaccharides to produce glucose. Drugs like Acarbose delay carbohydrate digestion and reduce blood glucose levels in the short term, leading to improved HbA1c levels in patients with type-2 diabetes.

Determination enzyme inhibitory activity

***In vitro* alpha amylase inhibitory assay [17]**

Alpha -amylase inhibitory activity was determined by the method described by Worthington Enzyme Manual (Worthington Biochemical Corp., 1993a) with slight modifications. Different concentrations of sample such as 62.5µg/mL - 1000µg/mL was prepared from a stock concentration of 10mg/mL and make up to 1000µl using 25mM phosphate buffer pH 6.9, containing 25µl of porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25°c for 10 min. After pre incubation, 25µl of 0.5% starch solution in 25mM phosphate buffer pH 6.9 was added. The reaction mixtures were then incubated at 25°c for 10 min. The reaction was stopped with 50µl of 96mM 3, 5 dinitrosalicylic acid colour reagent. The micro plate was then incubated in a boiling water bath for 5 min and cooled to room temperature. Absorbance was measured at 540nm using a microplate reader (Erba, Lisascan). The blank with 100% enzyme activity was prepared by replacing the plant extract with 200 µl of buffer. A blank reaction was similarly prepared using the plant extract at each concentration in the absence of the enzyme solution. A positive control sample was prepared using Acarbose (62.5 µg/ml–1000 µg/ml) and the reaction was performed similarly to the reaction with plant extract as mentioned above. The α-amylase inhibitory activity was expressed as percent

inhibition and was calculated using the equation given below: The % α-amylase inhibition was plotted against the extract concentration and the IC_{50}values were obtained from the graph.

The alpha - amylase inhibitory activity was calculated according to the equation given below:

Inhibition (%) = (A control – A sample) / A control × 100

Where

A control was the absorbance of the control (without ZnL complex);

A sample was the absorbance in the presence of ZnL complex.

In vitro alpha glucosidase inhibition assay [18] [19]

Alpha glucosidase activity was measured by the determination of reducing sugar arise from hydrolysis of sucrose by alpha glucosidase enzyme. The effects of samples were assayed according to the method Matsui et al., with slight modifications. Different concentrations of sample such as 125µg/mL-2000µg/mL from a stock concentration of 10mg/mL were taken and was incubated for five minutes before initiating the reaction with substrates sucrose(37mM), in a final reaction mixture of 1mL of 0.1 M phosphate buffer (pH 7.2). The reaction mixture was incubated for 20 and 30 min at 370C and the reaction was stopped incubating in a boiling water bath for 2 minutes. A tube with phosphate buffer and enzyme was maintained as control. The tubes were added with 250µL of glucose reagent and incubated for 10 minutes followed by measuring absorbance at 510nm using a microplate reader (Erba, Lisascan).

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

Measurement of glucose uptake in HepG2 cells

The preventive effects of phytoconstituents on insulin signalling and on both glucose production and uptake were studied in insulin-responsive human HepG2 cells treated with high glucose. HepG2 cells were used in this study due to their common physiologic function to lipid or glucose metabolism with normal hepatic cells. The effect of compounds on insulin resistance was comparable to that of metformin, a biguanide agent that reduces hyperinsulinaemia and improves hepatic insulin resistance.

In vitro cytotoxic activity by MTT assay [19] [20]

The *in vitro* cytotoxic activity of the ethanolic root extract of *Dracaena terniflora* Roxb. was assessed by MTT Assay (3-(4,5-dimethythiazol- 2-yl)-2,5-diphenyl tetrazolium bromide) using Hep G2cell line. The 50% cytotoxic concentration (CTC_{50}) was determined by estimating mitochondrial synthesis using tetrazolium assay.

Culturing and maintenance of Hep G2 cells [21]

Hep G2 (Human Hepatic Cells) cells were initially procured from National Centre for Cell Sciences (NCCS), Pune, India and maintained Dulbecco's modified Eagles medium, DMEM (Sigmaaldrich, USA). The cell line was cultured in 25 cm^2 tissue culture flask with DMEM supplemented with 10% FBS, L-glutamine, sodium bicarbonate (Merck, Germany) and antibiotic solution containing: Penicillin (100U/ml), Streptomycin (100µg/ml), and Amphotericin B (2.5µg/ml). Cultured cell lines were kept at 37ºC in a humidified 5% CO_2 incubator (NBS Eppendorf, Germany).

The viability of cells were evaluated by direct observation of cells by Inverted phase contrast microscope and followed by MTT assay method.

Cells seeding in 96 well plate

Two days old confluent monolayer of cells were trypsinized and the cells were suspended in 10% growth medium, 100µl cell suspension ($5x10^3$ cells/well) was seeded in 96 well tissue culture plate and incubated at 37ºC in a humidified 5% CO_2 incubator.

Preparation of compound stock

1mg of sample was weighed and dissolved in 1mL DMEM using a cyclomixer. The sample solution was filtered through 0.22 µm Millipore syringe filter to ensure the sterility. Ethanol was added to induce toxicity.

Hepatotoxicity Evaluation [21]

After attaining sufficient growth, Alcohol (40%) was added to induce toxicity and incubated for one hour, freshly prepared each compounds in 5% DMEM were five times serially diluted by two fold dilution (100µg, 50µg, 25µg, 12.5µg, 6.25µg in 500µl of DMEM) and each concentration of 100µl were added in triplicates to the respective wells and incubated at 37ºC in a humidified 5% CO_2 incubator. Non treated control cells were also maintained.

Hepatotoxicity Assay by Direct Microscopic observation

Entire plate was observed after 24 hours of treatment upto 72 hours in an inverted phase contrast tissue culture microscope (Olympus CKX41 with Optika Pro5 CCD camera) and microscopic observation were recorded as images. Any detectable changes in the morphology of the cells, such as rounding or shrinking of cells, granulation and vacuolization in the cytoplasm of the cells were considered as indicators of cytotoxicity.

Hepatotoxicity Assay by MTT Method

15 mg of MTT (Sigma, M-5655) was reconstituted in 3 ml PBS until completely dissolved and sterilized by filter sterilization. After 24 hours of incubation period, the sample content in wells were removed and 30µl of reconstituted MTT solution was added to all test and cell control wells, the plate was gently shaken well, then again incubated at 37ºC in a humidified 5% CO2 incubator for 4 hours. After the incubation period, the supernatant was removed and 100µl of MTT Solubilization Solution (Dimethyl sulphoxide (DMSO), Sigma Aldrich, USA) was added and the wells were mixed gently by pipetting up and down in order to solubilize the formazan crystals. The absorbance values [optical density (OD)] were measured by using microplate reader at a wavelength of 540 nm (Laura B. Talarico et al., 2004).

The percentage of growth inhibition was calculated using the formula

% Growth inhibition = (Mean OD of normal control − Mean OD of test group/Mean OD of Normal control) × 100

CTC 50 (50% cytotoxic concentration), the concentration of the test drug needed to inhibit cell growth by 50% is generated from the dose- response curves for test samples. The dose-response curve was generated using % growth inhibition on Y axis and the extract concentration (µg/ml) on X-axis.

Measurement of glucose uptake in HepG2 cells [22]

The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution (Invitrogen) for 2 minutes and passaged to T flasks in complete aseptic conditions. The cells were then subcultured to a 24 well plate. After attaining 80% confluency, the cells were kept in DMEM without glucose for 1 hour. Then the cells were treated with ethanolic root extract of *Dracaena terniflora* Roxb. (DTR) at a final concentration of 25µg/mL, 50µg/mL and 100µg/mL from a stock solution of 1mg/mL and incubated for 24 hours in DMEM containing 300mM glucose. An untreated control without glucose was also maintained. After incubation cells were isolated by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200µl of cell lysis buffer (1MTris Hcl, 0.25M EDTA, 2M NaCl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and the glucose uptake was estimated using glucose kit method (Erba Mannheim, Germany) and the absorbance was read at 505nm using a UV visible spectrophotometer (Agilent, USA). All experiments were carried out in triplicates and mean value was used to find the glucose uptake (%) The glucose concentration of the wells with cells was subtracted from the glucose of the blank wells to obtain the amount of glucose consumption.

$$\text{Total Glucose in mg/dL} = \frac{\text{Absorbance of Test}}{\text{Absobance of Standard}} \times 100$$

$$\% \text{ Glucose uptake} = \frac{\text{OD of Test} - \text{OD of Control}}{\text{OD of Test}} \times 100$$

Data analysis

All samples were analyzed in triplicates and the results were expressed as mean ± SD.

RESULT AND DISCUSSION

From the preliminary phytochemical screening, it was observed that ethanolic root extract consists of active constituents such as alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, proteins, coumarins and triterpenoids and absence of anthocyanins and glycosides. Since flavonoids and phenolic compounds in addition to other phytoconstituents play a major role in diabetic research, determination of total flavonoid and phenolic contents also emphasise their role in antidiabetic activity. Ethanolic root extract showed the flavonoid content of 72 mg quercetin equivalent/g of roots and a phenolic content of 31 mg gallic acid equivalent/g of roots.

In vitro alpha amylase inhibition assay

In alpha-amylase inhibition assay of Ethanolic root extracts of *Dracaena terniflora* Roxb, was evaluated as illustrated in Table: 1, Concentrations of 62.5,125, 250, 500, 1000 µg/mL of ethanolic root extract of *Dracaena terniflora* Roxb. were selected for testing the alpha amylase activity. The result of study showed that, there was a dose-dependent increase in percentage inhibitory activity against α -amylase enzyme. The Ethanolic extract showed a significant 50% α-amylase inhibitory (IC_{50}) activity at a concentration of 336 µg/ml and the results are comparable with that of the standard Acarbose which showed 50% inhibition at a concentration of 189 µg/ml. (Fig: 1)

In vitro Alpha glucosidase inhibition assay

α- Glucosidase plays a central role in modulating postprandial hyperglycemia, which breaks down α-1, 4-glucosidic linkages of disaccharides, resulting in simpler sugars. A previous study reported the established α-glucosidase inhibitors and their effects on delaying the expeditious generation of blood glucose after food uptake [23]. Alpha glucosidase inhibitory activity for the ethanolic root extract of *Dracaena terniflora* Roxb. is presented in Table 2. Varying concentration of the extract viz, 125, 250, 500, 1000 and 2000µg/ ml are taken and assayed for the α-glucosidase inhibitory activity. The extracts exhibited a dose dependent increase in percentage inhibitory activity against alpha glucosidase. The 50% alpha glucosidase inhibitory activity (IC_{50}) for the ethanolic extract of *Dracaena terniflora* Roxb. was found to be 789.184 µg/ml, while IC_{50} for standard Acarbose was 506.59 µg/ml. Thus the drug extract showed reasonable alpha glucosidase inhibitory effect when compared with the standard drug Acarbose. (Fig: 2).

In vitro cytotoxicity assay of ethanolic root extract of *Dracaena terniflora* Roxb.

The percentage cell viability with respect to the normal control (NC) cell lines (HEPG2) at different concentrations of ethanolic root extract of *Dracaena terniflora* Roxb. was determined and the results are shown in Figure: 3.The normal control cells showed 100% cell viability. The ethanolic root extract of *Dracaena terniflora* Roxb. at concentrations 100 µg/ml, 50 µg/ml, 25 µg/ml and 12.5 µg/ml and 6.25 µg/ml showed 72.98%, 78.05%, 83.89%, 88.01% and 98.95 %cell viability, respectively. The inhibitory concentration (CTC_{50}%) value of ethanolic root extract of *Dracaena terniflora* Roxb. was found to be 188.81µg/ml. In MTT assay, the cell viability was >70 % up to a concentration of 100µg/ml. Thus, DTR root extract showed less cytotoxic effect to hepatic cells due to the presence of phytoconstituents. Therefore, these extract concentrations were selected to further evaluate the cytoprotective activity against ethanol-induced cell damage. (Calculated using ED50 PLUS V1.0 Software)

Glucose Uptake in HepG2.

HepG2 cells are widely used for biochemical and nutritional studies as a cell culture model of human hepatocytes since they retain their morphology and most of their function in culture Thus, this cell line has been extensively used to study the hepatic glucose production and the modulation of the insulin pathway *in vitro* . Insulin resistance in insulin sensitive organs results in metabolic disorder such as hyperglycaemia, hyperinsulinaemia and hyper

triglyceridaemia, which are common features of type 2 diabetes. Insulin resistance in liver cells mainly causes impaired glycogen synthesis, failed to suppress glucose production, which is the major contribution to hyperglycaemia [24]. The results obtained for glucose uptake in HepG2 cells in the presence of the ethanolic root extract of *Dracaena terniflora* Roxb. at 25, 50 and 100 μg/ml are presented inTable:.3. Metformin was used as the standard. At 25 mg/ml, the glucose uptake was 6.49 ± 0. 276% and was increased to 31.27 ± 0. 251 % at 100 mg/ml concentration. Thus the drug extract showed a concentration dependent increase in glucose uptake when compared with standard Metformin. This suggests that the ethanolic root extract of *Dracaena terniflora* Roxb. mimics metformin by increasing glucose uptake in the liver. Metformin belongs to the category of the biguanide class of oral hypoglycemic. It exerts its hypoglycemic effect through activation of the AMP activated protein kinase (AMPK) in the liver, which in turn may lead to various pharmacologic effects, including inhibition of glucose, lipid synthesis, and also improved hepatic sensitivity to insulin [25].

CONCLUSION

In conclusion the result of the present study revealed that ethanolic root extract of *Dracaena terniflora* Roxb. exhibited potential antidiabetic activity focusing on the inhibitory effects on alpha -amylase and alpha –glucosidase and also demonstrated remarkable ability to enhance the uptake of glucose into the muscle cells and adipose tissues. . The inhibition of alpha-amylase and alpha-glucosidase would defer the degradation of carbohydrate, which causes a diminishing in the assimilation of glucose; therefore the rise of postprandial blood glucose level decreases. [26] Phytochemical screening indicated the presence of alkaloids, flavonoids, phenols, saponins, steroids, tannins, terpenoids, carbohydrates, proteins, coumarins etc which may be responsible for this restorative activity. Natural polyphenols have been accounted to inhibit the activity of carbohydrate hydrolyzing enzymes like alpha –amylase and alpha –glucosidase. Thus data obtained from this study suggest that ethanolic root extract of *Dracaena terniflora* Roxb. exerts an inhibitory effect on α-glucosidase and α-amylase and was also nontoxic at tested concentrations. The results displayed by the ethanol root extract are interesting enough to stimulate further in vivo experiments. In addition, these results support the traditional use of this plant in the management of diabetes. Further studies on experimental animals and humans are justified along with the identification of pure bioactive compounds.

REFERENCES

1. Saxena A. Diabetes-metabolic impact on kidneys and nutritional status. J Renal Nutr Metab 2018; 4:68-9
2. Diagnosis and Classification of Diabetes Mellitus American Diabetes Association Diabetes Care. 2009; 32(Suppl 1): S62–S67
3. Bathaie S, Mokarizade N, Shirali S. An overview of the mechanisms of plant ingredients in the treatment of diabetes mellitus. J Med Plant. 2012; 4(44):1–24.
4. Hui H, Zhao X, Perfetti R. Structure and function studies of glucagon-like peptide-1 (GLP1): the designing of a novel pharmacological agent for the treatment of diabetes. Diabetes Metab Res Rev. 2005; 21:313–31.
5. Kooti W, Farokhipour M, Asadzadeh Z, Ashtary-Larky D, Asadi-Samani M. The role of medicinal plants in the treatment of diabetes: a systematic review. Electron Physician. 2016 Jan 15; 8(1):1832-42.
6. Shukia, R., Sharma, S.B., Puri, D. et al. Medicinal plants for treatment of diabetes mellitus. Indian J Clin Biochem, 2000; 15, 169–177
7. Muhammad Jahangir Hossen., Md Bashir Uddin., Syed Sayeem Uddin Ahmed., Zhi-Ling Yu., Jae Youl Cho., *Pak Vet J*. 2016; *36(2)*, 127-133.
8. M. P. Raghavendra., A. G. Devi Prasad., T. B. Shyma., *IJPSR*. 2015;6(8), 3617-3625.
9. P.S Udayan., Satheesh George., K V Tushar., Indira Balachandran., Indian *journal of traditional knowledge*. 2005; 4(2), 159-163
10. Bidhan Mahajon., Remadevi R., *International Ayurvedic Medical Journal. IAMJ 3(6)* 2015; 1828-1847.

11. Satya Narayin., Usha singh., *Scientific Publisher India*, 2007, 15
12. Sadasivam. S., Manickam. A., (1996) Biochemical Methods for Agricultural Sciences, New Age International (P) Ltd., New Delhi.
13. Harborne JB. *Methods of plant analysis,* Chapman and Hall, London 1973.
14. Singleton V L., Orthofer R et al., *Enzymol*, 1999, 299, 152-178.
15. Jin-Yuarn Lin., Ching-Yin Tang., *Food Chemistry*. 2007, 101, 140-147.
16. Shakkeela Yusuf., Reshma., Puthenkalam Melvin., Fathima Abdul Jaleel., Sreeja PR., Jyoti Harindran., *RJPBCS* .2018, 9(4),766-772.
17. Worthington V (1993) Worthington Biochemical Corp. Freehold NJ, pp: 293-294
18. M.S. Deutschländer, M. van de Venter, S. Roux, J. Louw, N. Lall ; Hypoglycaemic activity of four plant extracts traditionally used in South Africa for diabetes, Journal of Ethnopharmacology; 2009. 124 619–624.
19. Matsui T *et al*. alpha **-**Glucosidase Inhibitory Action of Natural Acylated Anthocyanins.1. Survey of Natural Pigments with Potent Inhibitory Activity. J. Agric. Food Chem. 2001; 49: 1948-1951.
20. Aswathy V. M, M. A Shajahan and Indulekha V. C, Hepatoprotective effect of triphala and its combinations- in hepg2 cell lines. Int. J. Adv. Res. 7(9), 346-352
21. Sherin Joseph, Dr. Abdul Vahab A., Dr. Jyoti Harindran and Dr. R Shankar Iyer; Evaluation of hepatoprotective activity of polyherbal (hepin) against alcohol induced hepatotoxicity in wistar albino rats; World Journal of Pharmaceutical Research Volume 7, Issue 17, 791-807,
22. J.R. Abima Shazhni *et al*. Insights of antidiabetic, anti-inflammatory and hepatoprotective properties of antimicrobial secondary metabolites of corm extract from *Caladium X hortulanum*. Saudi J Biol Sci.2018; 25:1755–1761.
23. Shuyuan Liu, Zhi Yu , Hongkai Zhu , Wei Zhang and Yuqiong Chen; In vitro α-glucosidase inhibitory activity of isolated fractions from water extract of Qingzhuan dark tea , BMC Complementary and Alternative Medicine (2016) 16:378
24. Qun Huang, Lei Chen, Hui Teng, Hongbo Song, Xiaoqi Wu, Meiyu Xu Phenolic compounds ameliorate the glucose uptake in HepG2 cells' insulin resistance via activating AMPK Anti-diabetic effect of phenolic compounds inHepG2 cells, Journal of Functional Foods 19 ,2015; 487–494
25. Idowu Jonas Sagbo, Maryna van de Venter, Trevor Koekemoer, and Graeme Bradley *In Vitro* Antidiabetic Activity and Mechanism of Action of *Brachylaena elliptica* (Thunb.) DC Evidence-Based Complementary and Alternative Medicine 2018, 13.
26. L.R. Rhabaso, J.L. Chiasson, Glucosidase inhibitors. In: R.A. Defronzo, E. Ferrannini, H.Keen, P. Zimmet, (Eds.), International Textbook of Diabetes Mellitus, Vol. 1, 3rd Ed, John Wiley and Sons Ltd., UK, 2004, 901-914.

Table: 1. *In vitro* alpha amylase inhibitory assay

Concentration µg/ml	**% Inhibition**	
	Ethanolic root extract of *Dracaena terniflora* Roxb.	**Acarbose (standard)**
62.5	12.06 ±0.040	21.28 ±0.10
125	24.35 ±0.036	40.73 ±0.026
250	40.74 ±0.027	54.23 ±0.021
500	65.09 ±0.025	77.65 ±0.036
1000	73.09 ±0.031	91.66 ±0.015

Table 2: *In vitro* Alpha glucosidase inhibition assay

Concentration µg/ml	%Inhibition	
	Ethanolic root extract of *Dracaena terniflora* Roxb.	Acarbose (standard)
125	09.42 ±0.015	16.97 ± 0.025
250	23.18 ±0.020	32.27 ± 0.036
500	37.16 ±0.015	49.42 ± 0.020
1000	54.25 ±0.021	70.35 ± 0.030
2000	73.44 ±0.010	79.68 ± 0.066

Table 3. *In vitro* glucose uptake of Hep G2 cell lines

Concentration in (µg/mL)	% of Glucose uptake	
	Ethanolic root extract of *Dracaena terniflora* Roxb.	Metformin (standard)
25	6.49 ± 0.276	48.89±0.0566
50	15.92±0.039	62.50±0.386
100	31.27±0.251	82.86±0.363

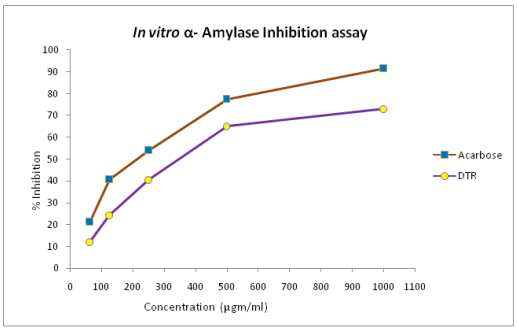


Figure. 1. *In vitro* alpha amylase inhibition assay of *Dracaena terniflora* Roxb.

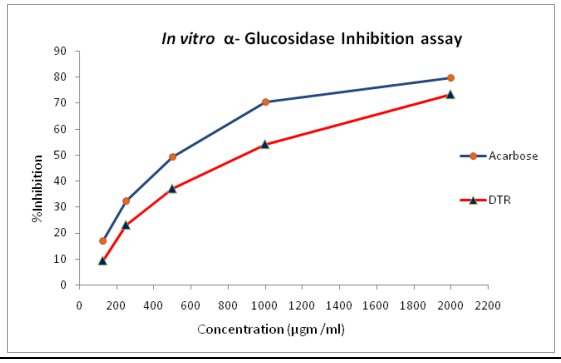


Figure. 2. *In vitro* Alpha glucosidase inhibition assay *Dracaena terniflora* Roxb.

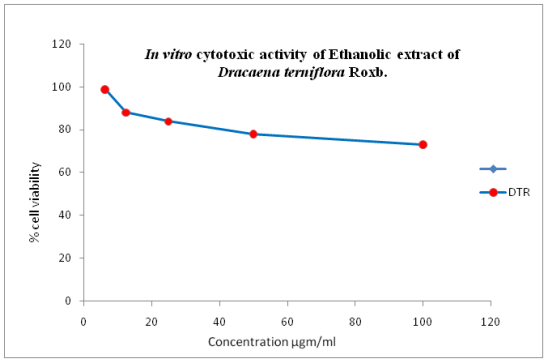


Figure. 3. *In vitro* cytotoxic activity of ethanolic root extract of *Dracaena terniflora* Roxb.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of *In vitro* Antioxidant Activity of Various Extract of Herbal Combination of Drug

Raghavendra Pai[1]*, Sanil Kumar R[2] and Sanil Kumar[3]

[1]Research Scholar, Annamalai University, Annamalai Nagar, Tamil Nadu, India
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar, Tamil Nadu, India.
[3]Accenture, Bangalore, India.

Received: 10 Nov 2020 Revised: 15 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
Raghavendra Pai
Research Scholar,
Annamalai University, Annamalai Nagar,
Tamil Nadu, India
Email : : paiannu143@gmail.com

ABSTRACT

The study was designed to examine the *in vitro* antioxidant activities of pet. ether, ethyl acetate and ethanolic extract of herbal combination of drug. The shade dried herbal drug *Trigonellafoenum graecum* and *Withania somnifera* powder was extracted with pet.ether, ethyl acetate and ethanol by continuous hot percolation method using Soxhlet apparatus 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 to 1000 µg/ml with reference natural standard rutin, quercetin, ascorbate and EDTA respectively and total phenolic and total flavonoid content was analysed. An IC_{50} value was found that ethanolic extract of herbal combination of drug is more effective all antioxidant activity. These *in-vitro* assays indicate that this herbal drug extract are better source of natural antioxidant, which might be helpful in preventing the progress of various oxidative stresses.

Keywords: Antioxidant, herbal combination of drug, *In-vitro* antioxidant, Total flavonoid content, Total phenolic content.

INTRODUCTION

Herbal medicine (also Herbalism) is the study of the botany and use of medicinal plants. The scope of herbal medicine is sometimes extended to include fungal and bee products, as well as minerals, shells and certain animal parts. Herbal medicine is also called phytomedicine or phytotherapy. Antioxidant activity is defined "as a limitation of the oxidation of proteins, lipids, DNA or other molecules that occurs by blocking the propagation stage

in oxidative chain reactions" and primary antioxidants directly scavenge free radicals, while secondary antioxidants indirectly prevent the formation of free radicals through Fenton's reaction. Free radical is defined as any atom (Eg. oxygen, nitrogen) have at least one unpaired electron in the outermost shell, and is accomplished of independent subsistence. Oxygen is the most significant element for life which is the major resource of free radicals. Oxygen is used by cell for generate energy, which leads to created free radicals are as end result of ATP (adenosine triphosphate) production by the mitochondria. Free radicals occurs not only normal cellular process always occurs upon revelation to certain chemicals such as polycyclic aromatic hydrocarbon, cadmium, lead, etc., radiation, cigarette smoke and high fat diet. A balance between formations of essential for normal cellular function. The herbal seed of *Trigonellafoenum-graecum* L. (fenugreek) is widely used for its medicinal properties all over the world and it is a very important spice in Indian culture. Around 260 species of *Trigonella*are diffused worldwide [7]. The genus name *Trigonella* means 'tri-angled', maybe because of triangular shape of its flowers, whereas the species name *foenum-graecum* means 'Greek hay' **[5]**. It is an annual crop and dicotyledonous plant belonging to the subfamily *Papilionaceae,* family *Fabaceae.* The herbal root of *Withania somnifera* Linn. commonly known as Ashwagandha, Indian ginseng, winter cherry is an important medicinal plant in the *solanaceae* family that has been used in ayurvedic and indigenous medicine for more than 3,000 years. Ashwagandha in Sanskrit means "horse's smell" probably originated from the odour of its root, which resembles that of sweaty horse. The species name *somnifera* means "sleep-making" in Latin, attributed to sedating properties. Ashwagandha is a small, branched, perennial woody shrub that grows usually about 2 feet in height and is naturally found in diverse areas ranging from Africa, the Mediterranean and East into India. The WHO has listed 21,000 plants, which used for medicinal purposes around the world. Among these, 2500 species are in India. There are about 800 plants which have been reported to show antidiabetic potential. Vast collections of plant-derived phyto active principles representing numerous natural bioactive compounds have established their role for possible use in the treatment of diabetes.

MATERIALS AND METHODS

Collection and authentication of plant material

The herbal drug was collected from Madurai, India. Taxonomic distinguishing proof was produced using The American College, Madurai, Madurai District, Tamilnadu, India. The herbal drug powdered materials were put away in a hermetically sealed holder. The herbal drug were shade dried and ground into fine powder. The powdered materials were stored in air tight polythene bags until use.

Preparation of plant extract

The equal amount of seed of *Trigonella foenum graecum* and root of *Withania somnifera* herbal drug 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 herbal drug was measured using the method described by [20]. 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.

$$Scavenging\ activity\ (\%) = \frac{A_{518}\,\text{Control} - A_{518}\,\text{Sample}}{A_{518}\,\text{Control}} \times 100$$

Nitric oxide radical scavenging activity

The ability of the herbal drug extracts to scavenge the nitric oxide radical activity were determined by the method described by [12]. 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 herbal drug extracts were described by [3]. 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 **[8]**.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 **[33]**. 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 herbal drug was evaluated by the transformation of Mo (VI) to Mo (V) to form phosphomolybdenum complex [25]. Ascorbic acid was used as standard. The antioxidant capacity was estimated using following formula:

$$\text{Total antioxidant activity} = \frac{A_{518}\ \text{Control} - A_{518}\ \text{Sample}}{A_{518}\ \text{Control}} \times 100$$

FRAP assay

The FRAP assay of herbal drug extracts were described by modified method of **[3]**. 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 herbal drugextract was measured using the method described by [19].

Estimation of total flavonoids content

Total flavonoid content of herbal drug extract was measured using the method described by [4].

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 48.92 % in pet.ether, 56.98% in ethyl acetate and 64.86% in ethanol when compared to rutin69.83% which is statistically significant at same concentration. The IC_{50} value was found to be 1030 µg/mlfor pet.ether, 780 µg/ml for ethyl acetate and 490 µg/ml for ethanolic extract of herbal drug 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 53.76% in pet.ether, 74.96% in ethyl acetate and 79.08% in ethanol when compared to quercetin98.01% which is statistically significant at same concentration. The IC_{50} value was found to be 910 µg/ml for pet. ether, 280 µg/ml for ethyl acetate and 175 µg/ml for ethanolic extract of herbal drug 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 55.28% in pet.ether, 60.84% in ethyl acetate and 66.84% in ethanol when compared to EDTA 97.90% which is statistically significant at same concentration. The IC_{50} value was found to be 850 µg/mlfor pet.ether, 480 µg/ml for ethyl acetate and 245 µg/ml for ethanolic extract of herbal drug 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 54.82% in pet.ether, 59.88% in ethyl acetate and 60.34% in ethanol when compared to ascorbate60.64% which is statistically significant at same concentration. The IC_{50} value was found to be 920 µg/mlfor pet.ether, 510 µg/ml for ethyl acetate and 475 µg/ml for ethanolic extract of herbal drug 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 53.46% in pet.ether, 56.42% in ethyl acetate and 62.74% in ethanol when compared to ascorbate60.64% which is statistically significant at same concentration. The IC_{50} value was found to be 870 µg/mlfor pet.ether, 660 µg/ml for ethyl acetate and 450 µg/ml for ethanolic extract of herbal drug 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 51.40% in pet.ether, 56.18% in ethyl acetate and 62.98% in ethanol when compared to ascorbate60.64% which is statistically significant at same concentration. The IC_{50} value was found to be 920 µg/ml for pet. ether, 750 µg/ml for ethyl acetate and 460 µg/ml for ethanolic extract of herbal drug 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 53.20% in pet.ether, 60.45% in ethyl acetate and 71.98% in ethanol when compared to ascorbate98.07% which is statistically significant at same concentration. The IC_{50} value was found to be 910 µg/mlfor pet.ether, 480 µg/ml for ethyl acetate and 245 µg/ml for ethanolic extract of herbal drug and for ascorbate it was 50 µg/ml.

Estimation of total phenol content

TPC showed a sharp pet.ether extract range of 2.139 mg/g, ethyl acetate extract range of 4.956 mg/g and ethanolic extract range of 8.320 mg/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 0.018 mg/g, ethyl acetate extract range of 0.298 mg/g and ethanolic extract range of 2.354 mg/g as concentration of plant extract varied from 50µg/ml to 1000µg/ml.

CONCLUSION

The present study was clearly indicated ethanolic extract of herbal drug 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 herbal drug extract.

REFERENCES

1. Ahmad Sulaeman and MahaniMahani. TrigonaPropolis and Its Potency for Health and Healing Process, *science direct journal and books,* Vol. 5(23), 2019, pp. 1-8.
2. AmuthaIswarya Devi J and KottaiMuthu A. (*In Vitro* antioxidant activity and total phenolic content of ethanolic extract of whole plant of *Lactucaruncinata*DC). *International Conference on Medical, Biological and Pharmaceutical Sciences (ICMBPS*), Vol. 5(7), 2014, pp. 72- 89.
3. Benzie IF, and Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": the FRAP assay, *Analytical biochemistry*, Vol. 239(1), 1996, pp. 70-76.
4. Cameron GR, Mitton RF and Allan JW. Measurement of flavonoids in plant sample, *Lancet,* Vol. 7(2), 1943, pp. 177-179.
5. Cortes Jofre M, Rueda JR, Corsini Munoz G, Fonseca Cortes C, Caraballoso M and BonfillCosp X. Drugs for preventing lung cancer in healthy people, *The Cochrane Database of Systematic Reviews*, Vol. 4(5), 2012, pp. 89-93.
6. Davidson MB, Navar MD, Echeverry D. U-500 regular insulin, Clinical Diabetes, Vol. 30(2), 2012, pp. 76-86.
7. Devi PS, Reddy AG, Rao G, Kumar CS and Boobalan G. Pharmacokinetic interaction of curcumin and glibenclamide in diabetic rats, *Vet World,* Vol. 5(8), 2015, pp. 508-511.
8. Elizabeth K, and Rao MNA. Oxygen scavenging activity of curcumin, *International Journal of Pharmaceutics*, Vol. 5(8), 1990, pp.237–240.
9. Evans WC. (1997), An index of medicinal plants, *A Text book of Pharmacognosy*, Vol. 7(5), 1997, pp. 12-14.
10. Finar G. Plants of economic importance. Medicinal Plants and Medicine in Africa, *Spectrum Books Ltd. Ibadan,* Vol. 7(8), 1986, pp. 150-153.
11. Florez H, Scranton R, Farwell WR, DeFronzo RA, Ezrokhi M. Randomized clinical trial assessing the efficacy and safety of bromocriptine QR when added to ongoing thiazolidinedione therapy in patients with type 2 diabetes mellitus, *J Diabetes Metab*, Vol. 2(5), 2011, pp.142-148.
12. Garrat DC. The Quantitative analysis of Drugs, *Chapman and Hall Ltd.,* Vol. 6(3), 1964, pp. 456-458.
13. Harborne JB. Phytochemical methods, London, *Chapman and Hall, Ltd.,* Vol. 8(6), 1973, pp. 49-188.
14. JaffnaaBanu M, Elavarasan G, AmuthaIswarya Devi J and KottaiMuthu A. (*In vitro* antioxidant activity of various extracts of aerial parts of *Premnatomentosa), InternationalJournal of Chemical and Pharmaceutical Sciences,* Vol. 6(2), 2015, pp. 578-584.

15. Jeyaraman Amutha Iswarya Devi, Velayutham Mani Mala and Narayanan Venkateshan. Evaluation of *invitro*antioxidant activities of various extract from leaf *cordia obliqua* willd, *World Journal of Pharmacy and Pharmaceutical Sciences*. Vol. 9(3), 2020, pp. 1-11.
16. Kalaskar MG, Shah DR, Raja NM, Surana SJ, and Gond NY. Pharmacognostic and Phytochemical Investigation of Ficuscarica Linn, *Ethnobotanical Leaflets*, Vol. 6(5), 2010, pp. 599-609.
17. Mace Gorbach SL. Anaerobic bacteriology for clinical laboraties, *Pharmacognosy,* Vol. 2 (3), 1963, pp. 89-91.
18. Madhavan V, Basnett H, Gurudeva MR and Yoganarasimhan SN. Pharmacognostical evaluation of *DroseraburmanniiVahl* (Droseraceae), *Indian journal of traditional knowledge,* Vol. 8(3), 2009, pp.326-333.
19. Malick CP and Singh MB. Plant enzymology and histo enzymology, *New Delhi: Kalyani Publishers,* Vol. 5(7), 1980, pp. 283-286.
20. Mensor LL, Menezes FS, Leitão GG, Reis AS, Santos TCD, Coube CS and Leitão SG. Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method, *Phytotherapy research*, Vol. 15(2), 2001, pp. 127-130.
21. Nakagami T, Yamamoto Y, Fukushima S, Oya J, Iwamoto Y. Assessment of cholesterol absorption and synthesis in japanese patients with type-2 diabetes and lipid-lowering effect of ezetimibe, *J Diabetes Metab*, Vol. 2(3), 2011, pp. 132- 139.
22. Narayanan V, Raju R, Lavakumar V, Jeyaraman A.I.D and Jeyakumar A. (Phytochemical and *in vitro* antioxidant activity of various bark extracts of*SyzygiumCumini* Linn), *Orient J Chem*, Vol. 33(5), 2017, pp. 67- 74.
23. Natarajan suresh, Shubhranshushekhar, AmuthaIswarya Devi J, Satheesh Kumar D and KottaiMuthu A. (Evaluation of *In-vitro* antioxidant potential of whole plant of various extracts of *Trianthemadecandra*), *American Journal of Phytomedicine and Clinical Therapeutics*, vol. 1(5), 2013, pp. 490-496.
24. Nichol A, Chandra Sekar M. Successful management of extremely insulin-resistant obese diabetic patient with insulin glargine, U-500 regular insulin and pramlintide, *J Diabetes Metab*, Vol. 2(3), 2011, pp. 139-143.
25. Parmar C and Kaushal MK. (Cordia obliqua Willd Fruits), *Sub-Himalayan Region*, Vol. 7(5), 1982, pp.129-136.
26. Poonam T, Prakash GP and Kumar LV. Influence of *Allium sativum* extract on the hypoglycemic activity of glibenclamide: An approach to possible herb-drug interaction, *Drug Metabol Drug Interact*, Vol. 2(8), 2013, pp. 225-230.
27. Ribeiro C, De AlencarMota C S, Voltarelli F A, De Araujo M B, Botezelli J D. Effects of moderate intensity physical training in neonatal alloxan- administered rats, *J Diabetes Metab*, Vol. 1(2), 2010, pp.102-107.
28. Riddle M, Frias J, Zhang B, Maier H, Brown C. Pramlintide improved glycemic control and reduced weight in patients with type 2 diabetes using basal insulin, *Diabetes Care*, Vol. 30(11), 2007, pp. 2794-2799.
29. Saini A, Devidayal, Verma S, Bhalla A K. Comparative efficacy of once daily insulin glargine with twice daily NPH insulin in children with type 1 diabetes, *J Diabetes Metab*, Vol. 2(3), 2011, pp. 124-128.
30. Samadi N, Safavi M, Mahmoodi M. Impact of quality of life education on self-concept among type 2 diabetes patients, *J Diabetes Metab*, Vol. 2(6), 2011, pp. 127-132.
31. Samala S and C Veeresham. Enhanced bioavailability of glimepiride in the presence of boswellic acids in streptozotocin-induced diabetic rat model, *Nat Prod Chem Res*. Vol. 5(3), 2013, pp. 123.
32. Venkatesh S, Reddy YR, Ramesh M, Swamy MM, Mahadevan N, and Suresh B. Pharmacognostical studies on Dodonaeaviscosa leaves, *African Journal of Pharmacy and Pharmacology*, Vol. 2(4), 2008,pp. 083-088.
33. Winterbourne CC, Hawkins RE, Brain M and Carrel RW. The estimation of red cell superoxide dismutase activity, *J Lab chem Med*., Vol. 85(2), 1975, pp. 337-341.

Table No. 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	12.65 ± 0.018	15.30±0.022	35.68 ± 0.044	18.85 ± 0.076
2	250	29.43 ± 0.025	28.46±0.016	46.34 ± 0.052	22.08 ± 0.054
3	500	37.86 ± 0.036	41.74±0.038	50.72 ± 0.048	52.21 ± 0.022
4	1000	48.92 ± 0.008	56.98±0.012	64.86 ± 0.035	69.83 ± 0.014
IC_{50}		**1030 µg/ml**	**780µg/ml**	**490 µg/ml**	**480 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	27.53±0.026	35.98 ± 0.030	39.28 ± 0.044	73.81 ± 0.006
2	250	36.41 ± 0.042	52.36 ± 0.028	53.51 ± 0.034	91.31 ± 0.011
3	500	45.32 ± 0.022	64.65 ± 0.038	66.42 ± 0.030	92.99 ± 0.024
4	1000	53.76 ± 0.018	74.96 ±0.014	79.08 ± 0.016	98.01 ± 0.012
IC_{50}		**910 µg/ml**	**280 µg/ml**	**175 µg/ml**	**60 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	21.54 ± 0.024	28.40 ± 0.030	32.65 ± 0.026	58.68 ± 0.007
2	250	29.76 ± 0.044	41.96 ± 0.028	50.42 ± 0.033	65.87 ± 0.018
3	500	38.92 ± 0.038	51.38 ± 0.022	59.34 ± 0.042	83.83 ± 0.012
4	1000	55.28 ± 0.018	60.84 ± 0.012	66.87 ± 0.016	97.90 ± 0.019
IC_{50}		**850 µg/ml**	**480 µg/ml**	**245 µg/ml**	**65 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	20.56 ± 0.036	24.68 ± 0.022	27.75 ± 0.040	26.87 ± 0.076
2	250	29.48 ± 0.042	38.25 ± 0.038	40.66 ± 0.026	30.30 ± 0.054
3	500	38.94 ± 0.028	47.96 ± 0.044	52.06 ± 0.036	55.23 ±0.014
4	1000	54.82 ± 0.020	59.88 ± 0.016	60.34 ± 0.024	60.64 ± 0.022
IC_{50}		**920 µg/ml**	**510 µg/ml**	**475 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	22.04 ±0 .042	28.24 ± 0.026	38.35 ± 0.032	26.87 ± 0.076
2	250	29.10 ± 0.038	38.56 ± 0.040	47.98 ± 0.026	30.30 ± 0.054
3	500	36.25 ± 0.025	47.23 ± 0.034	59.08 ± 0.030	55.23 ±0.014
4	1000	53.46 ± 0.030	56.42 ± 0.018	62.74 ± 0.022	60.64 ± 0.022
IC_{50}		**870 µg/ml**	**660 µg/ml**	**450 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	20.52 ± 0.042	23.12 ± 0.032	30.14 ± 0.024	26.87 ± 0.076
2	250	28.34 ± 0.025	36.41 ± 0.040	44.36 ± 0.032	30.30 ± 0.054
3	500	35.68 ± 0.033	47.64 ± 0.024	54.18 ± 0.026	55.23 ±0.014
4	1000	51.40 ± 0.020	56.18 ± 0.016	62.98 ± 0.018	60.64 ± 0.022
IC_{50}		**920 µg/ml**	**750 µg/ml**	**460 µg/ml**	**410 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 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	24.68 ± 0.028	31.64 ± 0.034	36.14 ± 0.042	72.04 ± 0.014
2	250	36.46 ± 0.032	38.62 ± 0.018	48.95 ± 0.024	82.05 ± 0.034
3	500	45.82 ± 0.022	51.48 ± 0.033	60.28 ± 0.040	86.04 ± 0.026
4	1000	53.20 ± 0.018	60.45 ± 0.016	71.98 ± 0.020	98.07 ± 0.041
IC_{50}		**910 µg/ml**	**480 µg/ml**	**245 µg/ml**	**50 µg/ml**

*All the values are expressed as mean ± SEM for three determinations

Table No. 8: Total phenol

S.No	Extracts	Total phenolic content (mg/g of Catechol)(±SEM)*
1	Pet. ether extract of combination of herbal drug	2.139 ± 0.032
2	Ethyl acetate extract of combination of herbal drug	4.956 ± 0.044
3	Ethanolic extract of combination of herbal drug	8.320 ± 0.028

*All the values are expressed as mean ± SEM for three determinations.

Table No. 9: Total flavonoids

S. No	Extracts	Total flavonoids content (mg/g) (±SEM)*
1	Pet. ether extract of combination of herbal drug	0.018 ± 0.004
2	Ethyl acetate extract of combination of herbal drug	0.298 ± 0.009
3	Ethanolic extract of combination of herbal drug	2.354 ± 0.015

*All the values are expressed as mean ± SEM for three determinations

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Blending Nature of Edible Oils at 180°C through Chemical Parameters and the Evaluation of the Chemical Compounds through FT-IR and DPPH Free Radical Scavenging.

Selvaraj Rama Jeba and K.Karthikeyan*

Department of Food Science and Nutrition, Periyar University, Salem, Tamil Nadu, India.

Received: 01 Dec 2020 Revised: 26 Dec 2020 Accepted: 12 Jan 2021

***Address for Correspondence**
K.Karthikeyan,
Assistant Professor,
Department of Food Science and Nutrition,
Periyar University, Salem, Tamil Nadu, India.
Email : ramajeba06@gmail.com

ABSTRACT

Profound processing subject's edible oils to high temperature intervals that are too long and oxygen induces their rapid degradation. The compounds that are most suspected of impairing the nutritional properties of the oils or inducing physiological adverse effects are oxidation products found in exploited frying fats and oils. Hot temperatures and diverse substances allow complex interactions to take place during the cooking cycle and numerous compounds form. Complex reactions involve not only the constituents of cooking oil, such as TAGs and other minor compounds, but also the constituents of fried food, such as air, lipids, sugars and proteins. The analysis therefore shows that the technique of spectroscopic recognition and the percentage of oxidation in mixing oils and their free radical scavenging actions by DPPH show the chemical alterations of the heating oil at 180 ° C in unique mixing ratios with groundnut, mustard and palm oils.

Keywords: Cooking oil, Chemical alterations, FT-IR spectroscopic, Temperature, Oil oxidation

INTRODUCTION

Long-chain oxidation of unsaturated fats triggers neuromyopathic inflammation, both in newborns and adults [1]. Oxidation of marine lipids results in a loss of nutritional value and enhancement of unsavory flavor. Lipid oxidation increases the accessibility period of various intricate food products and nutritional sustenance estimation by restricting the content of simple polyunsaturated unsaturated fats [2]. Deep heating, which involves the immersion of foodstuff in fat at higher level up heating process ranging from 150 to 190 ° C, is one of the greatest common cooking approaches for achieving desirable product attributes [3]. The oil undergoes a series of reactions during the heating cycle that can create toxic or carcinogenic compounds. Initially, the development of peroxides and conjugated diene or conjugated triene is due to the main oxidation free fatty acids. ketones, aldehydes, cyclic

compounds and alcohol are subsequently produced as secondary oxidation products [4]. Of all the frying oils, those with a high content of oleic acids likepalm and sunflower oils have improved wellbeing profiles and constancy. Reasonable statistics on the output of a whole new source of deep-frying oil with prevailing vegetable oils is relevant in the global context [5]. Consequently, the present work was carried out during deep-frying processes for comparative evaluation of algal oil with the most widely used vegetable oils (from palm and sunflower). The effect of deep-frying on the characteristics of algae, palm and sunflower oils such as viscosity, density, acid value, total polar compounds, refractive index, peroxide value, oil absorption, acidity, color, free fatty acid percentage, fatty acid profile and radical scavenging activity was evaluated. Based on previous studies [6], for the textural and sensory perceptions assessment of deep-frying oil, potato sticks were used as a food matrix. The use of vegetable oil has been one of the oldest and most common methods of preparing food for deep frying. The attractive taste of fried foods, golden brown color, and crispy texture make deep-fried foods a very common trade. The rate of oxidation depends on the level of unsaturation and increases, with the double responsibility of unsaturated fats being increased [7]. Frying is a method of immersing food in hot oil, with contact between heat, air, and food at a temperature between 180^0C and 260^0C. Food surface area, Frying time, food moisture content, and form of cooking oil affect oil self-oxidation. Chemical processes are usually observed during the deep-frying cycle deterioration of fatty acids, and these generate volatile or non-volatile compounds.

Throughout cooking, most volatile compounds evaporate, and the remaining volatile compounds in oil undergo more chemical oxidation reactions or are consumed in fried foods. The non-volatile compounds in the oil change the oil's chemical and physical attributes, as well as processed food consistency. The Peroxide value (PV) is used as an indicator of the degree to which rancidity reactions during deep-frying have happened. This major oxidation material, such as fatty acid compounds with hydroperoxide, is also analyzed using the FT-IR spectroscopy process. At the point where air reacts with unsaturated lipids, lipid peroxidation offers a broad assortment of oxidation items [6]. The primary autoxidative drug peroxides are extremely unstable and are transformed into secondary products spontaneously. Cleavage off the carbon bonds adjacent to the hydroperoxy group results in fatty acids such as linoleic acid and linolenic acid. Hydrocarbons and short-chain aldehydes are the main chain-cleavage products formed from hydroperoxides. This converted lipids of groundnut, Palm, mustard and sunflower oils into simple hydrocarbon and aldehydes were studied comparatively by analyzing different parameters. To ensure the safety and quality of deep-fried foods it is essential to investigate and track the deterioration of oils during the deep-frying cycle. Free radicals and oxidants allow oils and fat to undergo peroxidation and macromolecules such as proteins and DNA oxidation also causes significant damage to body cells [8]. Radicals are chemical species containing one or more unpaired electrons, and free radicals are a radical that instantly springs from the molecular system from which they are produced [9].

METHODS AND MATERIALS

Sample preparation for Heating

Oil : Mostly available and common in India is unrefined Groundnut oil and mustard oil was used to experiment. The oil was collected from the Local oil mill near Salem, India.

Heating materials: A 3-liter size deep-fat domestic fryer pan was used. Fryer's full capacity oil was collected and heated continuously for 3 hours a day at 180 ° C, and the sample was taken for analysis. The cycle was continued to heat oil for up to 3 days i.e. a total of 9 hours. Till the end of the 9th hour, no new fresh oil is applied.

Experimental Analysis of Oil Exposed To Heated At 180°C

Acid value (AV): The acid value [Cd 38-63] was calculated by the number of milligrams of potassium hydroxide needed in 1 gram of the test sample to neutralize the free acids [10].

Iodine number (IV) : odine value (IV) was calculated by reacting to the experiment with the iodine monochloride (ICl) excess solution in glacial acetic acid. Unreacted iodine monochloride interacted with potassium iodide and converted to iodine, the intensity of which was estimated by sodium thiosulphate titration [11].

Peroxide value : The peroxide value (PV) was measured using the sodium thiosulfate solution as a titrating agent towards the developed iodine in the sample, after reaction of the iodine salt (KI) peroxides in the sample [12].

***p*- Anisidine value** : The sample's p-anisidine value was calculated using isooctane as the substance below 350 nm of wavelength [12].

Total oxidation (Totox): The anisidine value is used to measure total oxidation in combination with peroxide value. Calculated total oxidation as TOTOX= 2PV+ p-AV [13].

Saponification number (SV): The sample was treated with alkali and the unreacted paraffins were titrated against 0.5 N hydrochloric acid for the saponification value (SV) [14].

Viscosity: One part of fluid was experiencing viscosity passing over another part of the fluid. viscosity [Tq 1a-64] was determined by using standard AOCS methods [15].

Density: Density [Cc 10c-95] was determined by using standard AOCS methods [15].

FTIR : The test was performed on a 4000-400 cm-1 Bruker-Tencer-37 FTIR-ATR spectrophotometer. Small amounts of oil extracts in a substrate were used for FT-IR spectra imaging. Total wavelengths of new groundnut and pure mustard oil were reported from 4000 to 400 cm-1 and extracted with OPUS computer software for three consecutive days of reheating. Chemically oils are glycerol esterifying compounds of fatty acids. The value if IR spectroscopy is to classify and receive the molecular structure information as various compounds are allocated to some band of absorption linked to the same structural group. The sample measurements were replicated three times [16].

Free radical scavenging assay through DPPH (2, 2′-diphenyl-1 - picrylhydrazyl)

Radical oil scavenging behavior, utilizing DPPH radical scavenging techniques, was evaluated and compared to that of Trolox. Depending on the process, the ability to scavenge free radicals from crude oil was evaluated [17].Using standard Trolox (0–300 μM), a calibration curve was prepared and within the specified range was dimensional. Radical scavenging process per ml of oils was described as equal to μMTrolox. The resistance percentage (antioxidant capacity) is determined by calculating the absorption coefficient at 517 nm, using the following formula, Inhibition (%) = $\left[\frac{A\ control\ -A\ sample}{A}control\right] \times 100$Where, A Monitoring is the process absorbance and A Specimen is the sample absorbance at 517 nm, respectively. The concentration of the sample needed to scavenge 50 percent DPPH revolutionaries is denoted by IC50 values. Antioxidant activity is done by comparing inhibition activity against different concentrations of oil [18].

Statistical analysis

For the experimental results, ANOVA was implemented. For research, the Microsoft Office Excels programme was used. For each study, three replications were performed.

RESULTS AND DISCUSSION

The quality of Groundnut oil and Mustard oil blended with palm oil was determined through the chemical properties like peroxide, iodine, *p*–anisidine, iodine, acid values with their density/viscosity flow nature have been studied in both heated (180 degree celsius) and unheated oils with the different blending nature. Additionally, the effect of temperature on these materials and the effect of warming many times up to their different breaking points using different oil were contemplated. Table.1. shows the effect of temperature on these structures and the impact of prolonged heating up to the boiling point has also been investigated.

Acid value

The acidity value is an indicator of the oils free fatty acid content. The blending ratios of all three-oil show in Fig.1. A) Mustard+Palm oil and B) Groundnut+palm oil are similar in reducing through heat point. Since palm oil is unrefined, its AV, respectively, is higher than peanut oil and rapeseed oil [19].

Iodine number

Iodine level is an unsaturation indicator in vegetable oilorfat. It regulates the consistency of oils to oxidation and permits for subjective resolution of the specific unsaturation of the fat. This poor value of iodine may have subsidized to its more noticeable oxidative validity. The oxidative and compound variations in oils within ability are represented by an extension in free unsaturated fat and a reduction in oils total unsaturation [20].The percentage of decreases in proportions of blending in Fig.2. a) and the proportion of blending is increases in Fig.2. b) is shown in Fig.2.a) Mustard+Palm oil and b) Groundnut+palm oil

Peroxide value

The unrefined vegetable oils, compared with refined oils, are distinguished by higher PV values. Peroxide (PV) confidence is used as a ratio of the degree to which rancidity behaviors happened in the early capacity as a measure of fat and oils consistency and reliability. Additionally, the peroxide confidence has been found to rise with the ability temperature, time and interaction with an oil test attitude. There is a gradual reduction in PV when a fat has been used in an alternative process for heating. The degree to which the oil has experienced rancidity is determined by peroxide value esteem [21]. The oil with a peroxide value between 1 and 5 mEq / Kg is small in oxidation and is at a normal oxidation level between 5 and 10 mEq / Kg [22]. The variations in peroxide value of the separate mixing Ratios of groundnut and mustard with palm native oil are seen in Fig.3. (a and b) during continuous heating at 180 degrees with 3 different cycles. The results suggest that in mustard with palm oil the highest increase in PV was detected than in groundnut with palm oil shown in figure. This may be attributed to the existence of natural antioxidants such as tocopherols in the mixture of oil and fatty acids.

p- Anisidine value

The shifts in the *p*-Anisidine value of all oils during the deep-heating period of three consecutive cycles are shown in Fig. 4. (a and b). A change in *p*-AV of all heating oils was identified as the deep-heating time was extended. This result may be attributed to the fact that less stable primary oxidized chemical (hydroperoxide) further liquified to form compound aldehyde. Molecules of aldehydes are secondary oxidized compounds that developed at heating temperatures. The value of *p*-anisidene, hence the aldehyde standard, expanded with an increasing number of heaters for all three oils with similar and proportional blending oils [23

ToTox value

The Totox value is a function of total oxidation, both primary oxidation, and secondary products. It is a combination of PV and p-. Totox value tests both hydroperoxides and their component breakdowns and offers a clearer indication of the gradual oxidative degradation of fat/oils. The result obtained from the determination of TV is shown in Fig. 5. (a and b). On the third day of heating, the television was increased for all the heating oils. As illustrated in Fig. 5 (b),

Palm oil and mustard oil showed the highest and lowest TV respectively, showing that palm oil displayed the lowest oxidative stability and that mustard oil displayed the highest oxidative stability relative to other oils. On blending the mustard and palm oil have the lowest TV. This observation could be explained by the high and low concentration of poly and monounsaturated fatty acids and the presence of natural antioxidants in oils [24].

Saponification value

The saponification value is an oxidation indicator during storage and shows oil degradation. From Fig.6. (a and b) the lower estimate of saponification esteems suggests a lower mean subatomic load of unsaturated fats or a lower volume of ester securities. This can mean that the fat particles have not connected [25]. The changes in mass are calculated depending on the Blending efficiency.

Viscosity

Oils are triglyceride (TG) blends, and their thickness is based on the concept of the TGs found in the liquid. Due to the complex course of action of unsaturated fats on the triglyceride particle's glycerol spine, the consistency shifted. For example, chain length and immersion/unsaturation[26], consistency is associated with the substance properties of the oils in this way. It clarifies that with an expansion in unsaturation the thickness and thickness decrease and rises with higher saturation and polymerisation. Thickness also relies on absolute temperature and heat. Absolute pressure does not affect the efficiency of the oils that are used for consumable purposes, but it is influenced by temperature. Outcomes arranged in Fig. 7 (a and b); It was discovered that the expansion of viscosities was observed during the third cycle while the reduction was observed at the diverse temperature during the first cycle. Mustard oil and palm oil of various amounts are more various experts because the quality of oil relies on the sub-atomic structure and decreases and unsaturated fats. Around the point where the temperature rises, the motor vitality further expands and has improved the particle production and decreases the intermolecular forces. The fluid layers effectively neglect each other and contribute to the decrease in thickness along these lines[27].

Density

With the elevation in temperature, the densities of the two oils were reduced just as with the use of common oil for warmth at individual temperatures. The oil densities are shown in Fig.8. (a and b) were reported with the framework of the first-day cycle of 0.937 g/mL, and the second, third-day period and the density figures are 0.917 g/mL and 0.912 g/mL at 180°C for separately. Browning of oils routinely undergoes large degradation and complex changes in compounds when warmed. The proximity of waterandair has intensified the crumbling of searing of fat/oils and contributed to anrise in the amount of polar atoms as indicated by the width of oils [28].

FT-IR

FT-IR spectroscopy, as the group factors within the wavelength are proportional to intensity, is a wonderful investigative device. Mid-IR spectra, as demonstrated by the presence and creation of the reference, were used to reflect edible fats and oils as they compare with the intensity and precision of the recurrence at which the highest band absorption or transmission exists. The oil's carbonyl functional group in the FTIR spectra appeared at the 1700–1600 cm^{-1} frequency region. In 1730–1680 cm^{-1} [29] the aldehydic C= O exhibited a stretched band, shown in Fig. 9 (a) and (b) close to and capable of overlapping with the absorption band of the ester carbonyl functional palm oil group in 1748–1746 cm^{-1}, especially because, under oxidative conditions, the frequency of this band may decrease to 1743 cm^{-1} as shown in Fig. 9 (c), depending on the absorption of carbonyl by saturated aldehydes[30].

The oil structure affects the band's precise locations and gives rise to a shift when the amount of unsaturated fats has changed [31], as well as aldehyde generation during oils auto-oxidation, which reduces the band frequency from 1746 cm^{-1} to below 1744 cm^{-1} Fig.10. (a and b) indicates the mixture of 25+75% of groundnut+palm and mustard+palm heated at 180°C, the band shows about 3467 cm^{-1} widening hydroperoxide intensity to OAH, 3009.71-3007.78 cm^{-1} as CAH expanding cis-twofold bond motion' CAH and 2924.85-2924.81 cm^{-1} shows aliphatic CH2 CAH

deviating and discreet stretching vibration. Aldehydes also exhibited weak absorption bands in the transmittance spectra 2700-2800 cm^{-1}, suggesting the presence of C-H stretching in aldehydes seen in Fig.11. (a and b), showing the blending of 50+50% of groundnut+palm and mustard+palm heated at 180°C **Fig.11.** Forecast of Blended oil Functional properties through Spectrum. (a) Groundnut+ Palmoil (50:50%) at 180°C temperature, (b) Mustard + Palmoil (50:50%) at 180°C temperature. The band about 3467 cm-1 doled out to OAH expanding hydroperoxide frequency, 3009.71–3007.78 cm-1 as CAH extending cis-twofold bond motion , CAH and 2924.85–2924.81 cm-1 shows CAH deviating and discrete extending vibration of aliphatic CH2. In the frequency region 2700–2800 cm–1, aldehydes also showed weak absorption bands which indicate the presence of C-H stretching in aldehydes shown in Fig. 11 (a,b). For consecutive days of heating at 180 ° C, FTIR spectra of palm, groundnut, and mustard oil modelspresented that there is a significant alteration in bandage between the blending of oil in equal ratios of 50 + 50 percent. As the proportion of the fatty acids varies, the oil configuration influences the particular location of the group of band and the yield of a move. The distribution of all oils displays hydroperoxide expanding vibration at a band of 3473 cm-1 allocated to O-H. Such mixes obtained with rehashed use of the browning oil as the hydroperoxides often decline, which may be due to hydroperoxide decline and ancillary oxidation inception. The fatty acids comprising the carbonyl group in vegetable oil are at the end of the mixture. In the frequency region 1700-1600 cm^{-1}, which belongs to C=O functional classes, all forms of edible oil display FT-IR spectrum. A sustained band of 1730-1680 cm^{-1} is shown in Fig.12. (a and b) in aldehydic C=O in auxiliary oxidative products formed at high temperature on fat dissolution, showing the blending of 75+25 percent of groundnut+palm and mustard+palm heated at 180°C. This perception illustrations that there is the proximity of a soaked aldehyde practical collection or additional optional oxidation compounds which makes a useful collection of triglycerides an absorbance cover with the expanding vibrations at 1745 cm^{-1} of the ester carbonyl. Different cases were seen at the point of breakage. As the ghastly local experiences, a few shifts in the oxidation progressions at room temperature and changes in temperatures, oils display a few areas of various disfigurations and twisting at 1461cm^{-1} of ACAH bowing vibrations of the aliphatic collections CH2 and CH3 and at 1375,15–1377,08$cm^{-1}$of ACAH bowing vibrations[32]. It is suspected that mustard oil is less deteriorated and creates less hydroperoxide three times after prolonged deep heating than other oils because it contains less polyunsaturated fatty acid concentrations. Palm oil, since it contains more accumulated polyunsaturated fatty acids, is depleted to a maximum of three times during deep heating than other oils. The blending of oils gives the better results when compare with other plain normal oils specially 50+50% (palm+groundnut) proportional ratio blends give the prolonged stability of oxidation when compare with others. Secondly 25+75 of palm+mustard gives increase in oxidative stability compare with 50+50%.

DPPH Assay

Free radicals created mostly during the heating process contribute to free radical chain oil degradation process. This free radical chain reaction describes aextensively recognized lipid peroxidation mechanism. To break the peroxidation chain response and strengthen the consistency and reliability of the oils throughout cooking, radical scavenging activity of oils can directly respond with and satiate peroxide radicals. DPPH radical assay is the most widely used method for evaluating radical scavenging behavior. In this process, DPPH is scavenged by oil via hydrogen donation which stabilizes the fragment of DPPH. The DPPH radical particle has a 517 nm absorbance which disappears after hydrogen radical acceptance from oil. As the heating cycle progressed shown in Fig. 13, the radical scavenging behavior of all oil samples had been increased. A similar trend was reported in the increase of radical scavenging reaction due to the heating procedure [33]. Significant improvement was experiential in all oils/fat under analysis in the radical scavenging behavior of the oils. A drastic shift in palm oil radical scavenging activity was detected from 307.70 to 43.64 μM / ml after the third heating cycle with the correlation coefficient of $p < 0.05$ in percent of DPPH scavenging activity as shown in Table. 2. The concentration and modification of the oils with the proportions (25+ 75, 50+ 50, 75+ 25) with their DPPH radical scavenging of Groundnut + Palm oil and Mustard + Palm oil shown in Fig. 14.

CONCLUSION

The outcome of this experiment suggested that the highest change in PV was observed in groundnut oil during the temperature level of up to 180 degrees of groundnut and mustard oil with the blending process of palm oil, while the lowest change in PV was observed in mustard oil. The significant increase in groundnut oil was observed, although the least significant change occurs in the mustard oil. Palm oil and mustard oil showed the highest and lowest Screen, respectively. This may be due to the presence of a high concentration of oxidized polyunsaturated fatty acids in palm oils while more monounsaturated fatty acids are found in mustard and groundnut oils. Groundnut oil with palm oil displays the lowest oxidative stability and palm oil mustard oil shows the highest oxidative stability during deep heating as opposed to other oils and their blends. The results of this review recommended that continued/repeated oil heating would decrease the protective impacts on well-being. As in groundnut fats/oil at the breaking fact, there is an extra crest at 3467.77 cm^{-1} which shows the framing of the optional oxidised object. The current investigation might be sent in numerous perspectives not exclusively to upgrade the nature of oil yet additionally give open mindfulness not to open eatable oils to high temperatures for extensive stretches ordinarily.
The results of this present research imply that antioxidant action has significant durability in each type of oil, which is influenced by the different antioxidant functions in them. Antioxidant efficacy is tested using ABTS and DPPH radical scavenging assay in mustard, groundnut, and Palm oil. From the measured concentration of inhibition for unheated and frequently heated oils, antioxidants (sesame lignans, tocopherol, etc.) performance and durability of mustard oils are expected to be stable in comparison with groundnut and palm oils. The free radical scavenging behavior in the Palm oil is relatively higher than other fat/oils, and it may be suggested for less adverse effect deep heating.

Conflict of interest statement

The author declared that no conflict of interest

REFERENCES

1. W. Wajner, and A. U. Amaral, Mitochondrial dysfunction in fatty acid oxidation disorders: insights from human and animal studies. *Bioscience reports*, 36 (1), e00281, 2016.
2. S. H. Hernandez-Lopez, J. G. Rodriguez-Carpena, C. Lemus-Flores, J. Galindo-Garcia, and M. Estevez, Antioxidant protection of proteins and lipids in processed pork loin chops through feed supplementation with avocado. *Journal of food science and technology*, 53(6), 2788 - 2796, 2016.
3. L. Yu, J. Li, S. Ding, F. Hang, and L. Fan, Effect of guar gum with glycerol coating on the properties and oil absorption of fried potato chips, *Food Hydrocolloids*, 54, 211 - 219, 2016.
4. B. Kowalski, Determination of oxidative stability of edible vegetable oils by pressure differential scanning calorimetry, *ThermochimicaActa*, 156, 347 - 358, 1989.
5. E. Tabee, M. Jagerstad, P. C. Dutta, Frying quality characteristics of french fries prepared in refined olive oil and palm olein, *Journal of the American Oil Chemists Society*, 86, 885 - 893, 2009.
6. E. Binkoski, P. M. Kris-Etherton, T. A. Wilson, M. L. Mountain, and R. J. Nicolosi, Balance of Unsaturated Fatty Acids Is Important to a Cholesterol-Lowering Diet: Comparison of Mid-Oleic Sunflower Oil and Olive Oil on Cardiovascular Disease Risk Factors. *Journal of the American Dietetic Association*, 105(7), 1080 - 1086, 2005.
7. J. Kerr, T. A. Kellner, and G. C. Shurson, Characteristics of lipids and their feeding value in swine diets. *Journal of animal science and biotechnology*, *6*(1), 30, 2015.
8. S. RubalyaValantina, and P. Neelamegam, Antioxidant potential in vegetable oil. *Research Journal of Chemistry and Environment*, 16(2), 87- 94, 2012b.
9. B. Aydenız, and E. Yilmaz, Performance of Different Natural Antioxidant Compound in Frying Oil. *Food technology and biotechnology*, 54(1), 21 - 30, 2016.

10. T. D. Crowe, and P. J. White, Adaptation of the AOCS official method for measuring hydroperoxides from small-scale oil samples. *Journal of the American Oil Chemists Society*, 78(12), 1267 - 1269, 2001.
11. SON, Standard Organization of Nigeria. *Standards for Edible Refined Palm Oil and Its Processed form*, pp. 2 - 5, 2000.
12. G. Barthel, and W. Grosch, Peroxide value determination: comparison of some methods. *Journal of the American Oil Chemists Society*, 51, 540 - 544, 1974.
13. F. Shahidi, and U. N. Wanasundara, Methods for measuring oxidative rancidity in fats and oils. *Food Lipids: Chemistry, nutrition, and biotechnology*, 17, 387 - 403, 2002.
14. H. K. Sharma, B. Kaur, B. Sarkar, and C. Singh, Thermal behavior of pure rice bran oil, sunflower oil, and their model blends during deep-fat frying. *Grasas y Aceites*, 57(4), 376 - 381, 2006.
15. Codex Alimentarius Commission, Joint FAO/WHO Food Standards Programme, and the World Health Organization, *Codex Alimentarius commission: procedural manual*. Food and Agriculture Org, 2007.
16. Rohman, and Y. C. Man, Fourier transform infrared (FTIR) spectroscopy for analysis of extra virgin olive oil adulterated with palm oil. *Food research international*, *43*(3), 886 - 892, 2010.
17. S. Sahreen, M.R. Khan, and R.A. Khan, Evaluation of antioxidant activities of various solvent extracts of *CarissaOpaca* fruits, *Food chemistry,* 122, 1205 - 1211, 2010.
18. R. S. Valantina, and P. Neelamegam, Selective ABTS and DPPH-radical scavenging activity of peroxide from vegetable oils. *International food research journal*, 22(1), 289, 2015**.**
19. K.Das, R. Babylatha, A. S. Pavithra, And S. Khatoon, Thermal Degradation Of Groundnut Oil During Continuous And Intermittent Frying. *Journal Of Food Science And Technology*, 50(6), 1186 - 1192, 2013.
20. E. G. Perkin, Effect Of Lipid Oxidation On Oil And Food Quality In Deep Frying. In: A. J. S. Angels, (Ed.), Lipid Oxidation In Food, Chapter 18, ACS Symposium Series No. 500 *ACS, American Chemical Society*, Washington DC, Pp. 310 - 321, 1992.
21. X. Yu, F. R. Van De Voort, And J. Sedman, Determination Of Peroxide Value Of Edible Oils By FTIR Spectroscopy With The Use Of The Spectral Reconstitution Technique. *Talanta*, 74(2), 241 - 246, 2007.
22. L. S. Maguire, S. M. Osullivan, K. Galvin, T. P. Oconnor, And N. M. Obrien, Fatty Acid Profile, Tocopherol, Squalene And Phytosterol Content Of Walnuts, Almonds, Peanuts, Hazelnuts, And The Macadamia Nut. *International Journal Of Food Sciences And Nutrition*, 55(3), 171 - 178, 2004.
23. M. Kozlowska, And E. Gruczynska, Comparison Of The Oxidative Stability Of Soybean And Sunflower Oils Enriched With Herbal Plant Extracts. *Chemickezvesti*, 72(10), 2607 - 2615, 2018.
24. D. A. Pratt, K.A. Tallman, And N. A. Porter, Free Radical Oxidation Of Polyunsaturated Lipids: New Mechanistic Insights And The Development Of Peroxyl Radical Clocks. *Accounts Of Chemical Research*, 44(6), 458 - 467, 2011.
25. K. J. Denniston, J. J. Topping, And R. L. Cariet, In: General Organic And Biochemistry, Fourth Ed. Mcgraw Hill Companies, New York, Pp. 432 - 433, 2004.
26. W. L. Clark, And G.W. Serbia, Safety Aspects Of Frying Fats And Oils. *Food Technology (Chicago)*, 45(2), 84 - 89, 1991.
27. J. Kim, D. N. Kim, S. H. Lee, S. H. Yoo, And S. Lee, Correlation Of Fatty Acid Composition Of Vegetable Oils With Rheological Behavior And Oil Uptake, *Food Chemistry*, 118, 398 - 402, 2010.
28. P. J. White, Methods For Measuring Changes In Deep-Fat Frying Oils. *Food Technology,* 45(2), 75 - 80, 1991.
29. F. R. Van De Voort, A.A. Ismail, J. Sedman, And G. Emo, Monitoring The Oxidation Of Edible Oils By Fourier Transform Infrared Spectroscopy, *Journal Of The American Oil Chemists Society*71:243 - 253, 1994.
30. E. N. Frankel, Lipid Oxidation, *Progress In Lipid Research,* 19, 1 - 22, 1980.
31. R. J. Hamilton, The Chemistry Of Rancidity, In Rancidity Of Foods, Edited By J.C. Allen, And R.G. Hamilton, *Elsevier Applied Science*, London, Pp. 14 - 16, 1989.
32. **M. D. Guillen, And N. Cabo, Some Of The Most Significant Changesin The Fourier Transform Infrared Spectra Of Edible Oils Underoxidative Conditions. J***ournal Of The Science Of Food And Agriculture,* **80, 2028 - 2036, 2000.**
33. N. Chammem, S. Saoudi, I. Sifaoui, S. Sifi, M. De Person, M. Abderraba, F. Moussa, And M. Hamdi, Improvement Of Vegetable Oils Quality In Frying Conditions By Adding Rosemary Extract, *Industrial Crops, And Product,* 74 (2015) 592 - 599, 2015.

Table.1. Proportions of Blending of collected oils

Blends	Proportions		
	Groundnut Oil	Palm Oil (control oil)	Mustard Oil
A.	100	-	-
B.	75	25	-
C.	50	50	-
D.	25	75	-
E.	-	-	100
F.	-	25	75
G.	-	50	50
H.	-	75	25

Table 2: Correlation % of inhibition with DPPH assay ($*p < 0.005$, $**p < 0.05$, $\#p < 0.001$**)**

Oil type	Proportions	DPPH Assay (p< 0.001)	
		Heated	Unheated
Groundnut oil + Palm oil	25+75	p < 0.005	p < 0.05
	50+50	p < 0.005	p < 0.05
	75+25	p < 0.005	p < 0.05
Mustard oil + Palm oil	25+75	p < 0.005	p < 0.05
	50+50	p < 0.005	p < 0.05
	75+25	p < 0.005	p < 0.05

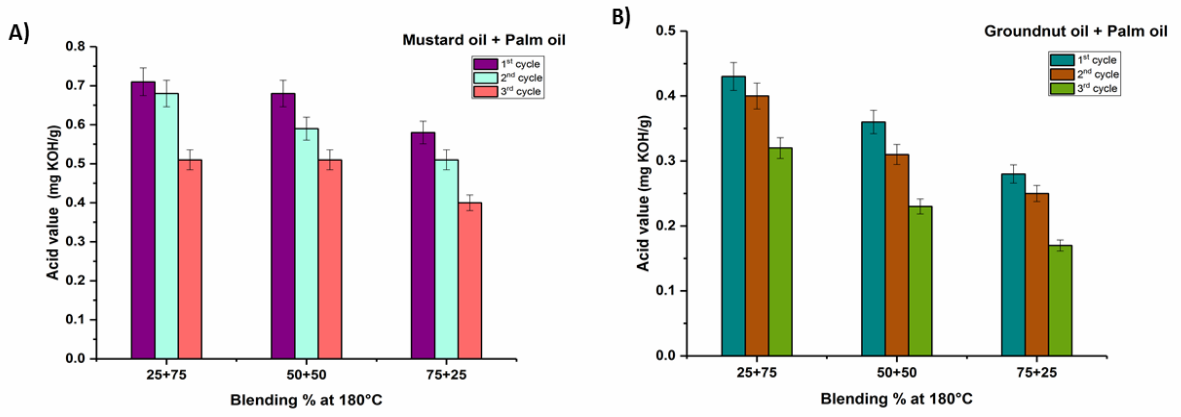


Fig.1. Changes in Acid value A) Mustard+Palm oil B) Groundnut+Palm oil with the Blending oil Proportions at 180°C

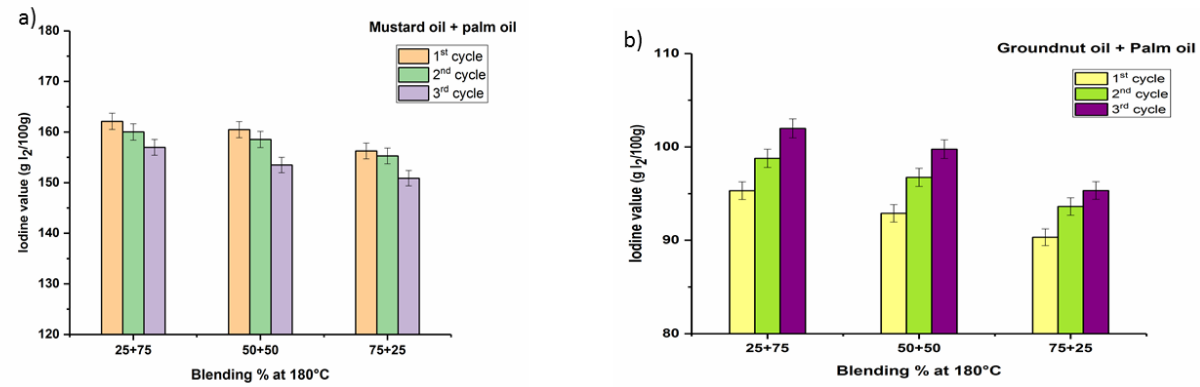


Fig.2. Changes in Iodine value a) Mustard+Palm oil b) Groundnut+Palm oil with the Blending oil Proportions at 180°C.

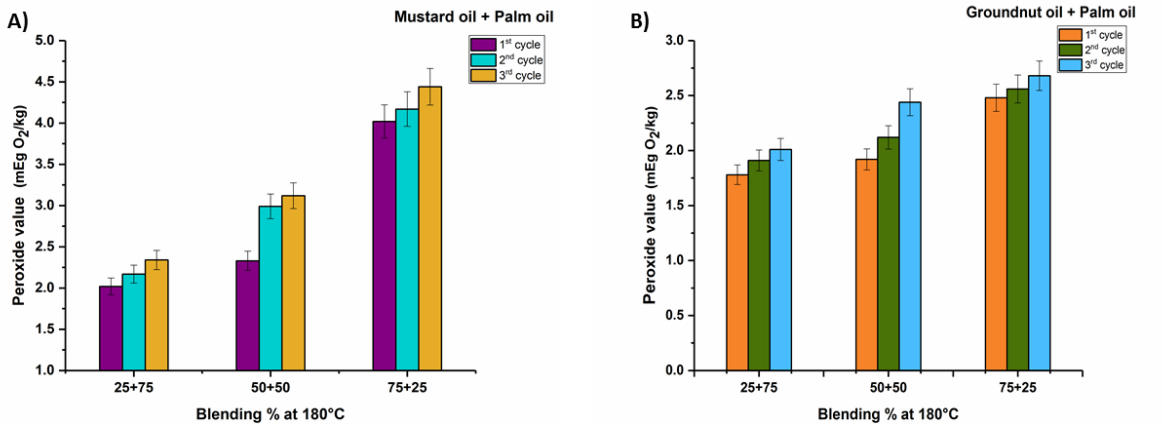


Fig.3. Changes in Peroxide value A) Mustard+Palm oil B) Groundnut+Palm oil with the Blending oil Proportions at 180°C.

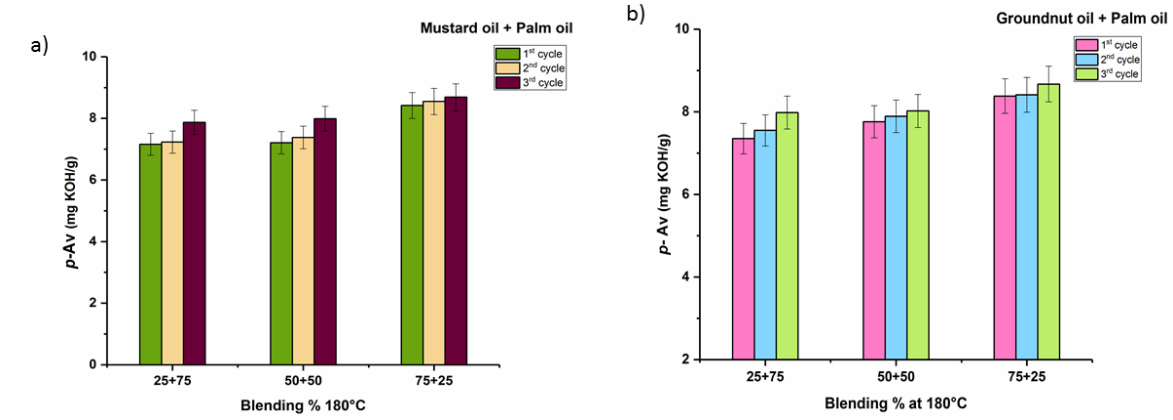


Fig.4. Changes in *p*- Anisidine value A) Mustard+Palm oil B) Groundnut+Palm oil with the Blending oil Proportions at 180°C.

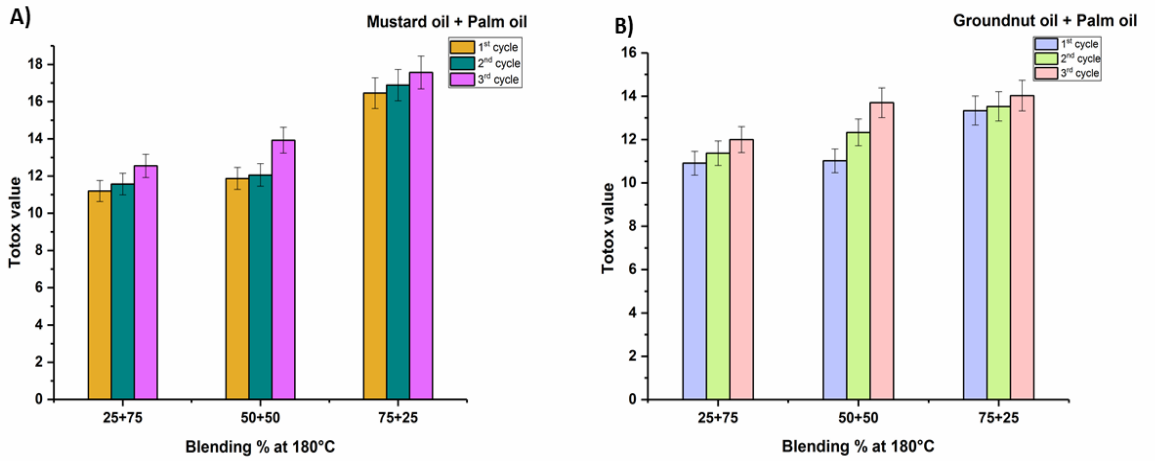


Fig.5. Changes in Totox value A) Mustard+Palm oil B) Groundnut+Palm oil with the Blending oil Proportions at 180°C.

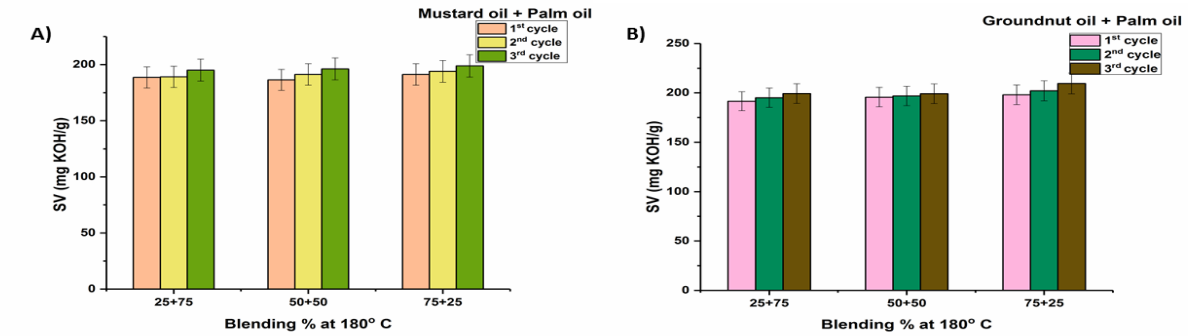


Fig.6. Changes in Saponification value a) Mustard + Palm oil b) Groundnut + Palm oil with the Blending oil Proportions at 180°C.

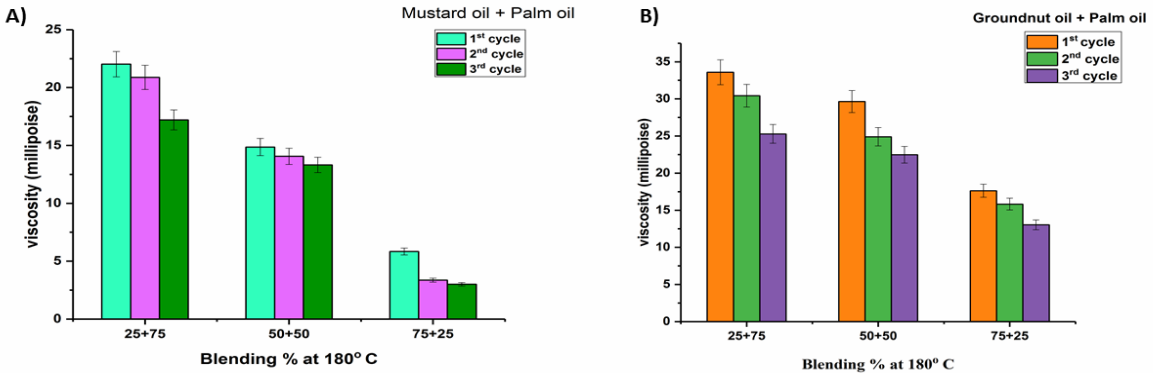


Fig.7. Changes in Viscosity value a) Mustard+Palm oil b) Groundnut+Palm oil with the Blending oil Proportions at 180°C.

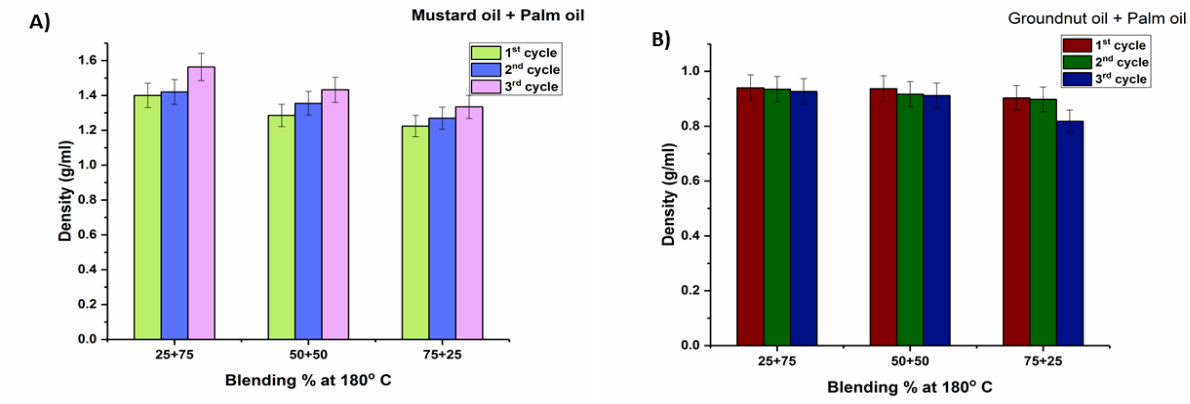


Fig.8. Changes in Density value A) Mustard+Palm oil B) Groundnut+Palm oil with the Blending oil Proportions at 180°C

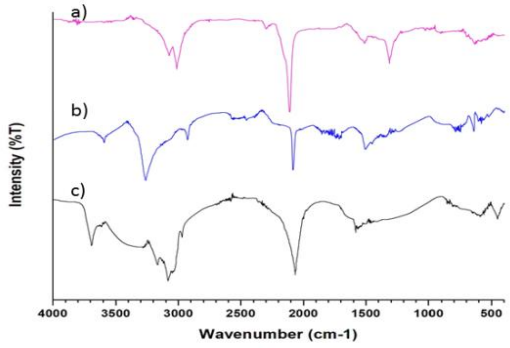


Fig.9. Forecast of initial oil Functional properties through Spectrum. (a) Groundnut oil at 180°C temperature, (b) Mustard oil at 180°C temperature, (c) Palm oil at 180°C temperature.

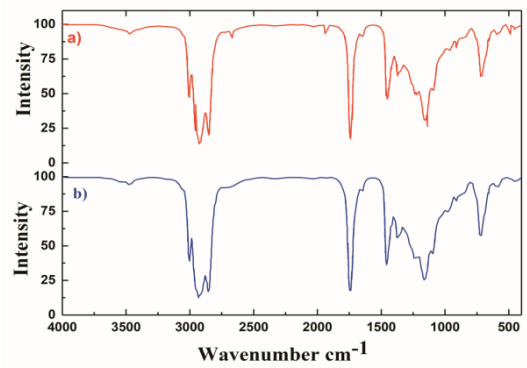


Fig.10. Forecast of Blended oil at 180°C temperature its Functional properties through Spectrum. (a) Groundnut+Palm oil (25:75%) (b) Mustard+Palm oil (25:75%) at 180°C.

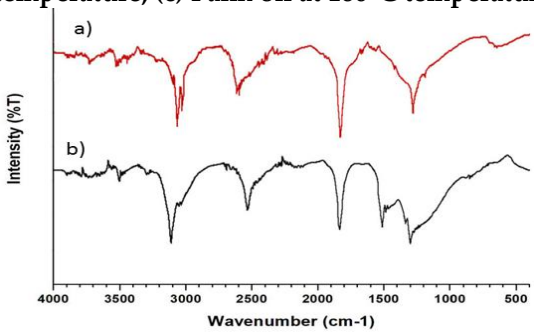


Fig.11. Forecast of Blended oil Functional properties through Spectrum. (a) Groundnut+ Palmoil (50:50%) at 180°C temperature, (b) Mustard + Palmoil (50:50%) at 180°C temperature.

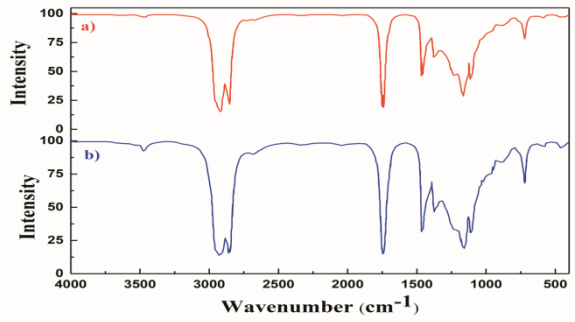


Fig.12. Forecast of Blended oil Functional properties through Spectrum. a) Groundnut+Palm oil (75:25%) at 180°C temperature, b) Mustard+Palm oil (75:25%) at 180°C temperature

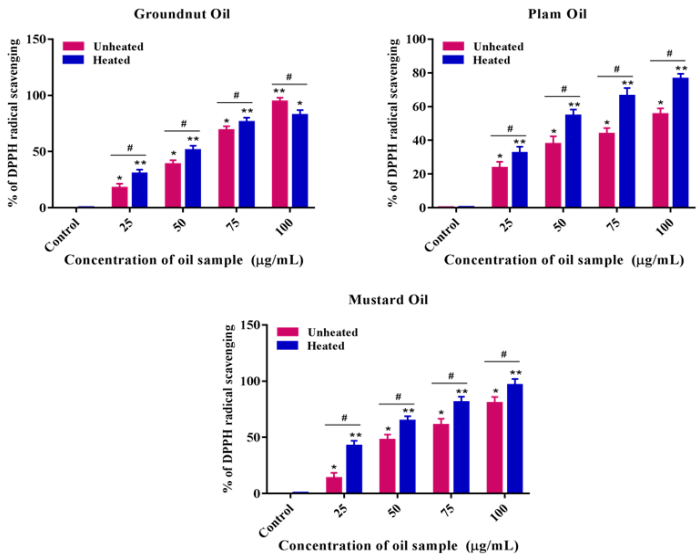


Fig. 13. Concentration and alteration of oils during heating/reheating with the proportions and their DPPH radical scavenging of Groundnut, Mustard and Palm oils.

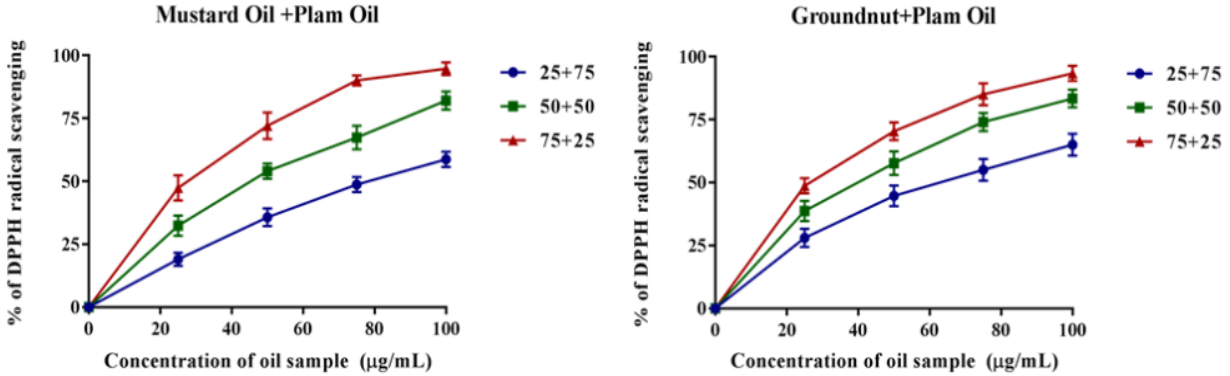


Fig. 14. Concentration and alteration of oils during Blending with the proportions (25+75, 50+50, 75+25) with their DPPH radical scavenging of Groundnut + Palm oil and Mustard + Palm oil.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Proximate Composition of Different Tissues of *Donax variabilis* in Tharangambadi Southeast coast of, TamilNadu, India

Thilagavathi M and Christy Ponni A*

Research Department of Zoology, TBML College, Porayar-609 307, Tamil Nadu, India. (Affiliated to Bharathidasan University, Tiruchirappalli – 24)

Received: 20 Nov 2020 Revised: 07 Dec 2020 Accepted: 18 Dec 2020

***Address for Correspondence**
Christy Ponni A
Research Department of Zoology,
TBML College,
Porayar-609 307, Tamil Nadu, India.
Email: zoology049@gmail.com

ABSTRACT

More importance has been given to marine molluscs, because they are both ecologically and economically important to humanity. Bivalve molluscs are important resources for marine fisheries; they are rich in biochemical compounds. This study deals with the biochemical composition of *D. variabilis* of male and female. During different seasons, the proximate composition of protein, carbohydrate and lipid content in different tissues (foot, muscle, mantle and gonad) was studied. The results of the proximate composition in *D. variabilis* showed that protein was high at 0.733±0.017 mg/g, followed by 0.228±0.018 mg/g of carbohydrates and 0.0221±0.01 mg/g of lipids. Because of its high-quality protein and well-balanced diets, the result shows that marine mollusc tissue (*D. variabilis*) is a valuable food recipe for human consumption.

Keywords: Biochemical, protein, carbohydrate and lipid.

INTRODUCTION

Seafood, due to its unique nutritional composition, is an important contributor to the diets of many people. These extremely nutritious shellfish resources are exploited by local people for food and shells are transferred for the preparation of lime to small-scale industries. Increasing demand for protein-rich foods and multiple molluscum shell uses in lime-based chemical industries have generated tremendous awareness of the advantages of exploiting and developing these resources. Some basic elements such as water, protein, lipids, carbohydrates and minerals for human diet are also known as percentage composition of the biochemical analysis (Ramakrishnan and Venkat rao, 1995). The availability of food with a high protein content is the biggest problem in some developing countries. Knowledge of the nutrition of edible living organisms is tremendously important as its biochemical analysis

(Nagabhushanam and Mane, 1978) reflects the nutritional value. Marine bivalve mollusks are tasteful and edible species will become more important next to fish and prawn. Marine molluscs are economically important species in the coastal region and are easy to cultivate. Marine molluscs are the main components of bivalve fisheries in coastal aquaculture (Jones and Alagarswami, 1973) and are an important source of nutrition for coastal people (Verlecar et al., 2006 and Parulekar et al., 1984). It is not only used as a food source, it has enormous amounts of natural bioactive compounds that are identified, isolated and vastly characterized for the treatment of human diseases (Fenical, 1993). Many researchers have now focused their research on molluscs to isolate secondary metabolites every day, although only marine molluscs have investigated some tiny portions of secondary metabolites. Studies on the nutritional value of edible gastropods and bivalves play an important role in meeting the growing population's nutritional demands in our country. The proper use of aquatic organisms by catching and cultivating fisheries will provide balanced nutritious food and malnutrition can be controlled. The percentage composition of five basic constituents, such as protein, carbohydrate, lipid, ash and water, generally means proximate composition. Depending on several variables such as species, size, gender, maturity, season and feeding regimes, the proximate composition varied widely (Xavier, 1996 and Ajaya, 2002). Among sea foods, molluscs are delicious foods rich in protein (Jagadis, 2005). Shellfish provide high quality protein with all the essential dietary amino acids for the maintenance and growth of essential fatty acids in the human body. Therefore, low-fat, high-protein foods that can be included in a low-fat diet should be considered shellfish. Therefore, the present study is framed by estimating the levels of protein, carbohydrate, lipid, moisture content, dry matter and water content to evaluate the nutritional value of the bivalves available, such as *Donax variabilis*.

MATERIALS AND METHODS

For biochemical analysis, the total protein, carbohydrate, lipid values for the various tissues such as gill, foot, muscle, and mantle of both *D. variabilis* male and female organisms were taken.

Collection of samples

Among the trash fishes, bivalves such as *D. varibilis* were collected. The samples collected were transported on ice to the laboratory. They broke the shells and removed the edible portion from the shell. To remove foreign material, it was thoroughly rinsed with deionized water and dried in a hot-air oven at 50 to 55 ° C. The well-dried samples were powdered and used for the estimation of protein, carbohydrate and lipid content.

Estimation of moisture

Using the AOAC (1980) method, the entire animal and organs were estimated. By following Lowry's method, the amount of protein present in the sample was estimated (Lowry et al., 1951). The procedure of Dubois et al. (1956) using phenol-sulphuric acid was followed for the estimation of total carbohydrate content. Following the gravimetric method of Folch et al., (1956), total lipids of the tissue samples were analyzed.

RESULTS

Biochemical Parameters

The total protein, carbohydrate, lipid values of both male and female organisms of *D. variabilis* for the various tissues such as gill, foot, muscle, and mantle are given in figures 1-6. The values of the different seasons such as Post monsoon, Summer, Premonsoon and Monsoon were analyzed and the results were analyzed for the different *D. variabilis* tissues.

Protein content

The highest protein content of gill tissue was observed in female *D. variabilis* as 0.733±0.017 mg/g during post monsoon season. In male *D. variabilis* maximum (0.656±0.041 mg/g) protein content was noted in gill tissues during post monsoon season and minimum protein content was noted as 0.573±0.004 mg/g during summer season (Fig. 1). Highest protein content value was noted in female *D. variabilis* as 0.807±0.012 mg/g in post monsoon, when compared to other seasons (Fig. 2). Least protein content value was noted in female *D. variabilis* as 0.736±0.007 mg/g during summer season. The protein content of muscle of male and female *D. variabilis* showed significant variations between seasons.

Carbohydrate content

Maximum carbohydrate content as 0.228±0.018 mg/g was recorded in female gill on monsoon season and minimum carbohydrate content as 0.209±0.012 mg/g was observed in summer season (Fig. 3). When the overall responses were analysed, the influence of various season on carbohydrate content was observed to be significant ($p < 0.05$). Maximum carbohydrate content of foot was 0.274±0.020 mg/g recorded in female foot in post monsoon season. Minimum carbohydrate content of foot was 0.249±0.007 mg/g recorded in monsoon season. The gonad carbohydrate showed statistically significant variations between seasons. Carbohydrate content of *D. variabilis* estimated on wet weight basis. Maximum carbohydrate content was 0.341±0.011 mg/g recorded in post monsoon season and minimum was recorded as 0.322±0.012 mg/g in summer season, which was compared with other season groups (Fig. 4). The muscle carbohydrate showed statistically significant values between the seasons ($p<0.05$). Maximum carbohydrate content 0.256±0.012 mg/g was recorded in female mantle on summer season.

Lipid content

The maximum lipid content in female was 0.0221±0.01 mg/g in gill of female in pre monsoon and minimum gill lipid in female was 0.0196±0.002 mg/g in monsoon season. Maximum lipid content in gill was 0.0225±0.001 mg/g recorded in male in pre monsoon season and minimum lipid content 0.0196±0.014 mg/g was recorded in male gill in monsoon season (Fig. 5). Maximum lipid content was 0.0228±0.003 mg/g in post monsoon and minimum lipid content was 0.0210±0.001 mg/g in summer season in male gonad. Maximum lipid content in liver was 0.0258±0.011 mg/g recorded in female muscle in post monsoon season. The minimum lipid content was 0.0236±0.012 mg/g recorded in same female muscle in monsoon season. Maximum lipid content value was noted in female liver of *D. variabilis* as 0.0221±0.018 mg/g in pre monsoon, and minimum lipid content was noted in female mantle as 0.0200±0.005 mg/g in monsoon season (Fig. 6). The lipid content in mantle showed significant variations ($p<0.05$).

DISCUSSION

The characteristics of the seasonal activities of bivalves are seasonal modifications in the biochemical composition. Differences in biochemical composition are influenced by various factors, such as hydrographic conditions, food availability, growth and reproductive cycle knowledge, which are essential for the interpretation of variations in tissue biochemical composition (Ansari, et al., 2000). In all aspects of cell structure and function, proteins are fundamental bimolecules. In the body, protein comes in the form of amino acids and other metabolites, which serve as the body's building blocks. The protein level of *D. variabilis* in the current study showed significant variation throughout the year in various organs such as mantle, gill, muscle, male and female mantle. Among the stations, the seasonal variation in the protein concentration of the mustache was linked to its natural reproductive cycle, the availability of food and climate change. Therefore, *D. variabilis* showed two spawning peaks, one during the post-monsoon period and one during the pre-monsoon period. A typical and general indicator reflecting the resting spawning cycle is the protein level (Mohan and Kalyani, 1989). According to Krishnakumar et al., Lee (2006), the maximum and minimum of protein correspond to the phases of growth / spawning and regression / resting. The protein content remained fairly high throughout all seasons according to Mane and Nagaphushanam (1983) and decreased only in the spawned gonad. In the current study, similar results have been observed. Joseph, M., 1998 observed an increase in protein content that was attributed to the spawning activity in a rhombea from June to

August. A similar trend of high protein levels during the period of maturation and a decreased level during spawning was also reported by Jayabal and Kalyani (1994). In the current study, male and female protein levels in all stations showed slight variations. Jayabal and Kalyani (1994) and Jonas Gunasekaran (2003) made a similar report for *Meretrix casta*. In the gill and mantles, ANOVA showed significant season-wise variation in the protein level. Seasonally, all organs have shown significant variation. Carbohydrates in the tissues of animals exist as protein bond sugars and glycogen. The best energy process for the living organism's body is protein-bound sugar (Vijayavel et al. 2007). In the current study, the value of carbohydrates was higher than that of lipids and lower than that of proteins. The seasonal variation in the content of carbohydrates is directly associated with the reproductive cycle (Xavier et al., 1996). Such a variation in the carbohydrate value in *P. viridis* is related to different metabolic activities. The carbohydrate of molluscs is mainly composed of glycogen, according to Wong and Cheung, (2001), and changes in the level of carbohydrates may be due to glycogen accumulation at distinct stages such as gametogenesis and spawning.

Low carbohydrate levels were mainly due to low temperatures in the monsoon (October-November) and unfavorable conditions causing stress on the mussels. An earlier study (Gabbot and Bayne, 2003) stated that muscle stress results in the use of carbohydrate reserves and a decrease in the ammonia-N reaction rate. He also stated that the natural gametogenic cycle in bivalves is closely related to glycogen storage cycles and subsequent de Nova lipid synthesis at the expense of the stored glycogen during vitellogenesis. In relation to gonad maturity, climatic changes, and food availability, carbohydrate values fluctuated between organs. Ansell et al. (1994) reported that there is a high percentage of carbohydrates and fat compared to any other organ in the gonad and digestive gland. Glycogen content was associated with the development of gonads and increased during the period of active gametogenesis, Arularsan et al., (2007) reported. The stationary variation in the level of carbohydrates may be due to differences in water depth and other water chemical characteristics, and so on. In Tranguebar coastal waters, similar reports were produced by Jonas Gunasekaran (2003). This study showed that the carbohydrate content in females was slightly higher than in males, which may be related to the reproductive cycle.

The content of lipids is an essential organic constituent of all animals' tissues and plays a key role in the metabolism of energy. Lipid is the body's best energy producer, alongside carbohydrates. Lipid, when carbohydrate levels are low, also provides food supply (Pazos et al. 1996, 1997). External factors, such as fluctuations in environmental conditions and qualitative and/or quantitative changes in food availability, or internal factors such as sexual maturation (Gardner and Riley, 1992), may affect the lipid composition of molluscs. The period of low lipid content coincided with post-spawning seasons, according to Qasim et al. (1997) and Parulekar et al. (1982), and it could be attributed to the depletion of energy resources for mussel spawning activities. John (1980) reported similar results on *Anadara rhombea*, Balasubrahmanian (1999) on *M. casta* and *M. meretrix* on Jayabal. Mane and Nagabhushanam (1983) indicated that in the spawned gonad, the fat content dropped sharply. The initiation of gametogenesis and the use of energy reserves for the development of gametes could also be another probable cause for low lipid values. The overall results of the proximate composition in *D. variabilis* showed that protein was high at 0.733±0.017 mg/g, followed by 0.228±0.018 mg/g of carbohydrates and 0.0221±0.01 mg/g of lipids. Because of its high-quality protein and well-balanced diets, the result shows that marine mollusc tissue (*D. variabilis*) is a valuable food recipe for human consumption.

ACKNOWLEDGEMENTS

The authors would like thank the Principal, TBML College, Porayar, Tamil Nadu, India for the encouragement and providing all necessary infrastructural facilities to perform the research for this study.

REFERENCES

1. Ajaya, B.D., 2002. Nutritional evaluation of molluscan seafood. Ph.D. Thesis, Annamalai University, India. pp: 129
2. Ansari, Z. A., A. H. Parulekar and S. G. P. Matondkar. 2000. Seasonal changes in meat weight and biochemical composition in the black clam *Villorita cyprinoides* (Grey). Indian J. Mar. Sci. 10:128–131
3. Ansell, A.D., 1994. Seasonal changes in biochemical composition of the bivalve Chlamys septemradiata the clyde sea area. Mar. Biol., 25(1): 85-99
4. AOAC, 1980. Official Methods of Analysis of the Association of Official Analytical Chemists. W. Horwitz (Editor), 13th edn, Washington, DC, 1018 pp.
5. Arularsan S., N. John Samuel, Eldose, P. Mani, B. Ravikumar and R. Murugesan, 2007. A comparative study of diversity of Rockey Shore molluscs of Tranquebar and Pondicherry coasts, south east cost of India. Indian J. Applied and pure. Bio. Vol., 22(2): 327-331
6. Balasubramanian, T., 1999. Impacts of coastal pollution. In: Proceedings of Indo-British integrated coastal zone management training short-course conducted by Institute for Ocean Management, Anna University, India
7. Dubois M, Gilles KA, Hamilton JK, Rebers PA, Smith F. Colorimetric method for determination of sugars and related substances. Anal Chem 1956; 28:350-356
8. Fenical W. Chemical studies of marine bacteria: developing a new resource. Chem Rev 1993; 93(5):1673-1683
9. Folch J, Less M, Stanley GHS (1956). A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem., 226: 497-509
10. Gabbot P.A and B. L. Bayan, 1973. Biochemical effects of temperature and nutritive sites on *Mytilus edulis* L.J.Mar. Biol. Assoc. U.K., 53: 269- 286
11. Gardner D and J.P. Riley, 1992. The component fatty acids of the lipids of some species of marine and freshwater mollusks. J. Mar. Biol. Ass. UK., 52: 827-838.
12. Jagadis I (2005). Biology of an intertidal clam *Gafrarium tumidum* (Roding) from south east coast of India. Ph.D., thesis, Annamalai University, p. 155
13. Jayabal, R and M. Kalyani, 1994. Allometric relationship in the hard clam *Meretrix meretrix* (Linnaeus) J. Mar. Biol. Assoc. India, 6 (1- 2), pp.319-322.
14. Jonas Gunasekaran, G., 2003. Biological studies on *Meretrix casta* (Chemnitz) in the Uppanar Estuary, Tranquebar, Tamilnadu, South India, Ph.D. Thesis, Bharathidasan University.
15. Jones S, Alagarswami K. Mussel fishery resources of India. Proc Symp On Liv Res India, Cochin: CMFRI Special Publ 1973; p 641
16. Joseph, M., 1998. Mussel and Oyster culture in the tropics. Tropical Mariculture, 309-358
17. Krishnakumar, P.K., G. Sasikumar, G.S. Bhat, D.P.K. Asokan, 2006. Biomarkers of environmental contaminants in field population of green mussel (*Perna viridis*) from Karnataka–Kerala coast (South West coast of India). Ecotoxicology, 15: 347–352
18. Lowry, O.H., Ronebrough, N.J., Farr, A.L., Randell, R.J., 1951. Protein measurement with Folin phenol reagent. J. Biol. Chem. 193, 265–276
19. Mane, U.H and R. Nagaphushanam, 1983. Seasonal changes in the Gonad and Associated Neurosecretion in the Green mussel *Perna viridis*. Proc. Symmp. Coastal Aquaculture, 2: 540-544
20. Mohan, D. and M. Kalyani. 1989. Seasonal variations in biochemical composition of green mussel *Perna viridis* (Linnaeus). Mahasagar-Bull. Natn. Inst. Oceanogr. 22:113–120
21. Nagabhushanam R, Mane VH. Seasonal variation in the bio- chemical composition of *Perna viridis* at Ratnagiri on the West Coast of India. Hydrobiologia, 1978; 57(3): 69-72
22. Parulekar AH, Nair A, Ansari ZA, Harakantra SN, Chatterji A. Ecology and culturing of edible bivalves in Goa. Ind J Mar Sci 1984; 13:190-192.

23. Pazas, A.J., C. Ruiz, O. Garcia-Martin, M. Abad and J.L. Sanchez, 1996. Seasonal Variations of the lipid content and fatty acid composition of *Crassostrea gigas* cultured in El Grove, Galicia, N.W. Spain Comp. Biochem. Physiol., 114B: 171-179.
24. Pazas, A.J., G. Roman, C.P. Acosta, J.L. Sanchez and M. Abod, 1997. Lipids classes and fatty acid composition in the female gonad of *Pectin maximus* in relation to reproductive cycle and environmental variables. Comp. Biochem. Physiol., ll713: 393-402
25. Qasim, S. Z., A. H. Parulekar, S. N. Harikantra, Z. A. Ansari and Nair, 1997. Aquaculture of green mussel *Mytilus Viridis* (L.) Cultivation on ropes from floating rafts. Indian. J. Mar. Sci., 6: 18-25
26. Ramakrishnan S, Venkat rao S. Nutritional Biochemistry. T.R. Publication, Chennai, 1995
27. Verlecar XN, Pereira N, Desai SR, Jena KB, Snigdha. Marine pollution detection through biomarkers in marine bivalves. Curr Sci 2006; 91:51-57
28. Vijayavel, K., A. Gopalkrishnan, Chezhian, and M. P. Balasubramanian, 2007. Biochemical constituents and bioaccumulation as biomarkers in the green mussel *Perna viridis* with reference to silver and chromium toxity. Toxicol. and Envirn. Chem., 89(2): 353-361
29. Wong, W.H and S. G. Cheung, 2001. Feeding rates and scope for growth of green mussels *Perna viridis* (L) and the relationship with food availability in Kat O, Hong Kong. Aquaculture, 193:123-137
30. Xavier R.M., 1996. Studies on the biochemistry and processing of edible meat of muricid gastropods *Chicoreus virgineus* (Roding, 1798) and *Rapana rapiformis* (Born, 1778).Ph.D. Thesis, Annamalai University, India.pp: 88.

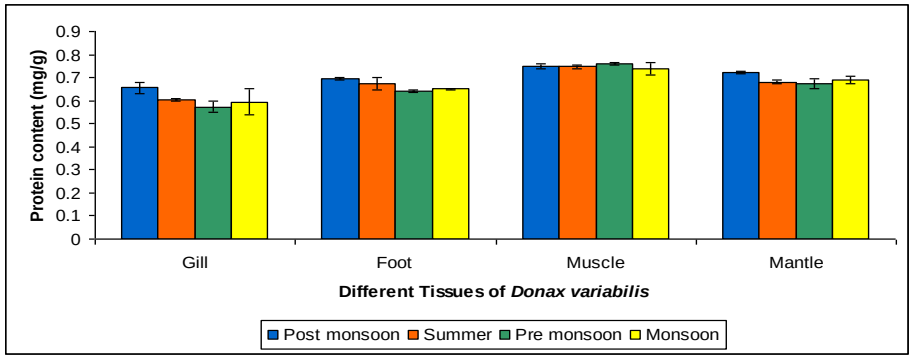


Fig : 1. Seasonal mean variations in protein content (mg/g) of male *Donax variabilis*

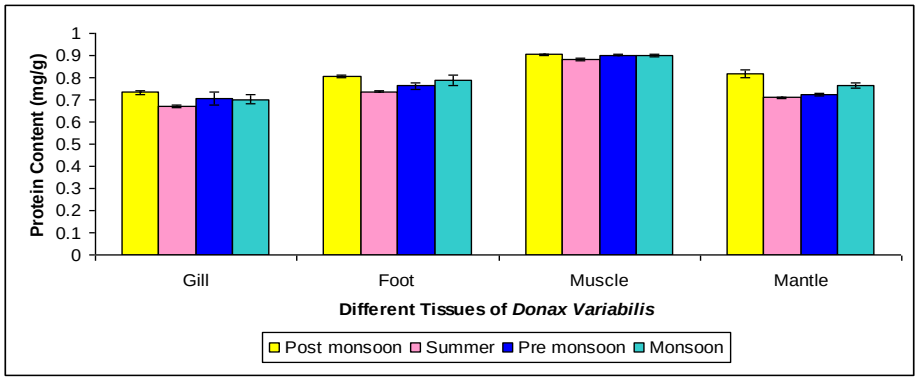


Fig : 2. Seasonal mean variations in protein content (mg/g) of female *Donax variabilis*

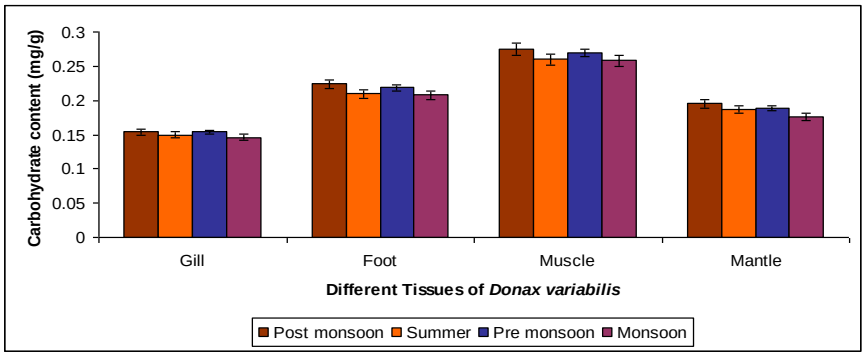


Fig : 3. Seasonal mean variations in carbohydrate content (mg/g) of male *Donax variabilis*

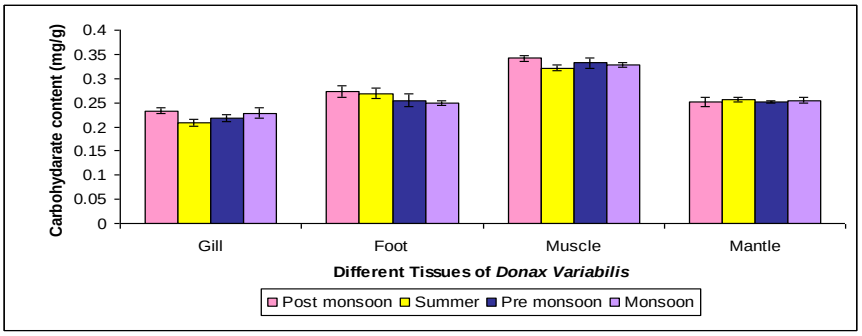


Fig : 4. Seasonal mean variations in carbohydrate content (mg/g) of female *Donax variabilis*

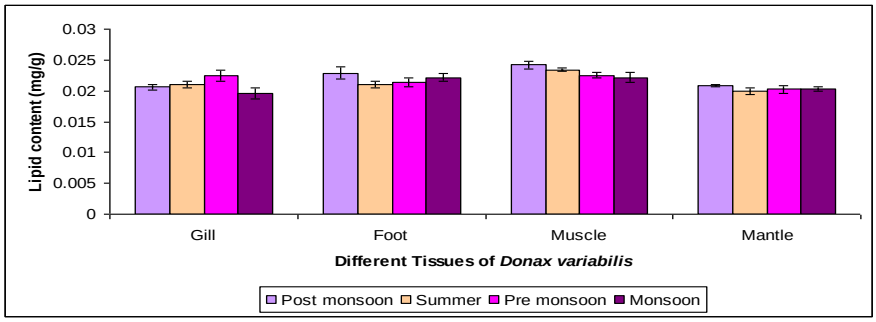


Fig : 5. Seasonal mean variations in lipid content (mg/g) of male *Donax variabilis*

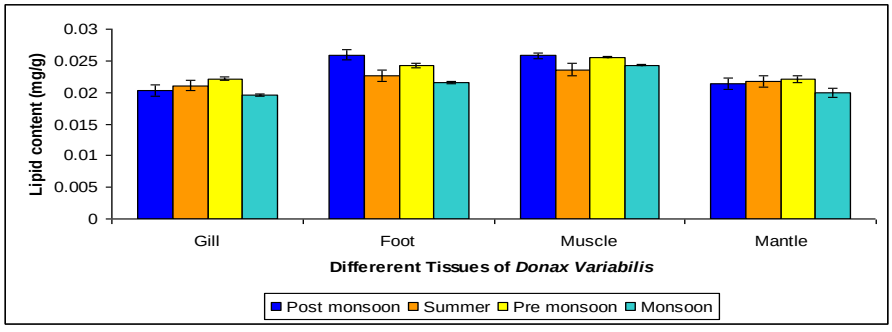


Fig : 6. Seasonal mean variations in lipid content (mg/g) of female *Donax variabilis*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

On Increasing the Accuracy of Multiple Crop Yield Quality Prediction using Hybrid Classification Algorithm

S. Manimekalai* and K. Nandhini

[1]Ph.D Research Scholar, PG and Research Department of Computer Science, Chikkanna Government Arts College, Tirupur, Tamil Nadu, India.
[2]Assistant Professor, PG and Research Department of Computer Science, Chikkanna Government Arts College, Tirupur, Tamil Nadu, India.

Received: 05 Nov 2020 Revised: 03 Dec 2020 Accepted: 29 Dec 2020

***Address for Correspondence**
S. Manimekalai
Ph.D Research Scholar,
PG and Research Department of Computer Science,
Chikkanna Government Arts College,
Tirupur, Tamil Nadu, India
Email : manimekalaipari@gmail.com

ABSTRACT

One of the most essential tasks in agricultural applications is predicting the quality of crop yield. Different data mining algorithms have been proposed with the use of chronological data about climatic conditions, soil parameters and so on. Among various data mining algorithms, Artificial Neural Network (ANN) has been used in both supervised and unsupervised learning to predict the quality of crop yield; but, it consumes high training time while using more number of hidden layers. Also, the simultaneous prediction of multiple crop yield quality within a single classifier is not suitable due to multi-class classification problem. As a result, this article focuses on increasing the prediction accuracy with the aid of hybridization of ANN and Ripper classification efficiently. At first, a training data which consists of different soil parameters for different types of crops is acquired from Tirupur district Soil Testing Laboratories. Then, the class imbalance problem is solved by generating the synthetic minority instances with the augmented Gram matrix based on the Weighted Kernel-based Synthetic Minority Oversampling Technique (WK-SMOTE). After that, the most optimal parameters are chosen by Krill Herd (KH)-based optimization algorithm. For each optimal parameter, the relative weights are computed using Rough Set (RS) theory that solves the multi-class classification problem and predicts the multiple crop yield quality simultaneously. These relative weights are added to the actual soil parameter values to get a new value and given as input to the ANN classifier which provides weight of each parameter. These weight values are fed to the Ripper classifier, namely Dynamic Multi-Class Neupper (DMCNeupper) classifier in which transformation function is used to optimize the kernel-defined feature space of ANN classification.

Finally, the experimental results show that the effectiveness of the proposed KHDMCNeupper classification-based crop yield prediction compared to the ANN classifier in terms of precision, recall, f-measure and accuracy.

Keywords: Agriculture, Crop Yield Prediction, Data mining, Artificial neural network, Soil parameters, Krill herd, Rough set theory

INTRODUCTION

Agriculture plays a vital role in most of the states like India where the demand of groceries has been magnified because of unconditional civilization. In earliest decades, people plant the crops in their own land and together natural crops planted that are used by completely different people, animals and birds since such crops can provide healthy life. In recent years, the agriculture is step by step degrading because of the innovative technologies and digitization. Also, people don't have the data regarding the harvesting of the crops in an extremely right time and at a suitable location. By victimization the sensible techniques, the seasonal weather conditions are being changed against basic properties like soil, water and ait that offer to insecurity of food. There's no accurate resolution and technologies by analyzing the issues like weather, temperature, etc., for avoiding the agricultural degradation. Many data mining algorithms [1] have been proposed on hypothesis of historical knowledge regarding weather, water level and crop growth level to increase the yield production. Most of the crop yield prediction systems are helpful for farmers to create choices regarding the soil types, irrigation level and types of crop to be harvested. Also, it is useful to predict the required information from a raw dataset to predict the yield quality issues and making a suitable solution. Within the past years, several classification algorithms have been developed to predict the crop yield quality. Among various data mining algorithms, Counter-Propagation Artificial Neural Network (CP-ANN) [2] has been used in both supervised and unsupervised learning to predict the quality of crop yield; but it consumes high training time while using more number of hidden layers. Also, the simultaneous prediction of multiple crops within a single classifier is not suitable due to multi-class classification problem. As a result, this article focuses on improving the accuracy of predicting the crop yield quality using soil parameters. Initially, a training dataset for different crops with various soil parameters is gathered. Then, the class imbalanced data problem is solved by generating the synthetic minority instances with the augmented Gram matrix based on the WK-SMOTE. Also, the most optimal parameters are selected by KH algorithm and RS theory is applied to compute the relative weights of the most optimal parameters for all crops. Then, these weights are added with the actual parameter values and given as input to the ANN that constructs the decision tree using DMCNeupper classifier. In DMCNeupper classifier, the transformation function is used to optimize the kernel-defined feature space for solving the multi-class classification problem and predicting the multiple crop yield quality simultaneously with increased prediction accuracy. Thus, the accuracy of predicting the crop yield quality is increased efficiently by using KHDMCNeupper classification algorithm. The remaining section of this article is structured as follows: Section II presents the previous researches related to the crop yield prediction. Section III explains the proposed crop yield prediction. Section IV illustrates the performance effectiveness of the proposed algorithms and Section V concludes the research work.

LITERATURE SURVEY

Dahikar & Rode [3] proposed a feed-forward back propagation ANN algorithm that uses soil and environmental parameters for predicting the crop yield. On the other hand, ANN was able to perform only with the statistical data and the performance was mainly depends on the user's ability. Ramesh & Vardhan [4] proposed the Multiple Linear Regression (MLR) and density-based clustering techniques for crop yield prediction. Nonetheless, few noisy data was taken as significant data that provides overfitting problem. Ganesh & Jayasudha [5] analyzed different data mining algorithms such as naive bayes, J48 and JRip algorithms to predict the soil fertility. However, the accuracy was still not effective. Chaudhary et al. [6] proposed an improved random forest classifier for solving the multi-class classification problem. But, an over fitting issue was occurred and it needs to choose the number of trees to achieve

better performance. Patel & Patel [7] proposed a RS theory to generate the classification rules from a set of agricultural data for predicting the crop yield. On the other hand, this method was not fit for dataset with number of attributes. Rajak et al. [8] recommended an ensemble method using SVM and ANN to predict the crop yield using soil, crop and environmental parameters. But, the performance was not effective since it uses only few parameters.
Silas & Nderu [9] proposed a K-means clustering and association rule mining technique for predicting a tea yield productivity in Kenya. Nevertheless, the clustering was time consuming while the dataset was large. Bhangale et al. [10] proposed a neural network for improving the crop yield prediction. But, the performance of this algorithm was not analyzed. Dolas & Joshi [11] proposed a modified decision tree classifier which is trained with C4.5 and CART for predicting the crops using soil parameters. Conversely, a further improvement was needed to schedule the irrigation process. Villanueva & Salenga [12] proposed a convolutional neural network algorithm for predicting the crop yield using bitter guard leaves which are gathered from Ampalaya farms.

Proposed Methodology

In this section, the proposed KHDMCNeupper classification-based crop yield quality prediction is explained in brief. The block diagram of this proposed technique is shown in Fig. 1. Initially, training dataset for six different crops such as rice, wheat, maize, sorghum (cholam), coconut and groundnut is collected which consists of different soil parameters. Then, WK-SMOTE is applied to solve the class imbalance problem and KH is applied to select the most optimal parameters. After that, RS theory is used to compute the relative weights of those optimal parameters and the computed relative weights are added with the actual soil parameter values to solve the multi-class classification problem. Moreover, the new soil parameter values are given to the ANN for obtaining the weight values of each parameter in which the kernel-defined feature space is optimized dynamically by using the transformation function. The estimated weight values are further used as input to the Ripper classifier to build the decision tree for predicting the multiple crop yield quality simultaneously *Data Acquisition.* The main aim of this article is increasing the accuracy of predicting the quality of multiple crops which are planted in the Tiruppur region of Tamilnadu. Therefore, the soil dataset for six different types of crops are acquired from the Soil Testing Laboratories in Tiruppur district. The collected soil parameters are pH value, Electrical Conductivity (EC), Organic Carbon (OC), Zinc (Z), Copper (Cu), Iron (Fe), Phosphorus (P), Manganese (Mn), Potassium (K), Sulphur (S), Calcium (Ca), Magnesium (Mg), soil temperature, Nitrogen (N) and Boron (B). The dataset with these soil parameters are collected from thirteen blocks of Tiruppur district such as Avinashi, Dharapuram, Kangayam, Gudimangalam, Kundadam, Mulanur, Madathukulam, Palladam, Pongalur, Tiruppur, Udumalpet, Uthukuli and Vellakovil.

Sampling & Kernel Space Optimization

To handle the class imbalance problem in the training dataset, WK-SMOTE i.e., oversampling method is applied that generates the synthetic minority instances to solve the binary classification problem consisting of the majority class C_{maj} and the minority class C_{min}. The novel minority instance is created between the minority instances a_p and a_q as:

$$a_{new} = a_p + (a_q - a_p) \times \zeta \qquad (1)$$

In Eq. (1), ζ is a random number to maintain the separability in the feature space. For each minority instances, the synthetic instances are generated along with the line segment of its k neighbors. The neighbors are computed by Euclidean distance since the distance metrics in the kernel-based ANN needs to be redefined for identifying the neighbors. Assume any two instances a_i and a_j that are transformed into the feature space as $\Phi(a_i)$ and $\Phi(a_j)$, correspondingly. The distance between these two instances in the feature space is computed as follows:

$$Dis^{\Phi}(a_i, a_j)^2 = \|\Phi(a_i) - \Phi(a_j)\|^2$$
$$= \mathbb{K}(a_i, a_j) - 2\mathbb{K}(a_i, a_j) + \mathbb{K}(a_i, a_j) \qquad (2)$$

By using Eq. (2), the k nearest minority instances of a seed instance in the feature space are computed. After that, one of the neighbors is chosen at random for each seed instance to generate a synthetic minority instance. Let the set of R minority data instances as C^{syn}, where i^{th} element of C^{syn} i.e., a_i^{pq} is created from the seed a_p and neighbor a_q as:

$$\Phi(a^{pq}) = \Phi(a_p) + \zeta^{pq}\left(\Phi(a_p) - \Phi(a_q)\right) \quad (3)$$

The inner product of each instances pair ($K^1 \in \mathbb{R}^{N\times N}$) is required for training the classifier where the data instance $K_{ij}^1 = \mathbb{K}(a_i, a_j) = \Phi(a)^T\Phi(a_j)$ for $a_i, a_j \in C$. The kernel matrix K^1 is increased for the new synthetic instances.

The kernel matrix K with R new minority instances is decomposed as:

$$K = \begin{bmatrix} K^1 & K^2 \\ K^{2T} & K^3 \end{bmatrix} \quad (4)$$

Where $K^1 \in \mathbb{R}^{N\times N}$ with data $K_{ij}^1 = \mathbb{K}(a_i, a_j), a_i, a_j \in S$;
$K^2 \in \mathbb{R}^{N\times P}$ with data $K_{ij}^2 = \mathbb{K}(a_i, a_j^{pq}), a_i \in S, a_j^{pq} \in S^{syn}$;
$K^3 \in \mathbb{R}^{P\times P}$ with data $K_{ij}^3 = \mathbb{K}(a_i^{lm}, a_j^{pq}), a_i^{lm}, a_j^{pq} \in S^{syn}$.

The data instances of K^2 and K^3 are computed as:

$$\mathbb{K}(a_i, a_j^{pq}) = (1-\zeta^{pq})\mathbb{K}(a_i, a_p) + \zeta^{pq}\,\mathbb{K}(a_i, a_p) \quad (5)$$

$$\mathbb{K}(a_i^{lm}, a_j^{pq}) = (1-\zeta^{pq})(1-\zeta^{lm})\mathbb{K}(a_p, a_l) + (1-\zeta^{pq})(\zeta^{lm})\mathbb{K}(a_p, a_m) + (\zeta^{pq})(1-\zeta^{lm})\mathbb{K}(a_q, a_l) + (\zeta^{pq})(\zeta^{lm})\mathbb{K}(a_q, a_m) \quad (6)$$

The increased kernel matrix K is computed by using Eqs. (4)-(6) only on the training data in C and the kernel function $\mathbb{K}(\cdot)$ with no precise mapping function $\Phi(\cdot)$. As a result, a suitable kernel function is used to train ANN classifier which has three layers such as input, hidden and output layer. Let the input vector $a \in \mathbb{R}^{N\times N}$. The output of ANN can map the input vector to a scalar, $b: \mathbb{R}^{N\times N} \to G$ which is achieved by employing the following equation:

$$b_j = \sum_{i=1}^{N} w_i \mathbb{K}_i(\|a - x_i\|) + \beta_j, \forall j = 1,2,\dots,N_0 \quad (7)$$

In Eq. (7), N and N_0 are the number of hidden and output layer neurons, correspondingly, $x_i \in \mathbb{R}^{N\times N}$ is the centroid for i^{th} neuron, w_i is the output layer weight for i^{th} neuron, β_j is the bias term for j^{th} output neuron and $\mathbb{K}_i$ refers the kernel function associated with i^{th} hidden neuron which is defined as:

$$\mathbb{K}(\|a - x_i\|) = (1-\zeta^{pq})\mathbb{K}(a, x_i) + \zeta^{pq}\,\mathbb{K}(a, x_i) \quad (8)$$

Therefore, the minority class in the training dataset is synthetically balanced and thus class imbalance problem is solved. Moreover, the feature space with the large kernel-space between clusters and close proximity of the classes is optimized to solve the multi-class classification problem. To achieve this, a transformation function is defined to generate a novel R-dimensional feature space. Assume that the total amount of soil parameters in the training dataset is $S = \{1,2,\dots,s\}$ and each parameter denotes a row feature vector. Each feature vector is placed in the U-dimensional Euclidean space in a S by U matrix. For each class $n \in \{1,2,\dots,N\}$, this matrix is converted to another S by R matrix B^n in the R-dimensional Euclidean distance such that the probability of $L \in \mathcal{R}^P$ being arranged exactly is superior to that of $K \in \mathcal{R}^P$. All feature vectors in class μ are placed in M_μ by U matrix V_μ. All feature vectors in classes other than μ are $(S - M_\mu)$ by U matrix $V_{\neg\mu}$. The converted form of V_μ and $V_{\neg\mu}$ in Z^n is M_μ by R matrix B_μ^n and $(S - M_\mu)$ by R matrix $B_{\neg\mu}^n$, correspondingly. Each feature vector of $B_{\mu=n}^n$ must be in a R-dimensional kernel space with radius $\Re_n$

and centroid X_n. The R-dimensional kernel space for class n is generated by using the linear transformation function (B^n) as:

$$B^n = AO_n \mathbb{K}_n(\|s - X_n\|) + HY_n \quad (9)$$

In Eq. (9), $A_{n,i}$ is a $U \times R$ matrix of weights, Y_n is a R-dimensional row vector of biases in the ANN classifier and matrix H refers a Q-dimensional column vector of ones used to provide O_n consistent with A. The coefficients in each function are optimized such that the overall Euclidean distance Dis_n between all training feature vectors in $B^n_{\mu=n}$ and their centroid X_n as a function of weight and bias matrices is as:

$$Dis_n = \sum_{i=1}^{M_n} \left\| A_{n,i}O_n + Y_n - X_n \right\| \quad (10)$$

In Eq. (10), $A_{n,i}$ is i^{th} training feature vector in A_n, $A_{n,i} \times O_n + Y_n = B^n_{\mu=n,i}$ is i^{th} training feature vector in $B^n_{\mu=n}$ and the centroid of all feature vectors in $B^n_{\mu=n}$ is given as:

$$X_n = \frac{\sum_{i=1}^{M_n} \|A_{n,i}O_n + Y_n\|}{M_n} \quad (11)$$

For each $n \in \{1,2,\dots,N\}$, the optimal values of O_n and Y_n are obtained by reducing Dis_n. Moreover, two sets of inequality constraints are defined as:

$$s_{1,i} = \left\| A_{n,i}O_n + Y_n - X_n \right\| - \Re_n < 0, i \in [1,2,\dots,M_n] \quad (12)$$

$$s_{2,\eta} = \Re_n - \left\| A_{n,i}O_n + Y_n - X_n \right\| < 0, \eta \in [1,2,\dots,M_{\neg n}] \quad (13)$$

In above equations, $s_{1,i}$ and $s_{2,\eta}$ are i^{th} and η^{th} inequality constraints in two sets (12) and (13), correspondingly and $M_{\neg n} = S - M_n$ is the total amount of training feature vectors in classes other than n. The constraint $s_{1,i}$ assures that $B^n_{\mu=n,x}$ remains within the R-dimensional kernel space. The constraint $s_{2,\eta}$ drives $B^n_{\neg(\mu=n),\eta} = V^n_{\neg(\mu=n),\eta}O_n + Y_n$, η^{th} training feature vector in $B^n_{\neg(\mu=n)}$, out of the kernel space. The kernel space radius $\Re_n$ offers a margin between the training feature vectors in class $\mu = n$ and the training feature vectors in classes other than $\mu = n$ in the R-dimensional Euclidean distance. The total number of inequalities related to (12) and (13) are M_n and $M_{\neg n} = S - M_n$, correspondingly. The radius $\Re_n$ must be optimistic and thus another inequality constraint is included to the optimization formulation as:

$$s_3 = -\Re_n < 0 \quad (14)$$

The weights, biases and radius are known as transformation parameters which are denoted as $T = \{O, Y, \Re\}$. These parameters are optimized by Adeli and Park model to get optimal transformation functions which generates a regulated feature space for N different classes. This optimization model is defined by using a fitness function and inequality constraints (10)-(14) as:

$$f(T_n^t) = -\nabla Dis(T_n^{t-1}) - F\left[\sum_{i=1}^{M_n} s_{1,i}^+(T_n^{t-1})\nabla s_{1,i}(T_n^{t-1}) + \sum_{i=1}^{M_{\neg n}} s_{2,\eta}^+(T_n^{t-1})\nabla s_{2,\eta}(T_n^{t-1}) + s_3^+(T_n^{t-1})\nabla s_3(T_n^{t-1})\right] \quad (15)$$

In Eq. (15), ∇ is the gradient operator, $Dis(T_n^{t-1}) = Dis_n$ where superscript t is the iteration number and $(\cdot)$ term is the penalty function, F refers a penalty multiplier related to the learning rate in ANN, $s_{1,i}^+(T_n^{t-1}) = max\{0, s_{1,i}^+(T_n^{t-1})\}$, $s_{2,\eta}^+(T_n^{t-1}) = max\{0, s_{2,\eta}^+(T_n^{t-1})\}$ and $s_3^+(T_n^{t-1}) = max\{0, s_3(T_n^{t-1})\}$. An iterative process is applied for finding the equilibrium of (15) where the set of transformation function parameters in t^{th} iteration is as:

$$T_n^t = \int f(T_n^{t-1}), t \in [1,2,\dots,I] \quad (16)$$

In Eq. (16), I is the total number of iterations. The integration in (16) is done by using the Runge-Kutta method. For each class n, this model employs an iterative process for finding the optimum transformation parameters such that N optimizations are performed to obtain N optimized transformation functions whereas I is large enough for removing any violation of constraints (12)-(14) and $T_n^I = \{O_n^I, Y_n^I, \Re_n^I\}$ is the optimized transformation parameters. After that, O_n^I and Y_n^I are used in (9) for generating n^{th} optimized transformation function as:

$$B_{opt}^n = AO_n^I \mathbb{K}_n(\|s - X_n\|) + HY_n^T \quad (17)$$

Then, N converted forms of $A\left(B_{opt}^1, B_{opt}^2, \dots, B_{opt}^N\right)$, each corresponding to a regulated kernel space are located in S by $N \times R$ matrix.

$$B^{total} = \left[B_{opt}^1, B_{opt}^2, \dots, B_{opt}^N\right] \quad (18)$$

Each row in B^{total} called a feature vector representation reflects all N converted forms of the corresponding row in A in one $N \times R$ dimensional row vector. If B_s^{total} is s^{th} feature vector representation in B^{total}, the conditional probability of L^{total}, the $(N \times R)$-dimensional feature vector representation form of a new feature vector belonging to the class of B_s^{total} is computed as:

$$\Phi(L^{total}) = \frac{1}{2\pi^{(U/2)}\sigma^U} exp\left(-\frac{\|L^{total} - B_s^{total}\|^2}{2\sigma^2}\right) \quad (19)$$

Then, the class of L^{total} is determined by substituting for $K = L^{total}$ and $J = B_s^{total}$ in the following formulae:

$$\mathcal{P}_\mu(K) = \frac{1}{M_\mu}\sum_{i=1}^{M_\mu} \Phi_i(K) \quad (20)$$

$$Class\ of\ K = argmax\{\mathcal{P}_\mu(K)\} \quad (21)$$

If $N \times R < U$, then the dimensionality of each training feature vector in A will be reduced. Thus, the feature space with the large kernel space between clusters and the close proximity of classes is optimized in ANN classifier to classify the training dataset. Moreover, the most optimal soil parameters are selected by using KH algorithm.

Feature Selection

After that, the most optimal features are selected by KH-based optimization algorithm. The KH imitates the characteristic of krill where each KH will generate its contribution in the moving process depending on its fitness function. It adopts the d-dimensional search space Lagrangian model as:

$$\frac{dX_i}{dt} = M_i + F_i + R_i \quad (22)$$

In Eq. (22), M_i is the krill individual motion induction, F_i is the foraging action and R_i is the random dispersion. Various parameters such as highest number of iteration max_{Itr}, highest induced velocity max_M, highest dispersion velocity max_R, highest foraging velocity max_F, number of krill Num, locality of krill X and number of features Num_F are initialized. Then, the locality of features is generated at random. For each locality of current features, the prediction accuracy of each krill is determined as fitness function.

Initiation of Krill Individual Motion

The mutual properties among all krill individuals direct to the motion because they constantly endeavor to maintain a high concentration. The motion due to another krill individual is determined as:

$$M_i^{new} = max_M \delta_i + w_{num} M_i^{old} \quad (23)$$

In Eq. (23), $w_{num} \in [0,1]$ is the inertia weight with the motion induced and M_i^{old} is the previous motion induced.

$$\delta_i = \delta_i^{local} + \delta_i^{target} \quad (24)$$

Here, δ_i^{local} is the neighborhood effect as:

$$\delta_i^{local} = \sum_{j=1}^{Num_n} \hat{K}_{i,j} \hat{X}_{i,j} \quad (25)$$

$$\text{Where } \hat{X}_{i,j} = \frac{X_j - X_i}{\left\|X_j - X_i\right\| + t} \quad (26)$$

$$\hat{K}_{i,j} = \frac{K_i - K_j}{K_{worst} - K_{best}} \quad (27)$$

In Eq. (27), K_{best} and K_{worst} are the best and worst krill individual values, correspondingly, K_i is the fitness value of i^{th} individual krill, K_j is the fitness value of j^{th} neighbor, t is a petite optimistic value and Num_n is the number of neighbors. For each krill individual, the sensing space is determined as:

$$dist_{s,i} = \frac{1}{5N} \sum_{j=1}^{N} \left\|X_i - X_j\right\| \quad (28)$$

In Eq. (28), N is the number of krill individuals and X_i and X_j are the correlated locality of i^{th} and j^{th} krill. If the space between X_i and X_j is smaller than the defined sensing space, then X_j is a neighbor of X_i. The consequence of target path δ_i^{target} is discovered by the best krill individual as:

$$\delta_i^{target} = A_{best} \hat{K}_{i,best} \hat{X}_{i,best} \quad (29)$$

In Eq. (29), A_{best} is the krill individual effectual coefficient with best fitness to i^{th} individual krill and computed as:

$$A_{best} = 2\left(r + \frac{I_a}{I_{max}}\right) \quad (30)$$

In Eq. (30), $r \in [0,1]$ is the arbitrary value, I_a is the current iteration number and I_{max} is the highest number of iteration.

Motion due to Foraging Action

Based on the locality of the food and past incident about the locality of the food, the foraging motion is given as:

$$F_i = max_F \varepsilon_i + w_F F_i^{old} \quad (31)$$

In Eq. (31), $w_F \in [0,1]$ is the inertia mass of foraging motion and ε_i^{best} is i^{th} krill's best fitness so far.

$$\varepsilon_i = \varepsilon_i^{food} + \varepsilon_i^{best} \quad (32)$$

The food attractiveness $\left(\varepsilon_i^{food}\right)$ is given as:

$$\varepsilon_i^{food} = A^{food} \hat{K}_{i,food} \hat{X}_{i,food} \quad (33)$$

In Eq. (33), A^{food} is the food coefficient as:

$$A^{food} = 2\left(1 - \frac{I_a}{I_{max}}\right) \quad (34)$$

The consequence of the best fitness of i^{th} krill individual is determined as:

$$\varepsilon_i^{best} = \widehat{K}_{i,ibest}\,\widehat{X}_{i,ibest} \quad (35)$$

In Eq. (25), $\widehat{K}_{i,ibest}$ and $\widehat{X}_{i,ibest}$ are i^{th} krill individual's formerly visited best fitness and locality, correspondingly. For each iteration, the food centroid is determined as:

$$X^{food} = \frac{\sum_{i=1}^{N}\frac{1}{K_i}X_i}{\sum_{i=1}^{N}\frac{1}{K_i}} \quad (36)$$

Physical Dispersion

Physical dispersion motion is defined in terms of the highest dispersion velocity and an arbitrary directional vector as:

$$R_i = max_R\left(1-\frac{I_a}{I_{max}}\right)\beta \quad (37)$$

In Eq. (37), $\beta \in [-1,1]$ is the arbitrary directional vector.

Updating Locality

For different number of features, the localities of krill individuals are updated. Besides, an efficiency of KH is increased by combining the crossover and mutation with KH algorithm.

Crossover

Let $x_{i,s}$ is the s^{th} element of X_i and determined by:

$$x_{i,s} = \begin{cases} x_{t,m}, & r_{i,m} < C_0 \\ x_{i,m}, & else \end{cases} \quad (38)$$

In Eq. (38), C_0 is the crossover likelihood and the range is equal to 0.2 $\widehat{K}_{i,ibest}$.

Mutation

$$x_{i,m} = \begin{cases} X_{gbest,m} +> \rho\left(x_{p,m} - x_{q,m}\right), r_{i,m} < M \\ x_{i,m}, else \end{cases} \quad (39)$$

In Eq. (39), M is the mutation likelihood and the range is equal to $\frac{0.05}{K_{i,ibest}}$. A krill individual's locality vector in $[tm, tm+\Delta tm]$ is determined as:

$$X_i(tm+\Delta tm) = X_i(tm) + \Delta tm\frac{dX_i}{dt} \quad (40)$$

This procedure is repeated until the number of iteration reaches the upper limit. At last, the optimal soil factors related with the overall best krill are chosen. For each crop, the weights of the chosen optimal soil factors are computed by RS theory with a decision matrix $E\left(e_{ij}, i = 1,2,\dots,m; j = 1,2,\dots n\right)$ where m is the number of crop to be taken for predicting yield quality and n is the number of soil factors. Each row of E is assigned to any one crop and each column to one soil factor. Thus, a component e_{ij} of E indicates the quality of i^{th} crop with respect to j^{th} soil factor. The virtual weight of each soil factor is computed by dominance-based RS theory as:

$$\gamma_{ej} = 1 - \frac{Card\left(\left\{a_i \in A | D^+_{E-e_j}(a_i) \subseteq D^+_E(a_i)\right\}\right)}{Card(A)} \quad (41)$$

In Eq. (41), A is the normalized matrix of E, $D^+_E(a_i)$ is the set of elements dominating a_i with regard to E and $D^+_{E-e_j}(a_i)$ is the set of elements dominating a_i with regard to $E - e_j$. Once the value of γ_{ej} is determined, the weight of each soil factor is determined by normalization as:

$$w_j = \frac{\gamma_{ej}}{\sum_{j=1}^{n} \gamma_{ej}} \quad (42)$$

Classification

Further, the obtained weight of each soil factor is multiplied with their actual values and given as input to the ANN classifier. The input layer of ANN is denoted as:

$$X_i = X_i w_j \quad (43)$$

Also, the hidden layer of ANN is denoted as:

$$H_i = \sum_{i=1}^{n} w_i X_i + b \quad (44)$$

In Eq. (44), w_i is the weight value of input layer and X_i is the new value of soil factor and b is the bias. The hidden layer of ANN is defined by the tan-sigmoid transfer function as:

$$Y_i = f(H_i) = \frac{2}{1+e^{-2H_i}} - 1 \quad (45)$$

The output layer of ANN is denoted as:

$$o_i = f(\sum_{i=1}^{n} w_h Y_i + b) \quad (46)$$

In Eq. (46), o_i is the output neurons value, $f(x)$ is the transfer function and w_h is the weight value of the hidden layer. Besides, the weight value of ANN is updated to reduce the error of i^{th} neuron in the output layer as:

$$W_{ij\ new} = W_{ij\ old} + \delta(t_i - o_i)\, X_i \quad (47)$$

In Eq. (47), t_i is the preferred target output, $W_{ij\ new}$ is the new weight to i^{th} neuron in the output layer from the j^{th} neuron in the preceding layer, $W_{ij\ old}$ is the old weight to i^{th} neuron in the output layer from the j^{th} neuron in the preceding layer and δ is the learning parameter. Based on the output neuron values, the soil factor with the highest weight value is preferred as root node in the Ripper classifier to construct the decision tree. In Ripper classifier, the training dataset is split into the growing and pruning set. A primary rule set is built that overfits the growing set by using heuristic method. Then, the extended rule set is continuously shortened by applying the set of pruning operators. At each step of shorten process, the pruning operator is selected that yields the highest error reduction on the pruning set. This process is repeated until the weight value obtained from the ANN output layer is higher than the user-defined threshold value. Thus, the set of classification rule is constructed and the yield quality of different crops is simultaneously predicted with the highest prediction accuracy.

Algorithm for KHDMCNeupper Classifier-based Crop Yield Prediction

Input: Soil parameters such as P, K, S, Ca, Mg, Zn, Cu, Fe and Mn
Output: Yield quality

- Collect the training dataset for different crops;
- $for(each\ soil\ parameter\ in\ the\ training\ dataset\ )$
- {
- Solve class imbalanced data problem using WK-SMOTE algorithm;
  //Sampling & Kernel space optimization algorithm

- Initialize $C^{seed}, C^{neighbor} = \{\}$;
- $for(l = 1\ to\ R)$
- {
- Randomly sample a_p from C^{min};
- Compute k-nearest minority neighbors of a_p;
- Randomly sample a neighbor as a_q;

- Include a_p, a_q to $C^{seed}, C^{neighbor}$, correspondingly;
- }
- Determine kernel matrix K;
- Solve ANN decision;
- Return target class of a;
- $for(each\ class)$
- {
- Initialize the transformation function parameters $O, Y, \Re$ arbitrarily;
- Calculate the fitness value and inequality constraints;
- Reduce the fitness value using Adeli and Park model;
- Update the transformation parameters;
- Determine the optimized set of transformation function;
- Adjust the feature space dynamically;
- Select the most optimal parameters using KH algorithms;
  //KH algorithm

- Initialize max_{Itr}, max_M, max_R, max_F, Num, X and Num_F;
- Generate the locality of krill herds (soil factor) at random;
- Calculate the fitness value of the current soil factor according to its locality;
- $While(I_a > I_{max})$
- {
- Arrange the krill populace from best to worst;
- $for(all\ krill\ individual)$
- {

- Determine the motion induced by the other krill individual;
- Calculate foraging action;
- Evaluate the physical dispersion motion;
- Integrate the genetic functions;
- Update localities for each krill individual;
- Evaluate each krill individual according to its new locality;
- }
- Arrange the krill populace from best to worst;
- Discover the current best krill individual;
- Choose the optimal soil factors related to the overall best krill;
- Compute relative weights of each selected parameters;
- Update the soil parameter value;
- Assign the new soil parameter values as input to the ANN classifier;
- Process the ANN hidden and output layers;
- Update the weight of each neurons to minimize the error;
- Obtain the soil parameter with the highest weight as output of ANN;
- Make a soil parameter with the highest weight as a root node;
- Construct a tree based on Ripper algorithm;
- Generate the classification rules for all crops;
- Predict the yield quality;
- }

RESULTS AND DISCUSSION

The effectiveness of KHDMCNeupper classification algorithm is evaluated by using MATLAB 2017b and compared with the Ripper, ANN and CP-ANN classification in terms of precision, recall, f-measure and accuracy. In this experiment, the training dataset collected from Soil Testing Laboratories for rice, wheat, maize, coconut, sorghum and groundnut crops are used. The performance metrics are described below:

- Precision: It denotes the yield prediction at True Positive (TP) and False Positive (FP) rates.
- $Precision = \frac{TP}{TP+FP}$ (48)

- Recall: It indicates the yield prediction at TP and False Negative (FN) rates
- $Recall = \frac{TP}{TP+FN}$ (49)

- F-measure: It is calculated by using both precision and recall as:
- $F - measure = 2 \cdot \left(\frac{Precision \cdot Recall}{Precision + Recall}\right)$ (50)

- Accuracy: It is the fraction of TP and True Negative (TN) to the sum of amount of cases examined. It is calculated as:$Acc = \frac{TP + TN}{TP+TN+FP+FN}$ (51)

Table I shows the comparison of performance metrics for both proposed and existing crop yield prediction algorithms for six different crops such as rice, wheat, maize, coconut, sorghum and groundnut.

Fig. 2-5 shows the comparison of proposed and existing crop yield prediction algorithms in terms of precision, recall, f-measure and accuracy, respectively. In the graph, different crops are taken in the x-axis and the precision values are taken in the y-axis. From this analysis, it is observed that KHDMCNeupper classification algorithm achieves the highest precision, recall and f-measure than the other crop yield prediction algorithms. Similarly, the accuracy of KHDMCNeupper classification algorithm is higher than all other crop yield prediction algorithms that indicate the KHDMCNeupper classifier achieves the highest performance for predicting the yield quality of multiple crops simultaneously

CONCLUSION

In this article, a KHDMCNeupper classification algorithm is proposed to predict the yield quality of multiple crops simultaneously. By using this single classifier, multiple types of crops are allowed to train and predict their yield quality at the same time with the highest prediction accuracy. As a result, the training efficiency of predicting multiple crops together can be improved by using the single classifier compared to the prediction of each crop independently. Also, the prediction accuracy is almost identical to the accuracy of predicting each crop individually. This classifier can be applicable in real-time application for predicting the yield quality of crops and suggesting the cultivators to increase the crop productivity.

REFERENCES

1. P. Priya, U. Muthaiah and M. Balamurugan, “Predicting yield of the crop using machine learning algorithm,” Int. J. Eng. Sci. Res. Technol., vol. 7, no. 4, pp. 1-7, 2018.
2. X. E. Pantazi, D. Moshou, T. Alexandridis, R. L. Whetton and A. M. Mouazen, “Wheat yield prediction using machine learning and advanced sensing techniques,” Comput. Electron. Agric., vol. 121, pp. 57-65, 2016.

3. S. S. Dahikar and S. V. Rode, "Agricultural crop yield prediction using artificial neural network approach," Int. J. Innov. Res. Electr. Electron. Instrum. Control Eng., vol. 2, no. 1, pp. 683-686, 2014.
4. D. Ramesh and B. V. Vardhan, "Analysis of crop yield prediction using data mining techniques," Int. J. Res. Eng. Technol., vol. 4, no. 1, pp. 47-473, 2015.
5. S. H. Ganesh and M. Jayasudha, "Data mining technique to predict the accuracy of the soil fertility," Int. J. Comput. Sci. Mob. Comput., vol. 4, no. 7, pp. 330-333, 2015.
6. A. Chaudhary, S. Kolhe and R. Kamal, "An improved random forest classifier for multi-class classification," Inf. Process. Agric., vol. 3, no. 4, pp. 215-222, 2016.
7. H. Patel and D. Patel, "Crop prediction framework using rough set theory," Int. J. Eng. Technol., vol. 9, no. 3, pp. 2505-2513, 2017.
8. R. K. Rajak, A. Pawar, M. Pendke, P. Shinde, S. Rathod and A. Devare, "Crop recommendation system to maximize crop yield using machine learning technique," Int. Res. J. Eng. Technol., vol. 4, no. 12, pp. 950-953, 2017.
9. N. M. Silas and L. Nderu, "Prediction of tea production in Kenya using clustering and association rule mining techniques," Am. J. Comput. Sci. Inf. Technol., vol. 5, no. 2, pp. 1-7, 2017.
10. P. P. Bhangale, Y. S. Patil and D. D. Patil, "Improved crop yield prediction using neural network," IJARIIE, vol. 3, no. 2, pp. 3094-3101, 2017.
11. V. M. Dolas and U. Joshi, "A novel approach for classification of soil and crop prediction," Int. J. Comput. Sci. Mob. Comput., vol. 7, no. 3, pp. 20-24, 2018.
12. M. B. Villanueva and M. L. M. Salenga, "Bitter Melon Crop Yield Prediction using Machine Learning Algorithm," Int. J. Adv. Comput. Sci. Appl., vol. 9, no. 3, pp. 1-6, 2018.

Table.1. Comparison of Performance Metrics

Algorithms	Types of Crops	Precision	Recall	F-measure	Accuracy (%)
Ripper	Rice	0.678	0.778	0.629	50.2
	Wheat	0.702	0.822	0.731	72.1
	Maize	0.756	0.863	0.764	76.3
	Coconut	0.812	0.797	0.804	93.4
	Sorghum	0.829	0.812	0.820	94.3
	Groundnut	0.866	0.819	0.843	94.1
ANN	Rice	0.904	0.798	0.926	93.5
	Wheat	0.934	0.848	0.941	94.1
	Maize	0.888	0.885	0.903	91.3
	Coconut	0.836	0.811	0.824	93.8
	Sorghum	0.842	0.837	0.840	94.7
	Groundnut	0.889	0.838	0.864	94.5
CP-ANN	Rice	0.919	0.820	0.953	93.5
	Wheat	0.925	0.861	0.943	93.2
	Maize	0.967	0.904	0.921	95.9
	Coconut	0.853	0.832	0.842	94.2
	Sorghum	0.865	0.859	0.862	95.1
	Groundnut	0.909	0.857	0.883	95.0
KHDMCNeupper	Rice	0.984	0.985	0.988	99.3
	Wheat	0.980	0.983	0.987	99.0
	Maize	0.986	0.985	0.991	99.2
	Coconut	0.975	0.975	0.974	97.3
	Sorghum	0.990	0.990	0.986	98.6
	Groundnut	0.980	0.980	0.980	98.1

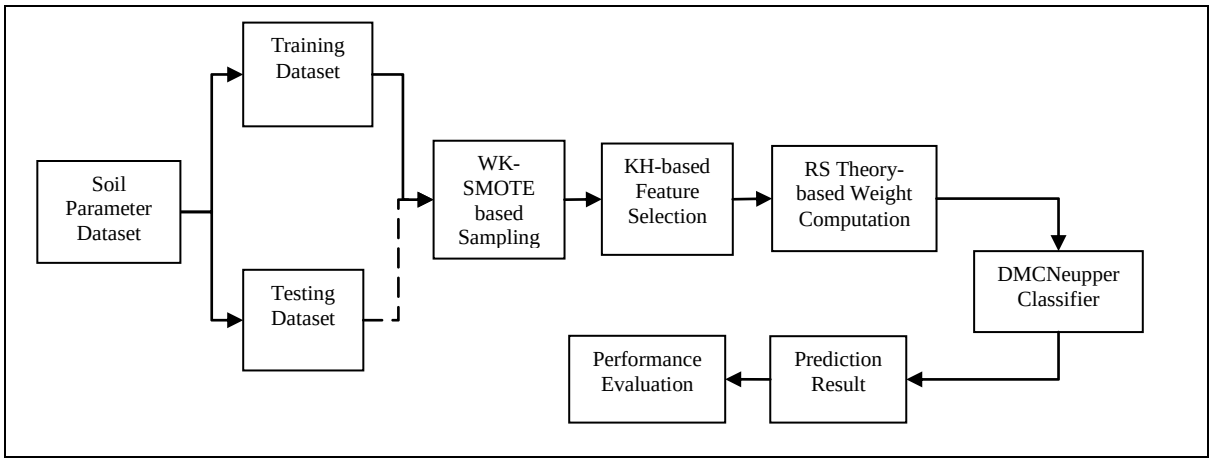


Fig. 1 Block Diagram of KHDMCNeupper Classification-based Crop Yield Prediction

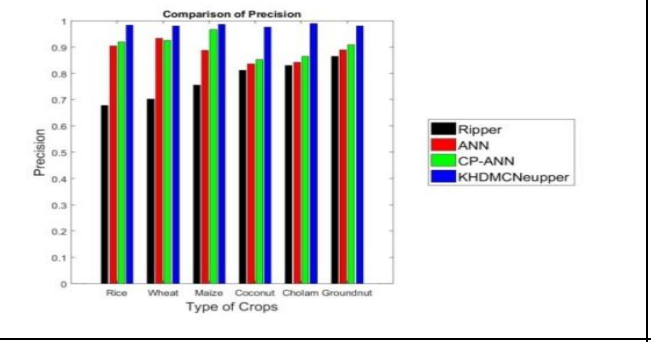


Fig. 2 Comparison of Precision

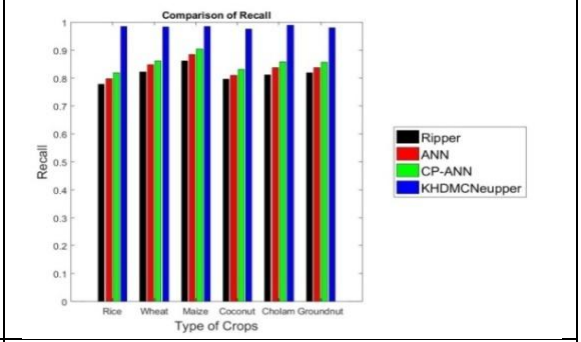


Fig. 3 Comparison of Recall

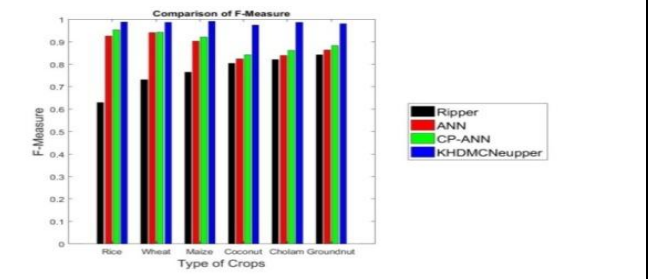


Fig. 4 Comparison of F-measure

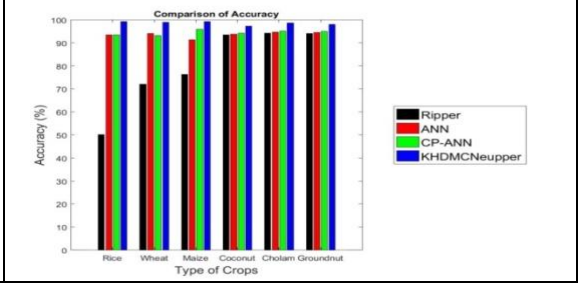


Fig. 5 Comparison of Accuracy

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Population and Distribution of Granivorous Birds in Agricultural Habitats at Selected Villages in Tharangambadi Taluk, Nagapattinam District, Tamil Nadu, Southern India

Vanitha K* and Thomas Nithiyanandam G

Department of Zoology, T.B.M.L. College (Affiliated to Bharathidasan University, Tiruchirapalli), Porayar, Tamil Nadu, India.

Received: 25 Nov 2020 Revised: 27 Dec 2020 Accepted: 02 Jan 2021

***Address for Correspondence**
Vanitha K
Department of Zoology,
T.B.M.L. College (Affiliated to Bharathidasan University, Tiruchirapalli),
Porayar, Tamil Nadu, India.
Email : joshi.chrisson@gmail.com

ABSTRACT

Granivorous birds play an important role in agriculture and they cause crop damage to lesser extent. It considered being species which feed on seeds, droplets and fruits of plants in general. In the present study 33 bird species were recorded at three villages in agricultural habitat. 22 crop damaging bird species including granivorous birds were recorded. Maximum population of bird species recorded were House sparrow, Baya weaver bird, Small green bee eater, House crow and Common myna. Minimum population of Asian koel, Indian Peafowl, Paddy field pipit, White bellied tree pie and Golden oriole were recorded. The density estimates among villages of all species were statistically significant (df = 2; F=0.004; P > 0.05). Shannon-Weiner diversity index, in different villages among seasons showed high values during Post-Monsoon (H= 2.5514; n= 22) and lower values during summer (H= 1.9018; n=22) seasons. The moderate diversity index was recorded during Pre-Monsoon (H= 2.1183; n=22) and Monsoon (H= 2.1032; n=22) seasons. Among the order of granivorous birds, Passeriformes have shown huge number of species raid the agricultural habitats of the study areas. It also showed variation among the species distribution in three different villages of Tharangambadi Taluk, Nagapattinam District. In the present investigation concluded that, the agricultural fields facilitate the food sources and refuge for various avian communities and mainly for the granivorous birds. So, it acts as a pest in the study area. It is not surprising that the best known impact of granivorous birds is not an economical one.

Keywords: Granivorous birds, population, agricultural fields, density, diversity

INTRODUCTION

Granivorous birds play an important role in agriculture and are very well studied by naturalist throughout India and its sub content [1]. Birds of agricultural areas, therefore, include granivorous, frugivores, insectivores, carnivores, nectarivores and omnivores. O' Connor and Shrubb [2] pointed out that the agricultural fields provide most appropriate food sources to the avian community including granivorous birds. Bird's positive and negative roles in agriculture production were well stated by many scientists. Granivorous birds are considered to be species which feed on seeds, droplets and fruits of plants in general. Not enclosed in such a definition, however, is feeding on (or the transportation of) pollen, although pollen of a number of plant species is able to germinate or produce seedlings [3]. Habitat is determining the avian distribution and number of birds in that particular area and is the key species in an agricultural ecosystem for maintaining the natural balance [4]. Various aspects of Granivorous birds such as its diet, foraging behaviour, damage on crops, population dynamics have been well documented in certain parts of India [5,6]. The distribution of bird species in agricultural fields depends more upon agriculture practices, vegetation structure, intensity of land use pattern, availability of water and using up of insecticides on agricultural land [7]. Many factors are influencing the avian density and diversity. Especially the granivorous bird species in agricultural areas are influenced by the several abiotic and biotic factors. Trees and shrubs have a particularly positive effect on bird diversity, and tree height, tree density, and plant diversity are important factors affecting species richness [8,9].

Vast knowledge and species conservation in a geographical area is a prerequisite for estimating the granivorous species in agricultural fields and would help us to know the population structure and developing avian species conservation issues [10]. Assessment of bird species assemblages which emphasized for monitoring ecosystem conditions and would help us identification of beneficial and pest species in many agricultural fields [11]. They further added, now a days agricultural fields and related habitats hampering due to various agricultural practices and using dangerous pesticides and fertilizers have been attributed as primary factor towards lowering the avian species diversity including granivorous birds. According to Conroy and Noon [12] to understand the conservation status of avian species in agricultural areas, we must have information on the distribution of the species, and occupancy of available and relative population estimates. There are about 8,640 species of birds worldwide, of which 2,060 species belonging to different families are found in India and Pakistan [13]. Apparently the Indian bird population has been dwindling due to direct and indirect impact from increasing human population and intensive modern agricultural practices [14]. Modern agricultural practices generate increasing land–use change worldwide [15]. The aim of the present study is to examine population and distribution of granivorous birds in agricultural habitats. The population of granivorous birds was carried out between months and seasons and to discusses the implications of these data for conservation activities and to recommend future surveys in other related areas. Hence the present study is designed with to make a survey of Granivorous birds in agricultural habitat, to list down the birds prevalent in the study area and to develop a check list of Granivorous birds.

MATERIALS AND METHODS

Study Area

The present study area is located in the in the Tharangambadi Taluk of Nagapattinam district. The district lies between $10^{o}25'$ and $11^{o}40'$ North Longitude and 79^{o} 49′ and 80^{o} 01′ East latitude of Tamil Nadu, India. Tharangambadi Taluk study area is a semi urban which is criss crossed by number of metal and kutcha roads. The study are bears various habitats such as agricultural area, shrubland, groove, human habitation, riverine bed, waste land etc. The river Cauvery runs through the study area which ends in Poompukar. The river Cauvery is chief water source of agricultural. This area receives north east monsoon during October to December. The Bay of Bengal existed east of just five Km from the study area. In the study area number natural water bodies existed viz., Wells, Ponds, Puddles. The villagers pump out water through motor for their agriculture activities also. People in the study area depend more upon the agricultural activities.

Paddy, banana, coconut, ground nut, sugar cane, cereals, pulses are grown here. Unaware farmers are using number of pesticides and insecticides for their crops and cut the trees for fire wood.

Granivorous bird survey in agricultural area

The present study was carried in agricultural habitats of three selected villages viz., Singanodai, Natchathiramalai, Thirukkadaiyur in Tharangambadi Taluk of Nagapattinam district, Tamil Nadu, India from January 2018 to December 2018. Though the study area bears number of habitats, the present study was attempted only in agricultural habitat. Data on Granivorous (crop damaging) birds were carried out for the conservation point of view with respect to Granivorous birds. Approximately 15 km^2 area was covered for intensive study. The months are passing through four seasons such as pre monsoon (June–August), monsoon (September – November), post monsoon (December – February) and summer (March – May). The Granivorous (crop damaging) bird survey was conducted on foot in agricultural habitat in three selected villages. Observations were made with 7x50 binocular, mainly in the morning and evening for Granivorous bird location and identification. Photographs were taken using Nikon 16x50CF binocular and Digital Still Camera (4x Zoom) were used for observations and recorded data. The bird species were confirmed by using field guides of Ali and Ripley [13] and Grimmett *et al*., [16]. No census was done on days with heavy rain and fog.

Abundance and density

The total Granivorous (crop damaging) birds seen in each month was calculated using the census data. Similarly the density of birds in each area, and individual abundance of selected species, was also calculated. The number of birds of each species is recorded in the data sheet. A bird call was considered to be equivalent to a single individual, and was used, along with sighting records, for density estimation. Common and scientific names of birds are based on Manakadan and Pittie [17]. The abundance of granivorous birds was estimated by adopting Line Transect Sampling method as mentioned by Laake *et al*., [18]. Totally 15 line transects of one km length and 30 mt. width on each side were laid in agricultural habitats in three villages. Each village five transects were laid and data were taken. Care was taken not to cross or overlap the transect. All transects were sampled immediately after the Sun rise and normally from 06.00 to 08.00 hrs. The data so obtained was extrapolated to estimate as density (birds/Sq km).

Data analysis

Univariate statistical analysis was conducted for data analysis by using SPSS version 16.0. Kruskal-Wallis one-way ANOVA was tested for population variation among seasons and villages. Results are reported as significant if they are associated with a value of $P < 0.05$. Diversity was calculated using Shannon-Weiner Index ($H` = \Sigma(pi \ln pi)$).

RESULTS

A large number of Granivorous birds, whereas low number of other birds was noted down. Among counts insectivorous, piscivorous, omnivorous, frugivorous, granivorous, carnivorous and nectarivorous birds were also included in agricultural habitat. These other bird species were timely visitors to the agricultural habitat for various purposes such as nesting, feeding, roosting etc. Paddy, Ground Nut, Green gram, Black gram, Cotton, Maize, Sesame, Sunflower, Banana, Sugar Cane and Red Beans crops were cultivated in the study area. During the present study a total number of 22 granivorous birds were documented, represented by 21 genera of 18 families from study areas. Some of them come under diversivority (omnivorous, frugivorous and insectivorous) categories. They cause crop damage also. The maximum population density of bird species recorded were House sparrow (1166.7 Birds/km^2) (23.6%) followed by Baya weaver bird (1100.0 Birds/km^2) (22.2%), small bee eater (400.0 Birds/km^2) (8.1%), House crow (416.7 Birds/km^2)(8.4 %), and Common myna (270.8 Birds/km^2) (5.5%). However, minimum population of bird species recorded were Asian koel (58.3 Birds/km^2) (1.2%), Indian Peafowl (41.7 Birds/km^2) (0.8%), Paddy field pipit (37.5 Birds/km^2) (0.8%), White bellied treepie (33.3 Birds/km^2) (0.7%) and Golden oriole (29.2 Birds/km^2) (0.6%). Among the families of birds, Corvidae and Columbidae were more number of species. IUCN status was showed

most of them least concern and few are under not evaluated (Table 1). The agricultural habitat afforded that much support like food, refuge and suitable environmental factors such as water, soil etc. The density estimates among villages of all avian species were statistically significant (df = 2, F= 0.004, P > 0.05). Among the order of granivorous birds, Passeriformes (13 species; 59%) has huge number of species followed by other order (Fig. 1).

Diversity Index

Variations in the diversity of Granivorous (crop damaging) birds, based on Shannon-Weiner diversity index, in different villages among seasons was carried out. The overall result revealed that the Diversity index showed high values during Post-Monsoon (H= 2.5514; n= 22) and lower diversity index was recorded during summer (H= 1.9018; n=22). The moderate diversity index was recorded during Pre-Monsoon (H= 2.1183; n=22) and Monsoon (H= 2.1032; n=22) (Table 2). The village wise differentiation among seasons, the results expressed that the high values was recorded in Natchathiramalai village (H= 2.9013; n=22) during Post-Monsoon. However, the low value was recorded in Thirukkadaiyur village (H= 1.0840; n=22) during summer (Table 2).

DISCUSSION

In the present investigation was carried out the density and diversity of granivorous birds in three different villages of Tharangambadi Taluk, Nagapattinam District. 22 granivorous birds were recorded through line transect methods. According to Gaston [19] that the Line Transect Method is the simplest method to get an index of bird population and can be carried out at any time of year. Density varied among the species as well as villages were studied. It might be due to the changes associated with variations in availability of food or unusually favorable climatic conditions in the study areas [20]. Human activity, pesticide use, alteration of vegetation pattern in the present study area might also be one of the reasons for such variations in the avian community. Among the order of birds recorded, the Passeriformes has huge number followed by other order. Turcek [3] contributed that, 63% of birds belong to order Passeriformes and 66% of bird species considered are also rather small, weighting less than 100 grms while 114 species birds recorded in European country. According to Urfi [21] diversity is the most frequently adopted criterion for evaluation of conservation schemes. Diversity index was calculated to know the diversification birds in the study areas. Overall diversity index was showed in post monsoon followed by pre monsoon, monsoon and summer. Diversity indices are directly correlated with the stability of the ecosystem and will be high in biologically controlled systems. All diversity indices have limitations because they attempt to combine a number of variables that characterize community structure. The evaluation of the study area shows the rich and undistributed species diversity of birds. In the present investigation the diversity index showed marked variations among months. Conservation of global biodiversity has become the issue of prime importance in recent decades [22- 24].

Distribution patterns of avian community have varied among the species depending on the biotic and abiotic environmental factors. The agricultural habitat of the present study area possessing various cultivation and the villages are is greenery and availability of food is sufficient for Granivorous birds. As a result, the Granivorous bird density is high in this area. Many species of birds found only in this habitat. The agriculture field has a good number of short trees, hedges, crops and insects and hence some species of population is found too low in agricultural habitat exhibited that they have alternate food habitual. Cutting of tree branches and firewood collection may be stopped in the study area. Public awareness programmes may be implemented to safe guard the Granivorous species in the present study area. The education in the value of conservation is the most economic way of protecting avian community. Using Pesticides and insecticides should be stopped in the agricultural fields as these substances would enter in to the body of all avian categories. Cutting of trees and removing of shrubs and herbs should be avoided. These may facilitate the food sources, roosting and refuge for various avian communities including Granivorous species.

ACKNOWLEDGEMENTS

We thank the Management and the Principal, T.B.M.L College, Porayar, Nagai District, Tamil Nadu, for providing necessary facilities to do this research work successfully. We thank the Professor and Head, Post Graduate and Research Department of Zoology for having provided logistic support for this research.

CONFLICT OF INTEREST

The author(s) declare(s) that there is no conflict of interest.

REFERENCES

1. Ali S. Sunder Lal Hora memorial lecture. Ornithology in India: its past, present and future; *Proc. Indian Natl. Sci. Acad.*, 1971; B37: 99-113.
2. O'Connor R, Shrubb M. *Farming and birds* (Cambridge: Cambridge University Press) (1986).
3. Turcek FJ. Granivorous birds in Ecosystems, Intern. Stud. Sparrows, 2010; 34: 5-7.
4. Haslem A, Bennett AF. Birds in agricultural mosaics: the influence of landscape pattern and countryside heterogeneity. Ecological Applications, 2008; 18: 185–196.
5. Bibby C, Jones M. Marsden S. Expedition Field Techniques: Bird Surveys, Royal Geographical Society, 1998. P. 143.
6. Balasubramanian P, Maheswaran B. Frugivory, seed dispersal and regeneration by birds in south Indian Forests. J. Bombay Nat. Hist. Soc. 2003; 100 (2&3): 411-431.
7. Luck GW, Daily GC. Tropical countryside bird assemblages: richness, composition and foraging differ by landscape context. *Ecol Appl*. 2003; 13: 235–247.
8. Thiollay JM. The role of traditional agroforests in the conservation of rain-forest bird diversity in Sumatra. *Conserv Biol*. 1995; 9: 335–353.
9. Harvey CA, Medina A, Sa´nchez ADM, Vilchez S, Hernandez B, Saenz JC, Maes JM, Casanoves F, Sinclair FL. Patterns of animal diversity in different forms of tree cover in agricultural landscapes. *Ecol Appl*. 2006; 16: 1986–1999.
10. Gopi Sundar KG Kittur S. Can wetlands maintained for human use also help conserve biodiversity? Landscape-scale patterns of bird use of wetlands in an agricultural landscape in north India. *Biological Conservation*. 2013; 168: 49–56.
11. Dhindsa MS, Saini HK. Agricultural ornithology: An Indian perspective. *Journal of Biosciences*. 1994; 19(4): 391–402.
12. Conroy MJ, Noon BR. Mapping of species richness for conservation of biological diversity: Conceptual and methodological issues. *Ecological Applications*, 1996; 6: 763-773.
13. Ali S, Ripley SD. A Pictorial Guide to the Birds of the Indian Subcontinent. Bombay Natural History Society, Oxford University Press, Bombay. 1983. P. 177.
14. Bibby CJ, Burgess ND, Hill DA. Bird census techniques. Academic Press Limited, London. 2000. P. 267.
15. Yoganand K., Davidar P. Habitat preferences and distributional status of some forest birds in Andaman Islands. *Journal Bombay Natural Society*, 2000; 97(3): 375-380.
16. Grimmett R, Inskipp C, Inskipp T. Birds of India, Pakistan, Nepal, Bangladesh, Bhutan, Srilanka and the Maldives. Princeton University Press, Princeton, New Jersey. 1999. P. 384.
17. Manakadan R. Pittie A. Standarised common and scientific names to birds of the Indian subcontirent. *Buceros*, 2001; 6(1): 1-38.

18. Laake JJ, Bckland ST, Anderson DR, Burnham KP. Distance user's guide. Version 2. Colorado coop. Fish Wildl. Res. Unit. Colorado State Univ. Fort. Collins. 1993. P. 72.
19. Gaston AJ. Census techniques for Himalayan pheasants including notes on individual species. *J. World Pheasant Assoc*. 1980; 5: 40-53.
20. Jarvinen A. Breeding strategies of hole nesting passerines in northern Lapland. *Ann. Zool. Fenn*. 1983; 20 (1): 129-149.
21. Urfi AJ. A bird-count at Nalsarovar: experiences of a participant. *Newsl. Birdwatchers*, 2000; 40: 33–34.
22. Parasharya BM, Dodia JF, Yadav DN, Patel RC. Sarus crane damage to paddy crop; *Pavo*. 1986; 24: 87-90.
23. Turner BL, Clark WC, Kates RW, Richards JF, Mathews JT, Meyer WB. The Earth as Transformed by Human Action. Cambridge University Press and Clark University, Cambridge. 1990.
24. Ehrlich PR, Wilson EO. Biodiversity studies: science and policy. *Science* 1991; 253: 758–762.

Table 1. Overall Density estimation of Granivorous (crop damaging) birds recorded in three different villages in the study area from January 2018 to December 2018.

S. No.	Common Name	Scientific Name	Order	Family	Villages			Overall /km²	%	IUCN status
					1*	2*	3*			
					Birds /km²	Birds /km²	Birds /km²			
1	Spotted dove	*Spilopelia chinensis (Scopoli, 1786)*	Columbiformes	Columbidae	87.5	25	175	95.8	1.9	LC
2	Blue rock pigeon	*Columba livia (Gmelin, 1789)*		Columbidae	125	100	275	166.7	3.4	LC
3	Indian roller	*Coracias benghalensis (Linnaeus, 1758)*	Coraciiformes	Coraciidae	50	100	125	91.7	1.9	LC
4	Small bee eater	*Merops orientalis (Latham, 1801)*		Meropidae	312.5	437.5	362.5	400	8.1	LC
5	Asian Koel	*Eudynamys scolopacea (Linnaeus, 1758)*	Cuculiformes	Cuculidae	50	62.5	62.5	58.3	1.2	LC
6	Indian Peaowl	*Pavo cristatus (Linnaeus, 1758)*	Galliformes	Phasianidae	50	25	50	41.7	0.8	LC
7	House sparrow	*Passer domesticus (Linnaeus, 1758)*	Passeriformes	Passeridae	1062.5	1125	1312.5	1166.7	23.6	LC
8	Baya weaver	*Ploceus philippinus (Linnaeus, 1766)*		Ploceidae	1350	1187.5	762.5	1100	22.2	LC
9	Paddy Field pipit	*Anthus rufulus (Vieillot, 1818)*		Motacillidae	25	62.5	25	37.5	0.8	LC
10	Black headed munia	*Lonchura atricapilla (Linnaeus, 1766)*		Estrildidae	200	125	187.5	170.8	3.5	LC
11	Common myna	*Acridotheres tristis (Linnaeus, 1766)*		Sturnidae	437.5	225	275	270.8	5.5	LC
12	Black drongo	*Dicrurus macrocercus (Vieillot, 1817)*		Dicruridae	62.5	87.5	50	66.7	1.3	LC
13	Common babbler	*Turdoides caudata (Dumont, 1823)*		Leiothrichidae	175	250	187.5	204.2	4.1	NE

14	Red-vented bulbul	*Pycnonotus cafer (Linnaeus, 1766)*	Passeriformes	Pycnonotidae	62.5	87.5	125	91.7	1.9	LC
15	Purple sunbird	*Nectarinia asiatica (Latham, 1790)*		Nectariniidae	87.5	125	25	79.2	1.6	NE
16	House crow	*Corvus splendens (Vieillot, 1817)*		Corvidae	550	262.5	437.5	416.7	8.4	LC
17	Jungle crow	*Corvus macrorhynchos (Wagler, 1827)*		Corvidae	150	100	125	125	2.5	LC
18	White bellied treepie	*Dendrocitta leucogastra (Gould, 1833)*		Corvidae	25	50	25	33.3	0.7	LC
19	Eurasian Golden oriole	*Oriolus oriolus (Linnaeus, 1758)*		Oriolidae	25	25	37.5	29.2	0.6	LC
20	Indian Pond heron	*Ardeola grayii (Sykes, 1832)*	Pelecaniformes	Ardeidae	175	125	25	108.3	2.2	LC
21	Little egret	*Egretta garzetta (Linnaeus, 1766)*		Ardeidae	187.5	112.5	100	133.3	2.7	LC
22	Rose ringed parakeet	*Psittacula krameri (Scopoli, 1769)*	Psittaciformes	Psittacidae	37.5	62.5	87.5	62.5	1.3	LC

Table 2. Shannon and Weiner diversity index of Crop damaging birds recorded in various seasons at three different villages.

S.No	Season	Name of the Village			Overall
		Singanodai	Natchathira malai	Thiruk kadaiyur	
1	Pre-Monsoon	1.8022	2.0154	1.8210	2.1183
2	Monsoon	2.7812	2.1034	2.0018	2.1032
3	Post-Monsoon	2.7203	2.9013	2.7519	2.5514
4	Summer	1.3210	1.7022	1.0840	1.9018

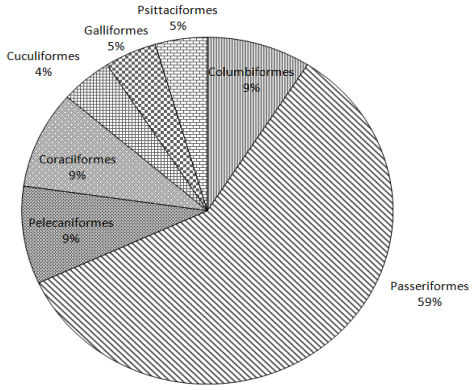


Fig. 1 Order wise granivorous bird contribution in agricultural field during the study period in the study area.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antibacterial Activity of Different Tissue Extracts of Marine Bivalve (*Donax variabilis*) against Selected Bacterial Strains

Thilagavathi M and Christy Ponni A*

Research Department of Zoology, TBML College, Porayar-609 307, Tamil Nadu, India. (Affiliated to Bharathidasan University, Tiruchirappalli – 24).

Received: 20 Nov 2020 Revised: 20 Dec 2020 Accepted: 05 Jan 2021

***Address for Correspondence**
Christy Ponni A
Research Department of Zoology,
TBML College, Porayar-609307, Tamil Nadu, India.
Email: zoology049@gmail.com

ABSTRACT

The objective of the present study is to evaluate the antibacterial activity of the different tissue extracts of *Donax variabilis* against human and fish pathogens. The different tissues of *D variabilis* (gill, foot and muscle) were separately immersed in methanol and steeped overnight in the cold at -20 °C. Antibacterial effects at different concentrations (20, 40, 60 and 80 µL) have been tested against four human pathogens. A greater degree of human pathogen bacterial inhibition was revealed by the gill and foot methanol extract. The gill and foot extracts of the bacterial strains showed a higher degree of inhibition. The highest zone of inhibition of the gill and foot methanol extract was shown against *P. vulgaris* and *E coli*. The broad antibacterial spectrum activity of *D. variabilis* tissue extracts. *D. variabilis* shows that it may have metabolites that are biologically active.

Keywords: *Donax variabilis*, bivalve, gill, foot, muscle, methanol extract

INTRODUCTION

The marine environment is an immense source for bioactive natural products to be discovered. A wide range of bioactive substances are isolated and characterized from food derived from the marine environment, several of which are highly promising for human and fish disease treatment. For the past two decades, because of single resistant determinants, the pharmaceutical industry has been relatively successful in overcoming problems. However, the use of many major classes of antimicrobial compounds has been limited by the advent of multiple resistant mechanisms. With the increased incidence of bacterial infections, the demand for effective and non-toxic antibacterial therapeutics has become even higher. The discovery of new antimicrobial compounds with less environmental and toxicological risks and no resistance created by pathogens is of vital interest (Chellaram et al., 2003). To date, approximately 7,000 marine natural products have been reported in marine invertebrates, 33 percent

from sponges, 18 percent from coelenterates (sea whips, sea fans and soft corals), and 24 percent from representatives of other invertebrate phyla such as ascidians (also known as tunicates), opisthobranch molluscs (nudibranches, sea hares, etc.), echinoderms (starfish, sea cucumbers, etc.) and echinoderms (starfish, sea cucumbers, etc) (moss animals). To date, 5070 Mollusca species have been recorded in India, of which 3,370 are from the marine environment (Venkataraman, 2005). Molluscs are considered to be one of the main sources of bioactive compounds that have anti-tumor, antimicrobial, anti-inflammatory and antioxidant activities (Anbuselvi et al. 2009; Chellaram et al., 2004; Benkendorff et al. 2011). Molluscs are also rich in nutrients that are useful to people of all ages (Anand and Edward, 2002). Mollusc-isolated compounds have also been used to treat rheumatoid arthritis and osteoarthritis (Chellaram and Edward 2009). Mollusc extracts have also shown antiviral and antibacterial activity against fish-pathogenic bacteria and may also be used in aquaculture (Darabpour et al. 2010).

Among marine invertebrates, the significant source of bioactive substances is molluscs. It is considered that bioactive compounds isolated from gastropods play a role in the chemical defense of animals against their predators. Molluscs are a common sight in the oceans and are an almost untapped resource for the discovery of new compounds. Several studies of antitumor, antileukemic, antibacterial and antiviral bioactive compounds from molluscs have been reported worldwide in Phyllidae sp. Bivalves (Chellaram and Edward, 2009) and gastropods (Kagoo and Ayyakannu, 1992; Ilagedone et al., 1999). Bioactive metabolites from molluscs such as sea hare (Schmitz, 1993), *Chromodoris* sp (Morris, et al., 1990), *Onhidella* sp (Ireland, et al., 1993) were isolated and structurally elucidated. There is currently an increasing interest in the bioactivity of molluscan extracts and secondary metabolites, although there is a small proportion of the total secondary metabolites investigated from molluscan species (< 1%). Some marine gastropods and bivalves have been of great interest to chemists of natural products, yielding a variety of chemical classes and several drug outcomes currently in clinical trials. Many molluscs mantle cavity produces mucus e.g. Muricid gastropods (rock snail) which defend the developing larvae against microbial infection (Benkendorff, et al., 2011). In recent years, human pathogenic microorganisms have developed resistance in response to the indiscriminate use of commercial antimicrobial drugs commonly employed in the treatment of infectious diseases (Elumalai, et al., 2011). Many classes of anti-tumor, anti-leukemic, antibacterial and antiviral bioactive compounds have been reported previously (Santhi et al., 2011; Anand and Edward, 2002; Rajaganapathi et al., 2001). In the present study, considering the importance of natural marine products, an attempt was made to investigate the antimicrobial activity of the various tissue extracts of *Donax variabilis*.

MATERIALS AND METHODS

Collection and extraction of samples: Live specimens of *D. variabilis* were collected and brought to the laboratory immediately and were removed by breaking the shells from their soft bodies. The muscle, foot and gill of the whole body of the sample (50 g) were cut into small pieces and the tissue sample was used with methanol solvent for extraction. The extracts were cold steeped at -18 °C overnight and filtered with filter paper from Whatman No. 1. The filtrate was poured into a previously weighted Petri dish and evaporated in the rotary evaporator to dryness.

Bacterial strains: For antimicrobial assay against human pathogens, methanolic crude extracts were used: *Escherichia coli*-MTCC 1687 (*E. coli*), *Proteus vulgaris*-MTCC 7299 (*P. vulgaris*), *Klepsiella pneumoniae*-MTCC 650 (*K. pneumoniae*), *Bacillus subtilis*-MTCC 4411 (*B. subtilis*). IMTECH, Chandigarh, India, obtained all of the pathogenic bacterial strains.

Agar well diffusion method: Firstly, using a sterile cotton swab in various plates respectively, the entire agar surface was streaked with the swab for 4 times with different bacteria. The inoculum was permitted to dry for 5 minutes. A well with a diameter of 6 to 8 mm is then aseptically punched with a sterile cork borer tip and different volumes (20 µl, 40 µl, 60 µl and 80 µl) of the tissue extract solution are introduced into the well at the desired concentration. Different concentrations of different volumes of tissue extracts, e.g foot, muscle and gill extract,

antibiotics (positive control Gentamycin (20 µg)) and methanol (negative control) were poured into 6 mm wells and plates were aerobically incubated for 18 hours at 37 °C (Sattanathan et al., 2015). The test materials with antimicrobial activity inhibited the growth of the microorganisms and the medium was visualized around a clear, distinct zone of inhibition. Growth appearance was considered bacteriostatic, whereas bactericidal effect was not considered as growth. By measuring the diameter of the inhibition zone expressed in millimeters, the antimicrobial activity of the test was determined with the help of a measuring scale, the diameter of the inhibition zone (DIZ) was measured. The area of inhibition for each sample was measured using the antibiotic zone scale. The lack of growth around the wells is an indirect measure of the growth capacity of microorganisms. This technique, however, offers many advantages over other techniques: simplicity, low cost, the ability to test huge numbers of microorganisms and antimicrobial agents, and the ability to interpret the results provided (Sattananthan et al., 2014).

Statistical analysis: Data from the triplicate experiments on the inhibitory effects of different tissues of *D. variabilis* were analyzed by one-way variance analysis (ANOVA) using SPSS software (21version) followed by Duncun's multiple range test (DMRT) and standard error ±. For the description of the significant levels, 'P' values at <0.05 were considered.

RESULTS

The antimicrobial activity results (Table 1-3; Fig 1-3). Maximum antibacterial activity was found in the present study on all bacterial strains of Gill, foot and muscle extracts, meaning variation in the inhibition zone was also shown. The highest activity was recorded for Gill extracts (80 µl) with 14±1.5 mm inhibition zone against *Proteus vulgaris* and the lowest activity was recorded for *K. pneunomiae* with 7±1.3 mm inhibition zone and for Gill extract with foot extract approach activity, but muscle extract showed different inhibition zone, muscle extract (20 to 80 µl) with a range of 2±1.4 to 10±2.1 mm on foot extract. The muscle extract was shown to have less antibiotic activity than Gill and Foot extracts in this research. In this case, both controls (Positive control-Gentamycin) recorded the maximum activity of antibacterial antibiotics on all bacterial strains and no negative control activity was observed (methanol). Whereas the gill and foot extracts showed great effects on the same bacteria and other strains of bacteria. The antimicrobial activity of bivalve extracts (*D. variabilis*) was compared with antibacterial antibiotics, showing that the extracts were more active than antibacterial antibiotics.

DISCUSSION

The study of the bioactivity of natural products and their possible pharmacological use has received considerable attention in recent years. Among marine invertebrates, the potential source of bioactive substances is molluscs. Bioactive compounds isolated from molluscs, in particular from gastropods, are considered to play a role in animals' chemical defense against their predators. Several promising lead compounds with anti-inflammatory activity in marine molluscs have been reported (Jayaseeli et al., 2001). However, no scientific research has been undertaken to substantiate the health benefits derived from molluscs in most cases, and the active ingredients in the taxa involved are typically unknown. The first attempt was initiated around the 1950s to locate antimicrobial activity in marine organisms (Kohn, 2001). A large number of marine organisms from a wide range of phylates have since been screened for antimicrobial activity (Saminathan, 1997). Many of these organisms were antimicrobial, although most of the marine-isolated antibacterial agents were not active enough to compete with classical antimicrobial agents obtained from microorganisms (Shanmuganadam, 1995). The highest activity against *P. vugaris* was found to be the lowest activity with muscle methanol extract against *Bacillus subtilis* and *E.coli* in the present study, with methanol extract from *Donax variabilis* gill and foot extracts. As noted in the methanol extract of *Donax faba* (Shanmuganadam, 1995) against the six human pathogens, the results are similar. Positive control methonal extract showed no activity against all strains of bacteria. Immanuel (2000) has prepared solvent extracts from *C. moneta* solvent extract shell powder and investigated the antibacterial effect against three opportunistic human pathogens, such as *P. vulgaris*,

Micrococcus sp. and Sabory discovered that the development of all three pathogens was inhibited. In this study, in almost all extracts, broad spectral antibacterial activity was recorded, which is the study's important finding. (Kagoo and Ayyakkannu, 1992) reported that *Chicoreus ramosus* hypobranchial gland exhibited a wide spectral activity against ten bacterial strains. Because of the tissue extracts, protine rich extracts are the broad spectrum of biological activity of crude extracts (Nagash, et al., 2010), but the muscle extract contains amino acid cysteine with antimicrobial activity (Akerkar, 2009). In order to compare the biological activity of crude marine extracts and antibacterial antibiotics, the extracts showed a clearer effect than the antibacterial antibiotic (Sattanathan et al., 2013). Very few investigations have been performed on the antibacterial properties of internal tissue organs such as *D. variabilis* bivalve extract, gill, foot and muscle methanol, but many of these studies are available for extracts from whole body tissue where the results could be evaluated with those of the current study (Jayaseeli et al .,2001). *B. subtilis* reported wide spectrum antibacterial activity for the methanol extracts when studying the antibacterial activity of *Donax variabilis* (gill, foot and muscle) against extracts four pathogenic bacteria such as *E. coli, K. pneumonia, P. vulgaris*. In conclusion, the current study shows that it is a good source of antibacterial activity agents and would replace existing antibiotics that are inadequate and cost-effective. In order to explore those bioactive compounds and convert them into usable drugs, further studies are essential.

ACKNOWLEDGEMENT

The authors are grateful to the principal, TBML College, Porayar, for providing this study with facilities.

REFERENCES

1. Akerkar, A.S., C.A. Ponkshe and M.M. Indap. 2009. Evaluation of immuno modulatory activity of extracts from marine animals. Indian Journal of Marine Sciences 38 :22-27.
2. Anand, P.T. and J.K.P. Edward, 2002. Antimicrobial activity in the tissue extracts of five species of cowries *Cyprea* sp. (Mollusca: Gastropoda) and an ascidian *Didemnum psammathodes* (Tunicata: Didemnidae). Indian J. Mar. Sci., 31: 239-242
3. Anbuselvi, S., Chellaram, C., Jonesh, S., Jayanthi, L., Edward, J.K.P. 2009. Bioactive potential of coral associated gastropod, *Trochus tentorium* of Gulf of Mannar, Southeastern India. J Med Sci, 9(5): 240–244.
4. Chellaram, C., Edward, J.K.P. 2009. Anti-nocicetive assets of coral associated gastropod, *Drupa margariticola*. Int. J. Pharmacol, 5: 236-239
5. Chellaram. C., Gnanambal, K.M.E., Edward, J.K.P. 2004. Antibacterial activity of the winged oyster *Pteria chinensis* (Pterioida: Pteridae). Indian J Mar Sci, 33(4): 369-372.
6. Benkendorff, K., McIver, C.M., Abbott, C.A. 2011. Bioactivity of the Murex Homeopathic Remedy and of Extracts from an Australian *Muricid* Mollusc against Human Cancer Cells. Evid Based Complement Alternat Med. 879-585.
7. Darabpour, E., Motamedi, H., Nejad, S.M.S. 2010. Antimicrobial properties of *Teucrium polium* against some clinical pathogens. Asian Pac J Trop Med, 3(2):124–127.
8. Elumalai, E.K., M. Ramachandran, T. Thirumalai, P. Vinothkumar, 2011. Antibacterial activity of various leaf extracts of *Merremiae marginata*. Gloer, Elumalai. Asian Pac J. Tropical Biomed, 1: 406-408
9. Ilagedone, M. R., Barreson, B.J., Scheuer, P. J., 1999. Bioactive natural products. Helvetica Chimica. Acta., 62: 2484-2486.
10. Immanuel, G.,Thaddaeus, B.J., Usha, M., 2000. Antipyretic, wound healing and antimicrobial activity of processed shell of the marine mollusc. Tropical. Biomed.,1643-1646.
11. Jayaseeli A, Anand TP and Murugan A, Antibacterial activity of 4 bivalves from Gulf Mannar. Phuket. Mar. Biol. Cent. Publ., 2001; 25: 215-217.
12. Kagoo, E.L., Ayyakannu, K., 1992. Bioactive compounds from *Chicoreusramosus* antibacterial activity-*in vivo.* Phuket. Mar. Biol. Cent. Spl. Publ., 11: 147-150

13. Kohn, A.J., 2001. Feeding in *Conus striatus* and *Conus catus*. Proc. Hawaiian Acd. Science., 31st Ann. Meet., 31.
14. Morris, S.A., De Silva, E.D., Anderson, R.J., 1990. Chromodorane diterpenes from the tropical doridnudibranch, Chromodoriscavae. Can. J. Chem., 69: 768-771
15. Nagash,Y.S., R.A Nazeer, and N.S. Sampath Kumar. 2010. In vitro antioxidant activity of solvent extracts of molluscs (*Loligo duvauceli* and *Donax strateus*) from India. World Journal of Fish and Marine Sciences 2:240-245.
16. Rajaganapathi, J., 2001. Antimicrobial activities of marine molluscs and purification of antiHIV protein. Ph.D. Thesis. India.
17. Saminathan, R., 1997. Biology and Pharmacology of the venomus cone snail *Conus lorosij*, Kiener from the southeast coast of India. M. phil. Thesis, Annamalai University, India.
18. Santhi, V., Sivakumar, V., Thilaga, R. D., 2011. Analgesic, anti-pyretic and anti-inflammatory activities of chloroform extract of Vava, prosobranch mollusc *Purpura persica.* Int. J. Pharmacol. Bio. Sci., 5: 9-15
19. Sattanathan, G., Rajesh.,R., 2013. Antibacterial activity of extract of *Chaetomorpha aerea* against fish pathogens. Ecology and Fisheries. 7 (1): 9-14.
20. Sattanathan. G., Ramasubramanian. V., 2014. Identification And Natural Control Measures of Bacterial Isolates from the Foot Crack of Human Being. International Journal of Institutional Pharmacy and Life Sciences. 4(3): 8-16.
21. Sattanathan. G., Gokulakrishnan, S., Rajesh. R., 2015. Efficacy of *Chaetomorpha aerea* against fish pathogens. LS-An International Journal of Life Sciences. 4(1): 37-40.
22. Schmitz, F.J., Bowden, B. F., Toth, S.I., 1993. Antitumor and cytotoxic compounds from marine Mar. Biotechnol., 1: 197-308
23. Shanmuganadam, P., 1995. Studies on the venom of *Conus figulinus*, lin (Mollusca: Gastropoda) from the Southeast coast of India. Ph.D. Thesis, Annamalai University, India.
24. Venkataraman K, Wafer M., 2005. Coastal and marine biodiversity of India. Indian J Mar Sci, 34(1): 57-71.

Table 1: Antibacterial activity of *(Donax variabilis*) gill extract against in selected bacterial strains

S.No	Bacterial strains	Concentration of extract / Zone of inhibition (mm)					
		20 µg/µl	40 µg/µl	60 µg/µl	80 µg/µl	Positive Control (Gentamycin) 20µg/µl	Negative Control (Methanol)
1	*E. coli*	3±0.2^{a}	5±1.4^{a}	10±0.9^{a}	13±1.9^{a}	10±2.2^{a}	R
2	*Proteus vulgaris*	4±1.3^{ab}	5±1.9^{a}	12±1.5^{b}	14±1.5^{b}	18±1.6^{ab}	R
3	*Klebsiella pneumonia*	R	R	5±1.2^{ab}	7±1.3^{a}	15±1.4^{b}	R
4	*Bacillus subtilis*	R	3±1.4^{a}	10±1.1^{b}	13±2.3^{b}	12±1.5^{a}	R

R – Resistant; The observed values were expressed as mean ± Standard deviation. Mean values with different superscripts are significantly ($p<0.05$) different from each other.

Table 2: Antibacterial activity of (*Donax variabilis*) foot extract against in selected bacterial stains

S.No	Bacterial strains	Concentration of extract / Zone of inhibition (mm)					
		20 µg/µl	40 µg/µl	60 µg/µl	80 µg/µl	Positive Control 20µg/µl	Negative Control (Methanol)
1	*E.coli*	R	4±1.5^{ab}	6±1.2^{b}	11±1.5^{ab}	16±2.5^{a}	R
2	*Proteus vulgaris*	5±0.9^{a}	7±1.2^{a}	11±1.8^{a}	14±.09^{a}	20±2.1^{ab}	R
3	*Klebsiella pneumonia*	5±0.8^{a}	6±1.5^{a}	10±2.1^{a}	13±1.9^{a}	18±1.8^{a}	R
4	*Bacillus subtilis*	R	R	R	5±1.7^{b}	19±2.3^{a}	R

R – Resistant; The observed values were expressed as mean ± Standard deviation. Mean values with different superscripts are significantly ($p<0.05$) different from each other.

Table 3: Antibacterial activity of (*Donax variabilis*) tissues extract against in selected bacterial strains

S.No	Bacterial strains	Concentration of extract / Zone of inhibition (mm)					
		20 µg/ µl	40 µg/ µl	60 µg/ µl	80 µg/ µl	Positive control 20µg/µl	Negative Control (Methanol)
1	*E.coli*	R	R	5±1.3 [a]	7±1.9 [a]	15±2.5 [a]	R
2	*Proteus vulgaris*	2±1.4 [a]	5±1.4 [ab]	5±2.5 [b]	10±2.1 [b]	18±2.2 [a]	R
3	*Klebsiella pneumonia*	R	R	5±1.9 [ab]	7±1.4 [a]	20±2.8 [b]	R
4	*Bacillus subtilis*	R	R	R	R	20±1.8 [a]	R

R – Resistant; The observed values were expressed as mean ± Standard deviation. Mean values with different superscripts are significantly ($p<0.05$) different from each other.

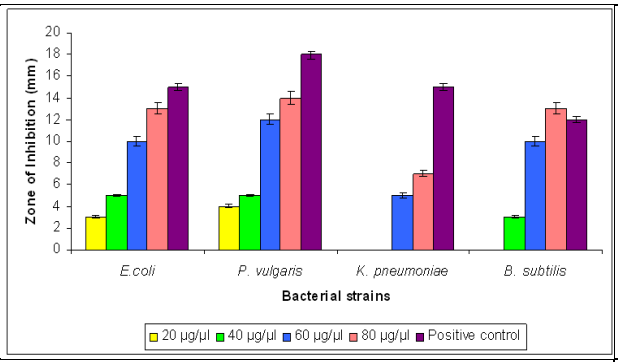


Fig. 1. Antibacterial activity of different concentrations of gill extracts of *D. variabilis* against selected bacterial strains.

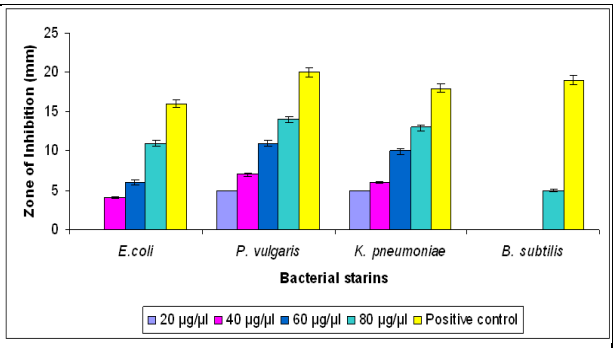


Fig. 2. Antibacterial activity of different concentrations of foot extracts of *D. variabilis* against selected bacterial strains.

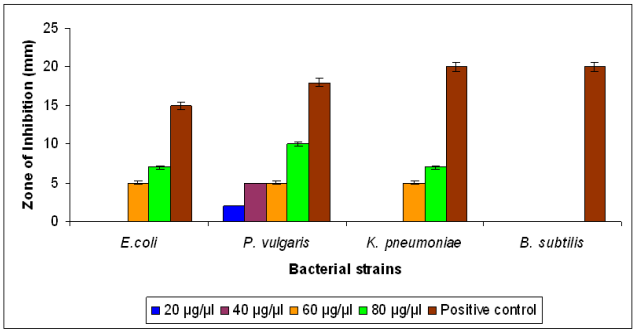


Fig. 3. Antibacterial activity of different concentrations of muscle extracts of *D. variabilis* against selected bacterial strains.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Predictors of Unsuccessful Interim Treatment Outcomes and Culture Conversion among Multidrug Resistant Tuberculosis Patients in Pakistan

Zafar Iqbal[1], Rabab Batool[2]*, Mazhar Ali Khan[3], Shaikh Zafar Ali[4] and Mohammad Asif[5]

[1]Department of Pulmonology, Lady Reading Hospital, Peshawar, Pakistan.
[2]Agha Khan University Hospital Karachi, Pakistan.
[3]Programmatic Management of Drug Resistant TB Unit, Lady Reading Hospital, Peshawar, Pakistan.
[4]King Fahad Hospital, Saudi Arabia.
[5]National TB control Program, Myanmar.

Received: 20 Nov 2020 Revised: 03 Dec 2020 Accepted: 18 Dec 2020

***Address for Correspondence**
Rabab Batool
Agha Khan University Hospital Karachi,
Pakistan.
Email: rabab.jafri88@gmail.com

ABSTRACT

To estimate the six months interim outcomes (IO), culture conversion (CC) time and factors associated with poor IOs and CC failure among Multidrug-Resistant TB (MDR-TB) patients who had previously been treated with second-line drugs (SLDs). A Prospective case series study was conducted at seven Programmatic Management of drug-resistant TB (PMDT) Units of Punjab, Pakistan. All bacteriologically confirmed MDR-TB patients (n=252) presenting at the PMDT units for disease re-treatment between March-2016 to January-2017 were included in the study. Data were statistically analyzed using SPSS version 20.0. Univariate and multiple logistic regressions were used to determine the risk factors responsible for poor IO. The factors associated with CC failure were assessed using the Cox proportional hazards model. The confidence level was taken at 95%, and a p-value < 0.05 was considered statistically significant. A total of 252 re-treatment MDR-TB cases were included. Of them, 40.1% experienced poor IO. Among the significant risk factors associated with poor interim outcomes were a higher number of drugs on the regimen (OR= 1.27, 95% CI: 1.03-1.58) and high sputum smear grading (OR=4.56, 95% CI: 3.30-18.71). Around 70.3% of patients experienced CC within the initial six months of treatment. The significant predictors of unsuccessful CC were older age (OR=0.98, 95% CI: 0.97-0.99), more number of SLDs patient was resistant to (OR=0.78, 95% CI: 0.62-0.98), the higher number of SLDs patient was previously exposed to (OR=0.90, 95% CI: 0.83-0.99), higher number of drugs on the regimen (OR=0.877, 95% CI: 0.80-0.95), treatment with Capreomycin (OR=0.58, 95% CI: 0.42-0.81), resistance to Fluoroquinolones (OR=1.53, 95% CI: 1.15-2.05) and higher baseline sputum smear grading (OR=0.56, 95%

CI: 0.40-0.77). The success rate of interim treatment outcomes was quite low and concerning. The identified risk factors included time to CC, number of SLDs the patient is exposed previously and higher number of drugs in the regimen.

Key words: Multidrug Resistant Tuberculosis, Second Line Drugs, Culture Conversion, Interim Outcomes, Pakistan

INTRODUCTION

A major obstacle in the successful control of TB that challenges the efforts of National TB Control Programmes (NTPs) throughout the World, is MDR-TB. It is defined as the condition in which *Mycobacterium* strains show resistance to two of the main anti-TB drugs, i.e. Isoniazid (INH) and Rifampicin (RMP). The management and treatment of MDR-TB are more complicated than drug-sensitive TB due to prolonged and extensive chemotherapy with SLDs, which are least effective and associated with adverse effects and poor outcomes [1-3]. Treatment of MDR-TB becomes challenging when it relapses or is declared failed. Restarting the treatment requires thorough judgement and poses complexity as these cases have previously been exposed to the MDR-TB regimen and have developed resistance. Hence, designing a regimen amongst a narrow list of drugs is complicated for a physician to prescribe more drugs to intricate the four effective medications on the regimen. Thus, in MDR-TB, a higher number of less effective drugs have more side effects and lower success rates [4-7]. Therefore, for effective treatment and successful outcomes, strict management throughout the treatment and assessment of possible predictors of unsuccessful outcomes. The most important and remarkable indicator for the effectiveness of treatment is Sputum culture (SC), which is performed monthly during the intensive phase (IP) and bi-monthly during the continuation phase (CP) of treatment [8]. It is essential to know the time to sputum culture conversion (SCC) as it is often used as an early predictive value for the final treatment outcome, especially among MDR-TB patients. Literature also suggests that the SCC delay for four-month is a precondition for suspecting treatment failure [9]. So it is crucial to know about the time of CC and factors responsible for it. Treatment category, co-infection with human immunodeficiency virus (HIV), severe radiological findings, a chronic level of disease, resistance to a high number of drugs at the baseline and delay of more than a month in the initiation of treatment are factors responsible for delaying CC [9-12]. Delay in SCC results in poor outcomes and is responsible for prolonging the treatment duration, resulting in economic wastage, poor adherence to treatment, and ultimately an unsuccessful outcome. From a public health perspective, reducing the SCC time is an important measure to control infection.

Different studies have reported CC as an essential predictor for treatment success [10, 13-15]. Successful outcomes have been observed in many countries among MDR-TB patients, where SCC is achieved in the initial months of their treatment. Similarly, it also plays a predictive role in the successful outcome of MDR-TB treatment. A study from Hong Kong suggested that CC in the initial two months predicted 100% successful treatment outcome in MDR-TB patients [16]. Similar results have been reported from the Dominican Republic [17]. Another study suggests that poor outcomes have been reported in MDR-TB patients who failed to achieve CC at the initial two months [18]. The delayed CC has been reported to be primarily associated with previous treatment, previous use of SLDs, severe stage of the disease, and resistance pattern of *Mycobacterium tuberculosis* (MTB) isolates. Identification of such factors responsible for delayed SCC may help in achieving better outcomes of MDR-TB treatment via better management, identifying the disease level in advance and selecting drugs of choice [13-15, 19-22]. Pakistan ranks fourth among the 22 high burden countries in the world for MDR- TB [23]. MDR-TB treatment is widely available in Pakistan through the programs implemented by the NTP and Global Fund. Despite the provision of free treatment, monthly sputum smear and culture monitoring and expansion of the services to more and far areas, studies investigating time to SCC are limited in Pakistan. Therefore our objective was to estimate the six months IO, the time to CC and associated risk factors with poor interim outcomes and failure to culture conversion among MDR-TB cases that had been previously treated with SLDs.

METHODOLOGY

Study Design and Setting

This prospective clinical case series study was conducted, including 252 bacteriologically confirmed MDR-TB patients presented for re-treatment at different PMDT sites including Ghulam Muhammad Mahar Medical College Hospital-Sukkur, Jinnah Postgraduate Medical College- Karachi, Chandika Medical College Hospital-Larkana, Fatima Jinnah Chest Hospital-Quetta, Institute Of Chest Diseases- Kotri, Peoples University Of Medical & Health Science-Nawabshah and Civil Hospital-Mirpurkhas, were enrolled (Figure 1). All patients with declared six months IO were included in this study, and the cases with either extrapulmonary disease, incomplete evaluation or those with missing cultures/smear were excluded. Information was collected using a pre-designed questionnaire inquiring patient's age, gender, site of enrollment, detailed history of previous treatment with anti TB drugs, medical history, history of first line drugs (FLDs), history of SLDs, previous treatment outcome with SLDs, baseline sputum smear microscopy and Drug-susceptibility testing (DST), monthly follow up sputum smear microscopy and culture results. Time to CC was calculated from the initiation of treatment till the patient had two consecutive negative cultures. The results of sputum smear microscopy results were graded as negative, scanty (1-9 Acid Fast Bacilli/100 High Power Fields), Positive 1+ (10–99 AFB/100 HPF), Positive 2+ (1–9 AFB/HPF) and Positive 3+ (>9 AFB/HPF).

Statistical Analysis

The collected data were statistically analyzed using SPSS version 20.0. All continuous variables were displayed using mean and standard deviation. For categorical variables, frequency and percentages were used for presentation. An Independent T-test was used to determine the effects of continuous variables on interim outcomes. The risk factors of poor interim outcomes were determined through univariate and multiple logistic regressions. Risk factors for failure to CC were assessed using the Cox Proportional Hazards Model. Only those variables were included in final multivariate analysis for which the p value was less than 0.25 except the age and gender. The confidence level was taken at 95%, and a p-value < 0.05 was considered as statistically significant.

2)

3) Ethical Considerations

The study was initiated after approval from the Institutional Review Board of Dow University of Health Sciences [Reference # IRB-79/DUHS/Approval/2016/278]. The study objectives were clearly explained to the enrolled patients, and written informed consent was taken from each patient before starting the treatment.

RESULTS

Out of the total 252 MDR-TB patients, 53.6% were male, and most (81.6%) of the enrolled cases were < than 45 years of age. Around 94.0% of patients' had received first-line TB drugs, and 52.0% were declared having failed in their first anti-TB treatment (Table 1). Comorbidities were found among 13.0% of patients of which 7.9% were diabetic (Table 2). The mean number of FLDs to which patients showed resistance was 4.2 ± 0.84, whereas, for SLDs, it was 0.72 ± 0.63. The study patients' treatment regimen included one type of injectable drug, where the majority (65.8%) was treated with Amikacin (AM). After DST, 54.1% of patients were identified with Fluoroquinolone (FQ) resistance (Table 2). Among study cases, 70.3% patients experienced CC within the initial six months of treatment. The mean time to CC was 62.17 ± 35.51 days. Moreover, 59.9% had favorable IO and 12.7% of the study cases died before IO, i.e. 6^{th} month of treatment. Among the IOs reported in the sixth month of the treatment, sputum smear or culture-negative was reported among 60.3% of the patients while sputum smear or culture positive among 23.4% of the patients (Table 3). Clinical characteristics, including time to CC, the number of SLDs to which patient was exposed in the past and the number of drugs in the treatment regimen showed significant association with interim outcomes (Table 4).

Univariate logistic regression showed that older age (OR=1.02, 95% CI: 1.00-1.04), patients previously exposed to SLDs were at the higher risk of poor IO (OR=1.30, 95% CI: 1.09-1.55) and those with a higher number of drugs on their treatment regimen showed greater odds value of poor interim outcomes (OR=1.27, 95% CI: 1.10-1.46). Patients treated with injectable drug Capreomycin were at a higher odd of poor interim outcome than those treated with Amikacin, (OR=3.01, 95% CI: 1.75-5.16). Patients with higher sputum smear grading at the baseline were also at the increased risk of poor interim outcome, i.e. the patients with baseline sputum smear grading Positive 3+/2+/1+ were more likely to have poor interim outcome than those who were sputum smear-negative at the baseline (OR=4.56, 95% CI: 2.30-9.00). However, after adjustment in multiple logistic regression, poor interim outcomes were significantly associated with a higher number of drugs on the regimen (aOR=1.27, 95% CI: 1.03-1.58) and higher sputum smear grading also significantly associated with poor interim outcomes (aOR=7.86, 95% CI: 3.30-18.71) (Table 5).

Cox proportional hazards regression was used to find the association among different patient characteristics and culture conversion. Older age (OR=0.98, 95% CI: 0.97-0.99), the number of SLDs patient was previously exposed to (OR=0.90 , 95% CI: 0.83-0.99), more number of SLDs patient was resistant to (OR=0.78, 95% CI: 0.62-0.98), higher number of drugs on the regimen (OR=0.877, 95% CI: 0.80-0.95), treatment with Capreomycin (OR=0.58, 95% CI: 0.42-0.81), resistance to Fluoroquinolones (OR=1.53, 95% CI: 1.15-2.05) and higher baseline sputum smear grading (Positive 3+/2+/1+) (OR=0.56, 95% CI: 0.40-0.77) were significantly associated with failure to CC within initial six months of treatment. However, in multivariate analysis, the patients who were sensitive to fluoroquinolones were more likely to experience CC than those who were resistant to Fluoroquinolones (OR=2.00, 95% CI: 1.23-3.24). The patients with higher sputum smear grading were 54% less likely to experience culture conversion within the initial six months of treatment with SLDs (OR=0.46, 95% CI 0.31-0.67) (Table 6).

DISCUSSION

The treatment and management of MDR-TB is intricate and challenging for the healthcare providers. There are different indicators for the magnitude of treatment's effectiveness, out of which SCC has been identified as the most important one. This study revealed that 70.3% of re-treatment MDR-TB patients experienced CC within initial six months of treatment which is comparable to a study from India [24] but lower than two other Indian studies displaying 98%, 87% [25,26], also studies from Peru (92.9%) [27] and South Africa (89%) [28]. This difference might be because patients in our study patients were already treated with SLDs, and they were enrolled for re-treatment which is comparatively difficult than the treatment of the normal MDR-TB patients.

In the present study, the median time to SCC was recorded as 62.17 days. This time was less than previously reported in the USA, which showed a median time of 93 days [31], a study from India, 91.3 days [24], and 91 days reported in a study from London [32]. But, on the other hand, Indonesia, Dominican Republic, and Peru, reported decreased SCC median time as compared to our findings i.e. 60 days, 59 days and 56 days respectively [17,27,33,34]. Treatment was easy among those patients who achieved SCC in less time as compared to those for whom SCC time was longer because duration of injectable drugs is directly associated with SCC time. Reducing SCC time is also important for reducing the rate of side effects of Injectable drugs and controlling the infection as sputum positive patients can continuously transmit the disease in the community.

SCC is an important factor and may be used as an early marker for final outcomes, the failure of SCC in the study cases might be due to different factors, e.g. age, bacterial load, previous use of SLDs, number of drugs in the regimen, use of capreomycin and sensitivity to FQ for most of our MDR TB patients. In this study, a negative association was found among older age groups and CC. Older age was significantly negatively associated with timely conversion, which is against a study's findings, including five different countries, which stated that the older age group is not significantly associated with timely culture conversion [24]. But a local study from Pakistan

supports our finding that older ages affect timely CC [35]. The possible reason behind this could be that immunity becomes compromised with poor absorption of drugs at an older age.

Bacterial load and previous exposure to SLDs remain important contributors to delayed SCC. Several studies proved that it takes time to recover from a severe state of disease. Also, if a patient has been exposed to any SLD previously, there is a possibility of resistance to those drugs [24, 27, 31, 33]. Another important factor is the different types of SLDs present in the treatment regimen. As full treatment depends on the regimen, management of treatment regimen must be according to DST result, and no such drugs are added to which resistance has been shown; this strategy of treatment is also discussed by other studies [21, 24, 27, 31, 34]. Another factor positively associated with delayed SCC was a high bacterial load. This finding is also consistent with the results found by Rie et al [12], Qazi et al [21]. and Caetano et al [36]. This may be because patients with a large number of MTB with a higher number of colonies take more time for SCC than those with fewer MTB colonies.

The rate of early conversion in re-treated MDR-TB observed in the current study was encouraging. These patients were previously treated for the same disease with the same SLDs and failed treatment, indicating that the MDR-TB management in the studied region was effective. The presence of trained and dedicated health care workers and trained treatment supporters play an important role in this regard. By the end of the study period, 59.9% of patients achieved successful treatment outcomes, whereas the remaining 40.1% declared unsuccessful interim treatment outcomes (23.4% remained positive, 12.7% died, and 4.0% died). Successful treatment outcomes for re-treatment MDR-TB cases in the present study area were much lower, i.e. it was below the international accepted treatment success rate of 75 – 90%. The percentage of the poor interim outcomes (40.1%) in the study cohort is higher than the percentage of poor treatment outcomes reported by other studies, i.e. 20.4% - 21.3% [13, 14, 19, 29, 30]. The reason for this discrepancy might be the irrational use of drugs in the cohort of the present study, as most of the study cases had unsuccessful outcomes in their previous SLDs treatment.

The death rate (12.7%) was much lower than the other comparative studies [29, 30, 37, 38]. Successful outcomes were lower, and the Interim Treatment failure rate (23.4%) was much higher than previous reports [1, 29, 37, 38]. But as these patients were re-treated, the possibility of immune-compromised and drug resistance significantly increased the failure rate. The loss of follow-up cases among the study cases is lower than previous reports [3, 14, 29, 30, 37, 38]. As in Pakistan, the treatment of MDR-TB is free of cost. The patient is treated according to PMDT rules with dedicated, experienced and well-qualified teams, monthly social support to the patient, and treatment supporter. Home visits play a significant role in lowering the lost to follow up rate. Moreover, if any patient delays or misses his/her monthly visit to the treatment site, the tracing process starts and end with the retrieval of that patient. Despite these efforts, few patients lost to follow up, and the reason behind this might be the prolonged treatment duration and lower socioeconomic status.

CONCLUSION

The high rate of unsuccessfully interim treatment outcomes rate among the studied cohort after treatment with the conventional MDR-TB regimen is quite concerning. The conventional MDR-TB regimen is not adequate to treat the MDR-TB patients with prior exposure to SLDs having poorer outcomes. However, the identified risk factors provides the healthcare providers with an opportunity to ensure improved clinical management by identify high-risk patients and enhancing the treatment success rates.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGMENT

Authors acknowledge the services of Getz Pharma and its Medical Affairs department for support in formatting manuscript.

FUNDING

None.

REFERENCES

1. Khan MA, Mehreen S, Basit A, Khan RA, Jan F, Ullah I, et al. Characteristics and treatment outcomes of patients with multi-drug resistant tuberculosis at a tertiary care hospital in Peshawar, Pakistan. Saudi medical journal. 2015;36(12):1463.
2. Batool R, Imran M, Kandhro AH, Salahuddin N, Uddin MKH. Resistance Patterns among Multidrug-Resistant Tuberculosis Patients: A Multi-Center Study from Pakistan. Int. J. Endorsing Health Sci. Res. 2017;5(4):07-11.
3. Dhingra VK, Rajpal S, Mittal A, Hanif M. Outcome of multi-drug resistant tuberculosis cases treated by individualized regimens at a tertiary level clinic. Indian Journal of Tuberculosis. 2008;55(1):15.
4. Javaid A, Khan MA, Jan F, Rauf M, Basit A, Mehreen S. Occurrence of adverse events in patient receiving community-based therapy for multidrug-resistant tuberculosis in Pakistan. Tuberkuloz ve toraks. 2018;66(1):16-25.
5. Javaid A, Khan MA, Mehreen S, Jan F, Khan MA, Ullah I, et al. Ototoxicity among patients receiving Multidrug-Resistant tuberculosis treatment; Experience from a tertiary care hospital. Pakistan Journal of Chest Medicine. 2018;23(2):31-8.
6. Kanji SI. Tuberculosis: Moving from cure to prevention in Pakistan. Int. J. Endorsing Health Sci. Res. 2015; 3(3):27-30.
7. Javaid A, Khan MA, Khan MA, Mehreen S, Basit A, Khan RA, et al. Screening outcomes of household contacts of multidrug-resistant tuberculosis patients in Peshawar, Pakistan. Asian Pacific journal of tropical medicine. 2016;9(9):909-12.
8. Falzon D, Jaramillo E, Scha HJ, Arentz M, Bauer M, Bayona J, et al. WHO guidelines for the programmatic management of drug-resistant tuberculosis: 2011 update. Eur Respiratory Soc; 2011.
9. Lee J, Lee BJ, Yoon HI, Lee CT, Lee JH. Influence of previous tuberculosis treatment history on acid-fast bacilli smear and culture conversion. The International Journal of Tuberculosis and Lung Disease. 2012;16(10):1344-8.
10. Kurbatova EV, Cegielski JP, Lienhardt C, Akksilp R, Bayona J, Becerra MC, et al. Sputum culture conversion as a prognostic marker for end-of-treatment outcome in patients with multidrug-resistant tuberculosis: a secondary analysis of data from two observational cohort studies. The Lancet Respiratory medicine. 2015;3(3):201-9.
11. Mulu W, Mekkonnen D, Yimer M, Admassu A, Abera B. Risk factors for multidrug resistant tuberculosis patients in Amhara National Regional State. African health sciences. 2015;15(2):368-77.
12. Kanda R, Nagao T, Van Tho N, Ogawa E, Murakami Y, Osawa M, et al. Factors affecting time to sputum culture conversion in adults with pulmonary tuberculosis: a historical cohort study without censored cases. PloS one. 2015;10(11):e0142607.
13. Holtz TH, Sternberg M, Kammerer S, Laserson KF, Riekstina V, Zarovska E, et al. Time to sputum culture conversion in multidrug-resistant tuberculosis: predictors and relationship to treatment outcome. Annals of internal medicine. 2006;144(9):650-9.
14. Kurbatova EV, Gammino VM, Bayona J, Becerra MC, Danilovitz M, Falzon D, et al. Predictors of sputum culture conversion among patients treated for multidrug-resistant tuberculosis. The International Journal of Tuberculosis and Lung Disease. 2012;16(10):1335-43.
15. Lu P, Liu Q, Martinez L, Yang H, Lu W, Ding X, et al. Time to sputum culture conversion and treatment outcome of patients with multidrug-resistant tuberculosis: a prospective cohort study from urban China. European Respiratory Journal. 2017;49(3):1601558.

16. Yew WW, Chan CK, Chau CH, Tam CM, Leung CC, Wong PC, et al. Outcomes of patients with multidrug-resistant pulmonary tuberculosis treated with ofloxacin / levofloxacin-containing regimens. Chest. 2000;117(3):744-51.
17. Rodriguez M, Monedero I, Caminero JA, EncarnaciÃ³n M, Dominguez Y, Acosta I, et al. Successful management of multidrug-resistant tuberculosis under programme conditions in the Dominican Republic. The International Journal of Tuberculosis and Lung Disease. 2013;17(4):520-5.
18. Salaniponi FM, Christensen JJ, Gausi F, Kwanjana JJ, Harries AD. Sputum smear status at two months and subsequent treatment outcome in new patients with smear-positive pulmonary tuberculosis. The international journal of tuberculosis and lung disease: the official journal of the International Union against Tuberculosis and Lung Disease. 1999;3(11):1047.
19. Alakaye OJ. Time to sputum culture conversion of Multidrug-Resistant tuberculosis in HIV positive versus HIV negative patients in Lesotho: University of Pretoria; 2018.
20. Magee MJ, Kempker RR, Kipiani M, Tukvadze N, Howards PP, Narayan KMV, et al. Diabetes mellitus, smoking status, and rate of sputum culture conversion in patients with multidrug-resistant tuberculosis: a cohort study from the country of Georgia. PloS one. 2014;9(4):e94890.
21. Ali S, Inam H, Abbasi Z, Hassan S. Tuberculous lymphadenitis with anthracosis: an unusual cause of unilateral vocal cord paralysis. Int. J. Endorsing Health Sci. Res.2019;7(2):108-112.
22. Shibabaw A, Gelaw B, Wang S-H, Tessema B. Time to sputum smear and culture conversions in multidrug resistant tuberculosis at University of Gondar Hospital, Northwest Ethiopia. PloS one. 2018;13(6):e0198080.
23. Javaid A. Multidrug-Resistant Tuberculosis: Current situation in Pakistan. Pakistan Journal of Chest Medicine. 2018;23(2):28-30.
24. Velayutham B, Nair D, Kannan T, Padmapriyadarsini C, Sachdeva KS, Bency J, et al. Factors associated with sputum culture conversion in multidrug-resistant pulmonary tuberculosis. The International Journal of Tuberculosis and Lung Disease. 2016;20(12):1671-6.
25. Singla R, Sarin R, Khalid UK, Mathuria K, Singla N, Jaiswal A, et al. Seven-year DOTS-Plus pilot experience in India: results, constraints and issues. The International Journal of Tuberculosis and Lung Disease. 2009;13(8):976-81.
26. Joseph P, Desai VBR, Mohan NS, Fredrick JS, Ramachandran R, Raman B, et al. Outcome of standardized treatment for patients with MDR-TB from Tamil Nadu, India. The Indian journal of medical research. 2011;133(5):529.
27. Tierney DB, Franke MF, Becerra MC, Viru FAA, Bonilla CA, Sajnchez E, et al. Time to culture conversion and regimen composition in multidrug-resistant tuberculosis treatment. PloS one. 2014;9(9):e108035.
28. Brust JCM, Gandhi NR, Carrara H, Osburn G, Padayatchi N. High treatment failure and default rates for patients with multidrug-resistant tuberculosis in KwaZulu-Natal, South Africa, 2000â€“2003. The International Journal of Tuberculosis and Lung Disease. 2010;14(4):413-9.
29. Javaid A, Ullah I, Masud H, Basit A, Ahmad W, Butt ZA, et al. Predictors of poor treatment outcomes in multidrug-resistant tuberculosis patients: a retrospective cohort study. Clinical Microbiology and Infection. 2018;24(6):612-7.
30. Khan MA, Mehreen S, Basit A, Khan RA, Javaid A. Predictors of poor outcomes among patients treated for multidrug-resistant tuberculosis at Tertiary Care Hospital in Pakistan. American-Eurasian J Toxicol Sci. 2015;7(3):162-72.
31. Shah NS, Pratt R, Armstrong L, Robison V, Castro KG, Cegielski JP. Extensively drug-resistant tuberculosis in the United States, 1993-2007. Jama. 2008 Nov 12;300(18):2153-60.
32. Rieu R, Chang C, Collin SM, Fazekas J, Dassanaike S, Abbara A, Davidson RN. Time to detection in liquid culture of sputum in pulmonary MDR-TB does not predict culture conversion for early discharge. Journal of Antimicrobial Chemotherapy. 2015 Dec 11;71(3):803-6.
33. Putri FA, Burhan E, Nawas A, Soepandi PZ, Sutoyo DK, Agustin H, Isbaniah F, Dowdy DW. Body mass index predictive of sputum culture conversion among MDR-TB patients in Indonesia. The International Journal of Tuberculosis and Lung Disease. 2014 May 1;18(5):564-70.

34. Kim J, Kwak N, Lee HY, Kim TS, Kim CK, Han SK, Yim JJ. Effect of drug resistance on negative conversion of sputum culture in patients with pulmonary tuberculosis. International Journal of Infectious Diseases. 2016 Jan 1;42:64-8.
35. Laserson, K.F., Thorpe, L.E., Leimane, V., Weyer, K., Mitnick, C.D., Riekstina, V., et al. (2005). Speaking the same language: treatment outcome definitions for multidrug-resistant tuberculosis. The International Journal of Tuberculosis and Lung Disease. 9(6):640-5.
36. Caetano Mota P, Carvalho A, Valente I, Braga R, Duarte R. Predictors of delayed sputum smear and culture conversion among a Portuguese population with pulmonary tuberculosis. Rev Port Pneumol. 2012;18(2):72–9.
37. Tang S, Tan S, Yao L, Li F, Li L, Guo X. Risk factors for poor treatment outcomes in patients with MDR-TB and XDR-TB in China: Retrospective multicenter investigation. PLoS One. 2013; 8(12): 1–8.
38. Orenstein EW, Basu S, Shah NS, Andrews JR, Friedland GH, Moll AP. Treatment outcomes among patients with multidrug-resistant tuberculosis: systematic review and meta-analysis. Lancet Infect Dis. 2009; 9(3): 153–161.

Table 1: Demographic characteristics and previous treatment history of the study participants (n= 252).

Demographic Characteristics		**N (%)**
Gender	Male	135 (53.6)
	Female	117 (46.4)
Age Groups (years)	5 – 24	92 (36.6)
	25 – 34	73 (28.9)
	35 – 44	41 (16.2)
	45 -54	28 (11.2)
	55+	18 (7.1)
Previous history of FLDs	Yes	237 (94.0)
	No	15 (6.0)
Outcome of previous FLD treatment	Cured	15 (6.0)
	Completed	29 (11.6)
	Failed	132 (52.0)
	Loss to Follow up	11 (4.4)
	Not Evaluated	65 (26.0)
Number of SLDs patient is exposed to; Mean ± SD (Range)		4.4 ± 13.4(4 – 5)
Outcome of previous SLD treatment	Cured	3 (1.1)
	Completed	6 (2.3)
	Failed	67 (25.2)
	Loss to follow up	45 (17.0)
	Not Evaluated	145 (54.4)

*First Line Drug (FLD); Second Line Drug (SLD)

Table 2: Clinical characteristics of study participants (n= 252).

Characteristics		N (%)
Registration Groups	Previously treated after relapse	8 (3.4)
	Previously treated after failure	64 (25.4)
	Previously treated after lost to follow up	43 (17.0)
	Not Evaluated	137 (54.4)
Presence of comorbid diseases	Yes	32 (13.0)
	No	220 (87.0)
Comorbid Diseases	Diabetes	20 (7.9)
	Hepatitis B	3 (1.1)
	Hepatitis C	5 (1.8)
	Depression	1 (0.3)
	DVT	1 (0.3)
	Renal Disease	1 (0.3)
	Epilepsy	1 (0.3)
Number of FLDs, patient showed resistant to; Mean ± SD (Range)		4.2 ± 0.84(4 – 5)
Number of SLDs, patient showed resistant to; Mean ± SD (Range)		0.72 ± 0.63(0 – 2)
Number of Drugs on the Regimen; Mean ± SD (Range)		7.2 ± 1.86(5 – 12)
Baseline Sputum Smear Grading Results	Negative	74 (29.4)
	Scanty	21 (8.0)
	Positive 1+	39 (15.4)
	Positive 2+	38 (15.1)
	Positive 3+	81 (32.1)
Injectable	Amikacin	165 (65.5)
	Capreomycin	87 (34.5)
Resistance to Flouroquinolone	Yes	136 (54.0)
	No	116 (46.0)

*First Line Drug (FLD); Second Line Drug (SLD)

Table 3: Mean time to culture conversion and interim outcomes

Variables		N (%)
Time to Culture Conversion (days)*; Mean ± SD (Range)		62.17 ± 35.51(30.0-69.0)
Interim Outcomes†	Negative	151 (59.9)
	Positive	59 (23.4)
	Died	32 (12.7)
	Lost to follow up	10 (4.0)
Interim Outcome Categories‡	Favorable	151 (59.9)
	Poor	101 (40.1)

* n for this category is 187 i.e., patient who experienced culture conversion within 6 months

† n for this category is 252 as interim outcomes are available for 252 patients, the reason for the missing values are either sample contamination or non-expectorant sputum on month 6.

‡ Negative interim outcomes recoded as favorable, and others were recoded as poor

Table 4: Association of patient's clinical characteristics with Interim Outcomes.

Characteristics	Interim Outcome		p-value
	Favorable Outcome 151(59.9%)	Poor Outcome 101(40.1%)	
	(Mean ± SD)		
Time to culture conversion (in days)	52.15 ± 30.75	72.97 ± 42.25	<0.001
Number of SLDs patient is exposed previously	4.16 ± 1.49	4.74 ± 1.52	0.003
Number of SLDs patient is resistant to	0.66 ± 0.62	0.78 ± 0.64	0.139
Number of Drugs in the Regimen	6.92 ± 1.84	7.79 ± 1.76	<0.001

*p-value calculated using Independent t-test

*Second Line Drug (SLD)

Table 5: Factors associated with poor interim outcome using Logistic Regression.

Characteristics	Poor Interim Outcome			p-value
	OR (95% CI)	p-value	aOR (95% CI)	
Age (years)	1.02 (1.00-1.04)	0.005	1.01 (0.99-1.03)	0.223
Gender				
Male	Ref		Ref	0.606
Female	0.87 (0.52 - 1.44)	0.589	1.19 (0.61-2.33)	
Outcome of previous FLD treatment				
Cured	Ref		Ref	
Complete	0.36 (0.09-1.43)	0.150	0.29 (0.04-1.79)	0.186
Failed	0.66 (0.21-2.00)	0.466	0.87 (0.18-4.08)	0.867
Lost to follow up	0.37 (0.06-2.03)	0.256	0.15 (0.01-1.29)	0.086
Not Evaluated	0.80 (0.25-2.55)	0.706	0.50 (0.10-2.54)	0.411
Number of SLDs patient is exposed to	1.30 (1.09-1.55)	0.004	1.25 (0.98-1.61)	0.070
Outcome of previous SLD treatment				
Cured/Complete	Ref		Ref	
Failed	0.94 (0.23 - 3.85)	0.935	1.32 (0.23-7.50)	0.747
Lost to follow up	0.60 (0.14 - 2.57)	0.499	0.80 (0.13-4.69)	0.805
Treatment not evaluated	0.37 (0.09 - 1.46)	0.158	1.02 (0.18-5.64)	0.982
Number of SLDs patient is resistant to	1.35 (0.90-2.02)	0.140	0.86 (0.39-1.92)	0.725
Number of Drugs on the Regimen	1.27 (1.10-1.46)	<0.01	1.27 (1.03-1.58)	0.024
Baseline Sputum Smear Grading Categories				
Negative	Ref		Ref	
Scanty	2.55 (0.84-7.76)	0.097	1.70 (0.32-9.01)	0.527
Positive 1/Positive 2/Positive 3	4.56 (2.30-9.00)	<0.01	7.86 (3.30-18.71)	<0.01
Injectable				
Amikacin	Ref			
Capreomycin	3.01 (1.75-5.16)	<0.01	1.34 (0.63-2.83)	0.439
Resistance to Fluoroquinolone				
Yes	Ref		Ref	
No	0.65 (0.39 - 1.09)	0.100	0.75 (0.28-2.02)	0.570

*OR: Crude Odds Ratio, CI: Confidence Intervals

aOR: Adjusted Odds Ratio, adjusted for all including study variables

*First Line Drug (FLD); Second Line Drug (SLD)

Table 6: Showing the association of patient characteristics and culture conversion using the Cox proportional hazards regression.

Characteristics	Culture Conversion			p-value
	OR (95% CI)	p-value	aOR (95% CI)	
Age (years)	0.98 (0.97-0.99)	0.037	0.99 (0.98-1.00)	0.323
Gender				
Male	Ref		Ref	
Female	1.15 (0.86-1.54)	0.318	0.92 (0.64-1.31)	0.643
Outcome of previous FLD treatment				
Cured	Ref		Ref	
Complete	1.60 (0.71-3.58)	0.251	1.34 (0.53-3.39)	0.532
Failed	1.41 (0.68-2.92)	0.346	0.92 (0.38-2.22)	0.865
Lost to follow up	2.35 (0.92-5.97)	0.072	2.51 (0.87-7.20)	0.086
Not Evaluated	1.95 (0.92-4.14)	0.078	2.07 (0.85-5.05)	0.108
Number of SLDs patient is exposed to	0.90 (0.83-0.99)	0.035	0.99 (0.89-1.11)	0.959
Outcome of previous SLD treatment				
Cured/Complete	Ref		Ref	
Failed	0.98 (0.38-2.50)	0.973	1.12 (0.38-3.28)	0.829
Lost to follow up	1.35 (0.52-3.49)	0.533	1.45 (0.48-4.34)	0.504
Treatment not evaluated	2.12 (0.86-5.20)	0.100	2.11 (0.74-5.96)	0.159
Number of FLDs patient is resistant to	1.11 (0.94-1.31)	0.198	1.07 (0.88-1.31)	0.460
Number of SLDs patient is resistant to	0.78 (0.62-0.98)	0.038	1.30 (0.89-1.90)	0.174
Number of Drugs on the Regimen	0.877 (0.80-0.95)	0.002	0.91 (0.83-1.01)	0.095
Baseline Sputum Smear Grading Categories				
Negative	Ref		Ref	
Scanty	0.61 (0.34-1.10)	0.103	0.62 (0.31-1.21)	0.163
Positive 1+/Positive 2+/Positive 3+	0.56 (0.40-0.77)	<0.01	0.46 (0.31-0.67)	0.000
Injectable				
Amikacin	Ref		Ref	
Capreomycin	0.58 (0.42-0.81)	0.001	0.79 (0.53-1.17)	0.250
Resistance to Fluoroquinolone				
Yes	Ref		Ref	
No	1.53 (1.15-2.05)	0.003	2.00 (1.23-3.24)	0.005

*OR: Crude Odds Ratio, CI: Confidence Intervals

aOR: Adjusted Odds Ratio, adjusted for all including study variables

*First Line Drug (FLD); Second Line Drug (SLD)

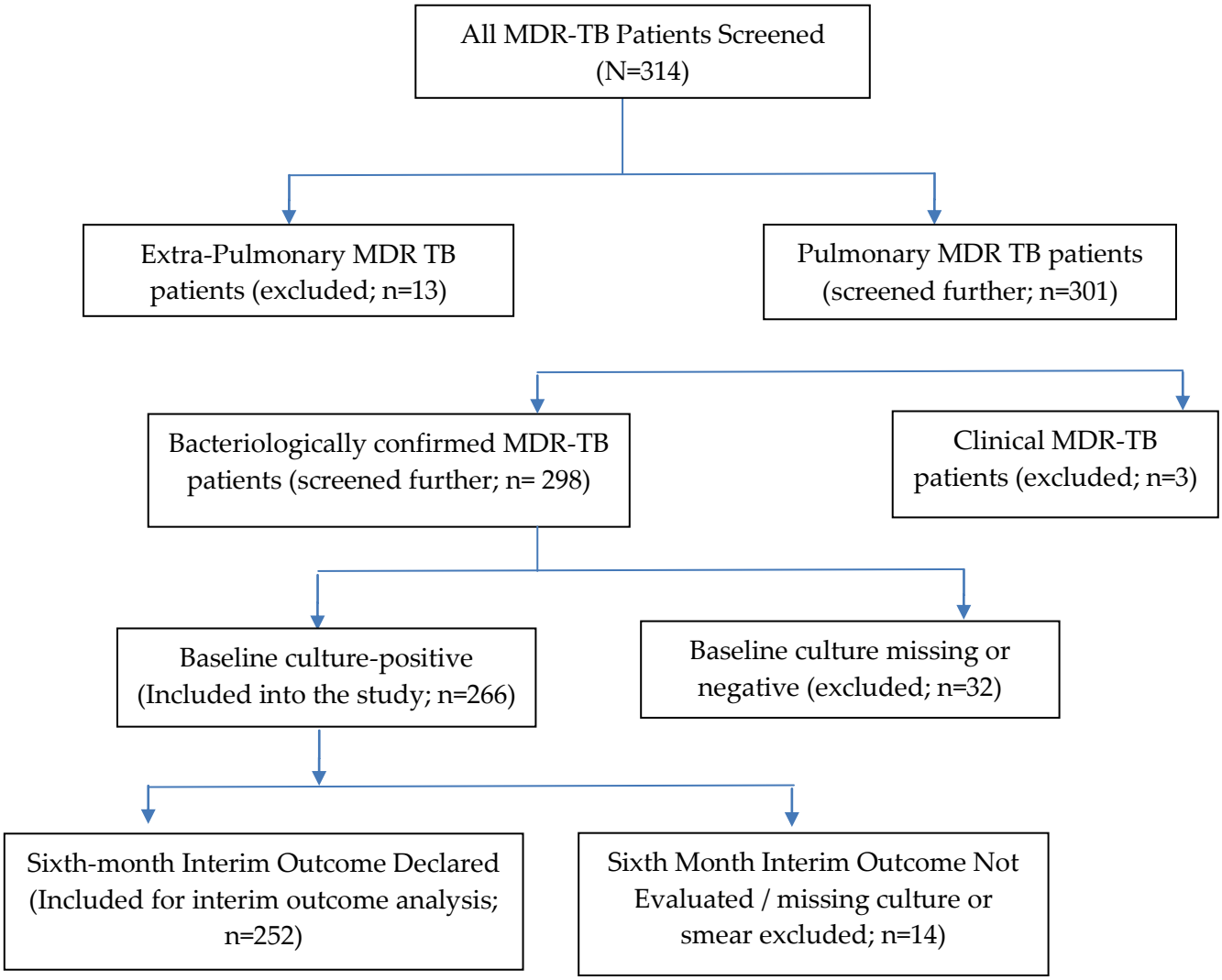


Figure 1: Flow chart for Enrolment, inclusion and exclusion of study cases.

RESEARCH ARTICLE

Heavy Metal Levels in the Sediment Samples of Gopashetty Koppa Pond, Shivamogga District, Karnataka

Ramesha Iyyanahalli*

Assistant Professor, Department of Zoology, Government Science College, Chitradurga, Karnataka, India.

Received: 19 Nov 2020 Revised: 21 Dec 2020 Accepted: 25 Jan 2021

***Address for Correspondence**
Ramesha Iyyanahalli*
Assistant Professor,
Department of Zoology,
Government Science College,
Chitradurga - 577501, Karnataka, India.
Email: ramlec.28@gmail.com

ABSTRACT

The present examination deals with the appraisal of heavy metals, such as iron, zinc, copper, lead, nickel, cadmium and manganese in the Gopishetty koppa pond sediment samples of Shivamogga taluk of Karnataka during the period 2017-18. Natural and anthropogenic activities leads to metal concentration in the water body. Iron content is maximum in the pond sediment followed by manganese and zinc. The metals are in the order of Fe>Mn>Zn>Cu>Ni>Pb>Cd. The results are compared with BIS and ATSDR guidelines according to norms**.**

Keywords: Heavy metals**,** Drinking Water Standards, Gopishetty koppa pond

INTRODUCTION

There is extraordinary worry over the expansion in contamination because of trace metals because of expanded industrialization (Patel et al, 2005; Tsai et al, 2002). The substantial metals in poisonous focuses can cause genuine medical issues, subsequently requiring precise and customary checking of their fixations in water bodies. A significant issue despite everything exists with the estimation and assessment of the toxicological parts of diligent synthetic compounds like overwhelming metals related with silt. A few investigations have been done on substantial metals circulation in the sediment of Indian rivers (Gurhan, 2007; Lokeshwari et al, 2006; Praveen Kumar, 2005).

The greater part of the formative exercises utilize characteristic assets as crude material and waste created is arranged into various ecological media. The indications of weight on the rare normal assets are apparent from the weakening air quality, soil debasement, dirtied rivers and streams and general status of condition in different locations. It is presently very much perceived that for economical turn of events and ideal utilization of characteristic assets, natural contemplations is required to be coordinated in arranging, structuring and execution of advancement

ventures can't be completely acknowledged except if they are ecologically and socially stable and feasible (Jayaram et al.,2016). Heavy metal toxicity has a serious concern all over the world as these heavy metals pose adverse effects on all forms of living organisms in the biosphere. Biomagnification of heavy metals along the food chains occurs leading to various health hazards to both humans and other living organisms. Heavy metals affect the structural, biological functioning of biomolecules (McCormick et al., 2005). Heavy metals are also known to interfere with synthesis and metabolism of the hormones (Manjappa and Puttaiah, 2005; Riddell et al., 2005; Gupta et al., 2009). Maximum level of trace metals in the sediments indicates good indication of man-induced pollution and attributed to anthropogenic activities rather than natural enrichment by geological weathering (Davies et al., 1991). Silt have the tendency to accumulation of heavy metals and adversely affect the benthic fauna (Nriagu,1988)..Keeping this in mind the present study is undertaken to know the heavy metal levels in the sediment samples of Gopishetty koppa pond of Shivamogga taluk of Karnataka.

MATERIALS AND METHODS

Study area

Shivamogga lies in the latitude 13°27'-14°39' N and between the longitudes 74°38' and 76°04' E at a mean height of 640 meters above sea level (National Informatics Centre,2007). The pinnacle Kodachadri slope at a height of 1343 meters above sea level is the most elevated point in this region. Streams Kali, Gangavati, Sharavati and Tadadi start in this region. The two significant waterways that course through this area are Tunga and Bhadra which meet at Kudli close Shimoga city to pick up the name of Tungabhadra, which later joins stream Krishna. The significant soil structures found in the Shivamogga region are red gravel, clay soil; red clay soil; lateritic gravel clay soil; lateritic clay soil; medium deep black soil; non-saline and saline alluvial-colluvial soil; brown forest soil (National Informatics Centre,2007). The selected area 'Gopashetty Koppa' is situated in Shivamogga city of Western ghats also called as the main opening of Western ghats of Malenadu. The district is characterized by high humidity (25-35^{0}C) and heavy rainfall season can be distinctly divided as summer (pre-monsoon), raining monsoon, winter (post monsoon). The pond is located in the Shivamogga city (Figure 1) at an altitude of 600 m. 38 m above the sea level. Presently the pond occupy basin about 55.60 hectare, the northern side. It is round in shape with the length of 600 mtrs. In the investigation silt tests from the pond has been gathered and analysed by following standard strategies recommended by APHA (1980), Trivedy and Goel (1984).

Preparation of sediment

Digestion strategy (Allen et al. 1986; Singh et al. 2010) was used to estimate the trace metals level in which 2 gm of residue test was warmed with 20 ml of acids (HNO_3, H_2SO_4 and HCl) in the proportion of 5:1:1 in an estimating container at 80 °C for 4–5 hr. Exactly when the sediments completely processed and leaves a straight forward arrangement, the example was cooled in room temperature and was sifted through Whatman filter paper into a pre-cleaned 100-ml volumetric jar. These examples were explicitly used for the examination of follow metals by Atomic Absorption spectrophotometer (AAS), fitted with a specific light of each metal using appropriate float clear. The glassware sets were totally cleaned with 10% HNO_3, lastly cleaned with water before the use. The instrument was aligned by running self-arranged standard arrangement of As, and furthermore drift blanks. The standard stock arrangement was having the concentration of 1000 mg/l. This arrangement was weakened up to the ideal focus to adjust the instrument. Precision and exactness of assessment were kept up by keeping up the repeated examination of the sample against Standard Reference Material (Singh et al.,2017).

RESULTS AND DISCUSSION

Table-1 and Figure 2 depicted heavy metal levels (μg/g) in sediment samples of Gopashetty koppa pond. The copper level varied from 14 to 57 μg/g. While, iron content fluctuated from 600 to 9800 μg/g. Zinc deviated between 19.6

and 67.5 µg/g. However, manganese level varied from 53.8 to 192 µg/g. Cadmium varied from 0.23 to 1.20 µg/g. Nickel content deviated 10 to 40 µg/g and lead in the sediment ranged from 11.5 to 28.5 µg/g respectively. The hard, alkaline water of old lead Belt rivers and streams is a major factor in determining speciation, solubility, mobility, bioavailability; and toxicity of the heavy metals of concern (Kabata - Pendias and Pendias, 1984). Sediments are known to contain considerably higher levels of Pb than corresponding surface waters, Results of analysis of Pb in sediments collected in 2017-18 from Gopashetty Koppa pond is shown in Table 1. Overall averages and ranges for Zn in sediments analysed during the present study is presented in Figure 1. For comparison, the ATSDR Toxicological Profile for Zinc (2002) cites evidence that concentrations of Zinc in Hamilton Harbour, Lake Ontario, sediments ranged from 1050 to 2900 µg/g (Mayer and Manning, 1990). In sediment of the Columbia river ranged from 45 to 51 µg/g, and in sediments from lake Roosevelt, Washington were 60-26,840 µg/g (Johnson *et.al.*, 1990). The latter lake receives discharges from a lead-zinc smelter and a refinery.

Results of analysis of Cu in sediments summarised in Table 1. The current Toxicological profile for Cu compiled by the ATSDR (2002) indicates that stream sediments from pristine areas generally contain < 50 µg/g of Cu. Copper level may reach several thousand µg/g in polluted sites as reported by Harrison and Bishop (1984). Surface sediment of Penobscot Bay, was 14.1 µg/g (dry weight) while sediments from estuaries and bays in other New England locations ranged from 4.4 to 57.7 µg/g (Larsen *et.al.*, 1983). Cu levels from 24 sites along the New Jersey coast ranged from <1 to 202 µg/g with a mean of 66 µg/g (Renwick and Edenbern, 1983). Cu in surficial sediments in lakes in the Sudbury region of northeastern ontario, where several smelters operate, decreased rapidly with increasing distance from the smelters. Three lakes 10 km from the Sudbury smelters contained Cu concentrations in sediment approaching 2000 µg /g (dry weight) (Bradley & Morris, 1986). In sediments of the Hudson river estuary, Cd concentrations in suspension were higher than in bottom sediments by a factor of 30 (Gibbs, 1994), Thornton (1992) reported a range of Cd content in marine sediments from 0.1 to 1.0 µg/g. In heavily industrialized areas, sediments contained 14-29 µg/g Cd. Table 1 shows levels of Cd in sediments of Gopashetty Koppa pond. Cd concentrations in surfacial sediments were elevated in the vicinity of industrial areas commonly approaching levels approximately on order of magnitude higher than geochemical background values.

Statistical Analysis

ANOVA and Post Hoc Tukey HSD (beta)

	Treatments					
	Cu (T1)	Fe(T2)	Zn(T3)	Cd(T4)	Pb(T5)	Total
N	12	12	12	12	12	60
∑X	343	38321	543.4	8.35	241.5	39457.25
Mean	28.5833	3193.4167	45.2833	0.6958	20.125	657.621
$\sum X^2$	11563	279704201	29156.64	6.5335	5191.25	279750118.4235
Std.Dev.	12.6452	3781.886	20.3373	0.2564	5.486	2074.0618
Source			***SS***	***df***	***MS***	
Between-metals			96466287.5017	4	24116571.8754	***F* = 8.43044**
Within-metals			157335921.2958	55	2860653.1145	
Total			253802208.7975	59		

The F-ratio value is 8.43044. p-value is .000022. The result is significant at p < .05.

Post Hoc Tukey HSD (beta)

The Tukey's HSD (honestly significant difference) procedure facilitates pair wise comparisons within your ANOVA data. The F statistic (above) tells whether there is an overall difference between sample means. Tukey's HSD test allows to determine between which of the various pairs of means - if any of them - there is a significant difference.

The value for Q indicates a significant result. Tukey's HSD is appropriate if the F-ratio score has not reached significance.

Pair wise Comparisons		**HSD.05 = 1947.3839 HSD.01 = 2362.1520**	**Q.05 = 3.9885 Q.01 = 4.8380**
T1:T2	M1 = 28.58 M2 = 3193.42	3164.83	Q = 6.48 (p = .00025)
T1:T3	M1 = 28.58 M3 = 45.28	16.70	Q = 0.03 (p = .00000)
T1:T4	M1 = 28.58 M4 = 0.70	27.89	Q = 0.06 (p = .00000)
T1:T5	M1 = 28.58 M5 = 20.13	8.46	Q = 0.02 (p = .00000)
T2:T3	M2 = 3193.42 M3 = 45.28	3148.13	Q = 6.45 (p = .00027)
T2:T4	M2 = 3193.42 M4 = 0.70	3192.72	Q = 6.54 (p = .00022)
T2:T5	M2 = 3193.42 M5 = 20.13	3173.29	Q = 6.50 (p = .00024)
T3:T4	M3 = 45.28 M4 = 0.70	44.59	Q = 0.09 (p = .00000)
T3:T5	M3 = 45.28 M5 = 20.13	25.16	Q = 0.05 (p = .00000)
T4:T5	M4 = 0.70 M5 = 20.13	19.43	Q = 0.04 (p = .00000)

CONCLUSION

The Gopashetty Koppa pond is small in size, with low variable flow conditions. Thus, a low dilution might result in enhanced trace metal concentrations because of the association with finer particles and sedimentation. Present water body was considered moderately polluted with respect to sediment Pb and Cd contents, which were comparatively higher than unpolluted sediments .The study results support the need of monitoring and evolution of metal loads input into the river system. This is important as the pond water is utilized for drinking, agricultural, , and industrial purposes, in addition to supporting the fishery. This has led into their use as waste depository sites, with possible reduction in water qualities.

REFERENCES

1. Allen SE, Grimshaw HM, Rowland AP (1986) Chemical analysis. In: Moore PD, Chapman SB (eds) Methods in plant ecology. Blackwell Scientific Publication, Oxford, London, pp 285–344.
2. APHA. Standard methods of the examination of water and wastewater (18th edn). American Public Health Association, New York (1998) 11-20.

3. ATSDR- Agency for Toxic Substances and Disease Registry. 1999. Toxicological profile for cadmium. Atlanta: US Department of Health and Human Services. NTIS Report No. PB/89/194476/AS
4. Bradley, R. W. and Morris J.R. 1986 Heavy metals in fish from a series of metal contaminated lakes near Sudbury, Ontario. Water Air Soil Pollut 27, 341.
5. Davies, C.A., Tomlinson, K. and Stephenson, T. 1991. Heavy metals in river estuary sediments. Environ. Technol. 12: 961-972.
6. Gibbs, R.J. 1994 Metals in the sediments along the Hudson River estuary. Environ Int 20(4), 507–516.
7. Gupta, A., Rai, D.K., Pandey, R.S., & Sharma, B. 2009. Analysis of some heavy metals in the riverine water, sediments and fish from river ganges at Allahabad. Environmental Monitoring and Assessment, 157, 449-458. http://dx.doi.org /10.1007/s10661-008-0547-64.
8. Gurhan yalcin M., Ibrahim Narin and Mustafa soylak.2007. Heavy Metal Contents of the Karasu Creek Sediments, Nigde, Turkey; Environ Monit Assess, V. 128, pp. 351–357.
9. Harrison, F.L.M. and Bishop D.J. 1984 A review of the impact of copper released into freshwater environments. Prepared for Division of Health, Siting and Waste Management, Office of Nuclear Regulatory Research, U.S. Nuclear Regulatory Commission. Washington, DC
10. Jayaram G. N. , Chandrashekarappa K. N. and Anantha Murthy K S. 2016. Heavy metal distribution in sediments around Kallur Mandli and Sagar road Industrial estate Shimoga, Karnataka, India. International Journal For Innovative Research In Multidisciplinary Field Volume - 2, Issue – 10 :360-365.
11. Johnson, A., Norton D, Yake B, et al. 1990 Transboundary metal pollution of the Columbia River (Franklin D. Roosevelt Lake). Bull Environ Contamin Toxicol 45: 703–710.
12. Kabata-Pendias, A and H. Pendias, 1984. Trace Elements in Soils and Plants. CRC PressInc.,Florida,USA.,ISBN-13:9780849366390,Pages:315
13. Lokeshwari H. and G. T. Chandrappa .2006. Impact of heavy metal contamination of Bellandur Lake on soil and cultivated Vegetation; Cur. Sci., v. 91, NO. 5, pp.10.
14. Larsen, P.F.,, Zdanowicz V, Johnson AC. 1983 Trace metal distribution in the surficial sediments of Penobscot Bay, Maine. Bull Environ Contamin Toxicol 31, 566–573.
15. Manjappa, S., and Puttaiah, E. T. (2005). Evaluation of trace metals in the sediments of river Bhadra near Bhadravathi town, Karnataka, India. Journal of Industrial Pollution Control, 21(2), 271–276.
16. Mayer, T. and Manning P.G. 1990 Inorganic contaminants in suspended solids from Hamilton Harbour. J Great Lakes Res 16: 299–318.
17. McCormick, Sd., O'Dea, M. F., Moeckel, A. M., Lerner, D. T and Bjornsson, B. T. (2005). Endocrine disruption of parr-smolt transformation and seawater tolerance of Atlantic Salmon by 4-nolyphenol and 17ß estradiol. General and Comparative Endocrinology, 142, 280– 288. http://dx.doi.org/10.1016 /j.ygcen.2005.01.015.
18. National Informatics Centre. "Geography of Shivamogga". *The Official website of Shimoga District*. District Administration, Shimoga. Retrieved 25 March 2007.
19. National Informatics Centre. "Traditional Soil Groups of Karnataka and their Geographic Distribution". *Official Website of the Department of Agriculture, Govt. of Karnataka*. Govt. of Karnataka. Retrieved 25 March 2007.
20. Nriagu, J. O. 1988. A silent epidemic of environmental metal poisoning. Environ. Poll. 50: 136 -161
21. Patel K. S., S, K. Shrivas, R. Brandt, N. Jakubowski, W. Corns & P. Hoffmann.2005. Arsenic contamination in water, soil, sediment and rice of central India; Jour. Envi Geoche. Hea., v. 27, pp.131–145.
22. Praveen Kumar M., Madhusudana Reddy T., Nithila P. and S. Jayarama Reddy.2005. Distribution of Toxic Trace Metals Zn, Cd, Pb, and Cu in Tirupati Soils, India; Electrochemical Research Laboratories, Department of Chemistry,Sri Venkateswara University, Tirupati, India; Symposium proceeding of Soil & Sediment Contamination, 14:471–478. 12.
23. Renwick, W.H. and Edenborn H.M. 1983 Metal and bacterial contamination in New Jersey estuarine sediments. Environ Pollut 5, 175–185.
24. Riddell, D. J., Culp, J. M., & Baird, D. J. (2005). Behavioral response to sub lethal cadmium within an experiment aquatic food web. Environmental Toxicology and Chemistry, 24, 431–441. http://dx.doi.org/10.1897/04-026R.1.

25. Singh A, Sharma RK, Agrawal M, Marshall FM (2010) Health risk assessment of heavy metals via dietary intake of food stuffs from the wast water irrigated site of a dry tropical area of India. Food Chem Toxicol 48:611–619.
26. Singh, H., Pandey, R., Singh, S.K. *et al.* Assessment of heavy metal contamination in the sediment of the River Ghaghara, a major tributary of the River Ganga in Northern India. *Appl Water Sci* 7, 4133–4149 (2017). https://doi.org/10.1007/s13201-017-0572-y
27. Thornton,I. .1992 Sources and Pathways of Cadmium in the Environment, Vol. 118 International Agency for Research on Cancer, Scientific Publications, 149–162.
28. Trivedy. R. K and Goel. P. K: Chemical and Biological methods for water pollution studies, Environmental publication, Karad, India. (1984).
29. Tsai .2002. Distribution of heavy metals in contaminated river sediment; Jour. Envi. Sci. eng., v.37, no. 8, pp. 1421–1439.

Table 1: Heavy metal concentration (µg/g) in the sediment of Gopishetty Koppa pond ,Shivamogga.

Months	Cu	Fe	Zn	Mn	Cd	Ni	Pb
January	16	600	19.6	86	0.23	17	11.5
February	15	980	20.6	82	0.53	18	14.5
March	14	1200	21.5	90.6	0.60	21	16
April	20	1500	25.6	111	0.50	20	17.5
May	29	9050	31.5	113.2	0.80	31	24
June	40	9500	67.5	136	1.0	34	23
July	57	1130	75.4	141	1.20	39	26
August	36	1270	61.4	192	0.86	40	25.5
September	27	1090	59.5	81	0.76	29	28.5
October	31	1076	60.2	67	0.73	18	17
November	37	9800	49.2	59	0.64	14	15
December	21	1125	51.4	53.8	0.50	10	23

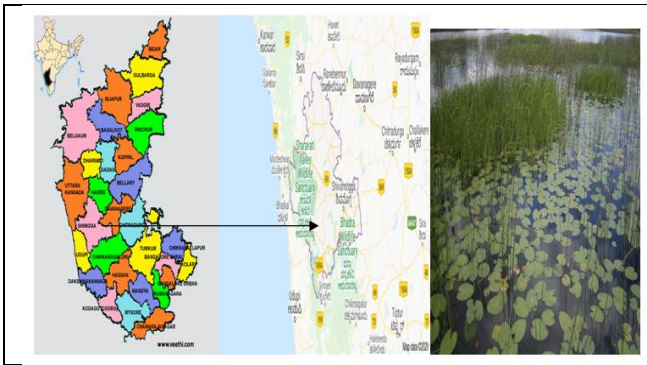


Figure 1: Study area map and a view of Gopashettykoppa pond (Source:www.veethi.com; in printerest.com)

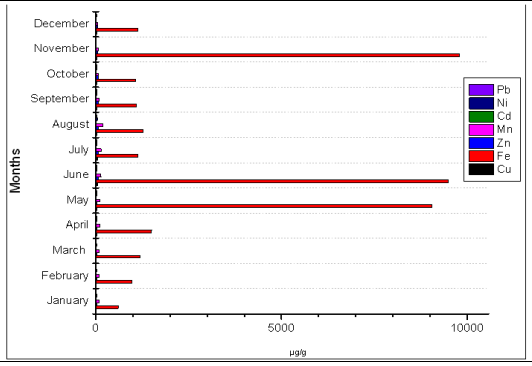


Figure 2: Heavy metal levels in the sediment of Gopishetty Koppa pond of Shivamogga taluk.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Water Quality Assessment during Pre-Monsoon and Post-Monsoon Seasons and its Impact on Human Health: A Case Study of Bhadra River Near Gondi Anicut, Bhadravathi Taluk, Karnataka

S.G. Dhananjaya

Assistant Professor and Head, Department of Zoology, Government Science College, Chitradurga-577501, Karnataka, India.

Received: 21 Nov 2020 Revised: 23 Dec 2020 Accepted: 25 Jan 2021

***Address for Correspondence**
S.G. Dhananjaya
Assistant Professor and Head,
Department of Zoology,
Government Science College,
Chitradurga-577501, Karnataka, India.
Email: dhananjayazoo@gmail.com

ABSTRACT

Indiscriminate disposal of sewage, sullage and human anthropogenic activities causes pollution of surface water as well as subsurface soil layers. An attempt has been made to assess the Physico-chemical water quality deterioration as its impact on human health. The present study was conducted in 10 sampling sites of Bhadra river near Gondi Anicut for periodic water quality assessment in summer and winter seasons. During 2016-17. Surface water samples were collected for analysis of various physico-chemical characteristics. The results of the analysis indicated the causes for water quality with deterioration in the surface water, seasonal trends in the level of contamination were identified and the water samples exceeding the limits prescribed by WHO and other agencies. Due to this elevated concentration of water quality parameters affect the health of the villagers residing in this watershed has been affected. The water in the downstream stretch of Bhadra river near Gondi village causes skin diseases, diarrhoea, dysentery, typhoid, water borne diseases, jaundice, hepatitis are found in the present study area.

Keywords: Surface water, Bhadra river, Water quality, diarrhoea, jaundice, hepatitis.

INTRODUCTION

About 80% of the world's surface is covered by water yet subjectively 97% of this huge regular asset falls unsuitable for human use (Rai, 2004). Water system of irrigation terrains represented 70% of the water utilized around the

world. In a few agricultural nations, water system speaks to up to 95% of water uses, and assumes a significant part in food formation and food security. Future farming advancement methodologies of the majority of these nations rely upon the likelihood to keep up, improve and grow flooded horticulture. Groundwater is the significant wellspring of new water accessible for water system. It is a significant sustainable asset having a few inborn preferences over surface water. The reliance on groundwater is expanding in numerous districts due to restricted surface water as enduring streams and regular disappointment of storm. It prompts overexploitation of the asset and consequently the spring are vigorously focused (Elampooranan, 1999). Proceeded with improvement and expanding utilization of groundwater joined with its reuse, the nature of ground water endures except if thought is given to ensuring it. Water is for all intents and purposes an all inclusive dissolvable and breaks down some of all that it interacts with. The quality prerequisite of surface and groundwater relies on its different uses like drinking, mechanical, and irrigational use. The chemical nature of the water is a factor which is of vital significance in its usage for water system, drinking and industrial usage. To build up quality rules, proportions of chemical, physical and bacteriological constituents should be indicated.

Water quality attributes of aquatic conditions emerge from a huge number of physical, chemical and natural associations. The water bodies – streams, lakes and estuaries are constantly exposed to a dynamic condition of progress concerning their geographical age and geo compound attributes. This dynamic equilibrium in the aquatic environment is steamed at human exercises brings about contamination which thusly shows drastically as fish kill, very bad taste of drinking water, unpleasant smells and unchecked development of aquatic weeds and so on. Nature of water is currently an incredible worry for naturalists just as the regular publics in all pieces of the world. The choice of World Health Organization (WHO's) 29th meeting (May 1976) accentuates that water for the buyers ought to be liberated from pathogenic life forms and harmful substances. Regardless of huge water assets in lakes and waterways and great monsoon, India faces enduring issues of floods and dry spells and high contamination of new water assets (De, 2000; Manivasakam, 1991; WBET, 1997). The water is necessary to human life is the fresh water and it is 2.8% of the total water resources on the earth. Of this fresh water, 2.2% is surface water and 0.6% is present in the form of ground water. It is estimated that about 0.4%, of total water resources available on planet earth is available for direct utilization by man, animals and plants. As such water is precious to man, Pollution of environment is unavoidable with the "development of industries in developing country like India (Prashant Shrivastava.2011). Based on this view, the effects of the human activities on the water quality of Bhadra river near Gondi Anicut was selected for the current study. The water is important to human existence is the new water and it is 2.8% of the all out water assets on the earth. Of this new water, 2.2% is surface water and 0.6% is available as ground water. It is assessed that about 0.4%, of all out water assets accessible on planet earth is accessible for direct use by man, creatures and plants. As such water is valuable to man, Contamination of air, water and soil is unavoidable with the "development of businesses in non-industrial nation like India (Prashant Shrivastava.2011). Keeping this in view, the impacts of the human activities on the water nature of Bhadra river close to Gondi Anicut was chosen for the present study.

MATERIALS AND METHODS

Study area

In Deccan plateau Bhadra is a major river in the Krishna Basin originating from Kuduremuka region of Western Ghats flowing towards east. The Bhadra river meets the river Tunga at Koodli village near Shivamogga and forms as Tungabhadra, a major tributary of the Krishna, which joins the Bay of Bengal. Gondi Anicut is constructed across river Bhadra, near Gondi village which is about 12 Km away from Bhadravathi city of Shivamogga District. It is situated at a latitude 13^0 46′ N and longitude of 75^0 41′ E. Gondi Anicut is positioned at 15 km downstream of Bhadra Reservoir. The water of the Gondi anicut is used for irrigation, bathing, washing and other human usages.

Water analysis

All the chemicals used were of analytical grade. Electrical conductivity was calculated with the help of digital conductivity meter. Water pH was determined with the help of pH meter. Dissolved oxygen was estimated as per Winkler's method. The remaining water quality parameters were measured as per the methods of APHA (1995) and Trivedy and Goel (1986).

RESULTS AND DISCUSSION

Results are depicted in Table 3-5 and Figure 2-3. Table 1 shows the methods followed for the estimation of water quality parameters .However, Table 2 shows the drinking water quality of Indian standards.

Pre-monsoon season

The water temperature ranged from 30 (S2) to 34^{o}C at site 6. While, turbidity and pH varied from 10 (S4)-18 NTU (S7) and 7.7(S5) to 8.4(S2,S7) respectively. Electrical conductivity of the water deviated from 40(S7) to 234 µmhos/cm (S9). TDS level ranged from 350(S4) to 460 (S10) mg/l. However, total hardness level fluctuated between 120 (S1) and 400 (S9) mg/l. While, calcium and magnesium contents ranged 40 (S1) -170(S10) mg/l and 35(S1) -145(S10) mg/l respectively. Nitrate of the water ranged from 1.0 (S1) to 4.9 (S7) mg/l. Chloride level ranged from 30(S8) to 305(S9) mg/l and sulphate content varied from 65(S5) to 102(S8) mg/l respectively.

Post Monsoon season

The water temperature fluctuated from 16.5 (S6) to 22^{o}C. While, turbidity and pH varied from 20 (S10)-38 NTU (S8) and 7.4(S2,S9) to 8.3(S1) respectively. Electrical conductivity of the water deviated from 95(S1) to 1420 µmhos/cm. TDS level varied from 250(S1) to 1320 (S8) mg/l. Nonetheless, total hardness level fluctuated between 115 (S1) and 420 (S8) mg/l. While, calcium and magnesium contents ranged 45 (S2) -190(S8) mg/l and 30(S2) -170(S8) mg/l respectively. Nevertheless, the nitrate level deviated between 0.95 (S4) and 5.8 (S7) mg/l. Chloride level varied 192(S6) to 300(S2) mg/l and sulphate content deviated from 87(S4) to 116(S7) mg/l respectively.

Health Hazards

The peoples of downstream area of Bhadra river near Gondi anicut village suffering from skin diseases (40%), diarrhoea & dysentery (30%) , water borne diseases (20%) jaundice and hepatitis (Figure 4).

Statistical analysis

Analysis of Variance for Health disorders

Based on the Interquartile range, the possible outliers in Group 1 values above 17.250 and below- 4.750. In Group 2 values above 12.750 and below -1.250. The possible outliers in Group 3 values above 12.250 and below -1.750. In Group 4 values above 16.000 and below -4.000 be considered. The outliers in Group 5 values above 17.500 and below -6.500 would be considered.

CONCLUSION

In this study, eleven water quality parameters along the river Bhadra near Gondi Anicut area at 10 different sampling sites during the Pre-monsoon and post-monsoon seasons of 2016-17 were assessed. The results revealed that except few parameters all the remaining water quality parameters were exceeding the desire prescribed limit of BIS standards. Therefore, it is concluded that river Bhadra in Gondi area is polluted and unsafe for domestic consumption. The deterioration of river water quality may be due to both point and non-point sources of pollution i.e. large as well as human activities including washing, bathing and agriculture sectors of the area.

REFERENCES

1. APHA, AWWA, WPFC:1995. Standard methods for the examinations for the water and waste water, 19th edition, DC, USA.
2. De A.K. 2000. Environmental Chemistry, fourth edition, New Age International Limited, New Delhi.
3. Elampooranan T, Rajmohan and Abirami L 1999. Hydrochemical studies of artesian well waters in cauvery deltaic area, south area, South India. *Indian J. Environ. Hlth.* 41, 107- 114.
4. Manivasakam,N.1991.Environmental Pollution, National Book Trust, first edition, second reprint, India.
5. Prashant Shrivastava.2011. Water quality assessment and its impact on human health: A case study of somni stream watershed patan block, Durg district, Chhattisgarh. Indian Journal of Science and Technology Vol. 4 issue 3:240-244.
6. Rai V.K.2004.Suitability of surface and groundwater for irrigation: A case study. *Natnl. Geographical J. India,VHU (up).* 50, 83-94.
7. Thillai Arasu ,P, S. Hema and M.A. Neelakantan.2007.Physico-chemical analysis of Tamirabarani river water in South India. Indian Journal of Science and Technology Vol.1 No.2 :1-6.
8. Trivedy, R.K. and Goel, P.K. 1986. Chemical and Biological methods for water pollution studies. Env. Publ. Karad, India.
9. Vineesha Singh and U.C.Singh.2008.Assessment of groundwater quality of parts of Gwalior (India) for agricultural purposes. Indian Journal of Science and Technology Vol.1 No 4 :1-5.
10. WBET.1997. Water and Basic Environmental Technology, Anmol Publications Pvt Ltd., New Delhi, first edition.
11. WHO.1984. Guidelines for drinking water quality (Vol. I), World Health Organisation, Geneva,

Analysis of Variance data

Case	Sum of Squares	df	Mean Square	F	P
Between	5.434	4	1.359	0.176	0.9494
Within or Residual	301.111	39	7.721	-	-
Total	306.545	43	-	-	-

Post Hoc Tukey HSD data is shown below

		Mean Difference	pTuckey
Group 1 (Skin diseases)	Group 2	0.778	0.975
	Group 3	1.000	0.939
	Group 4	0.333	0.999
	Group 5 (Jaundice/Hepataitis)	0.444	0.997
Group 2 (Diarrhoea)	Group 3	0.222	1.000
	Group 4	-0.444	0.997
	Group 5	-0.333	0.999
Group 3(Dysentry)	Group 4	-0.667	0.987
	Group 5	-0.556	0.993
Group 4 (Typhoid)	Group 5	0.111	1.000

Data table				
Group	**Mean**	**Std Dev**	**N**	**df**

			Mean Difference	pTuckey
Group 1	6.333	3.162	9	8
Group 2	5.556	2.506	9	8
Group 3	5.333	2.121	9	8
Group 4	6.000	3.071	8	7
Group 5	5.889	2.934	9	8

Table 1: Method of determination of water quality parameters

Sl.No.	Parameters	Methods
1.	Temperature	Thermometer
2.	pH	pH metry
3.	Electrical conductivity	Conductometry
4.	Total dissolved solids	Evaporation method
5.	Alkalinity as $CaCO_3$	Titrimetry
6.	Total hardness	EDTA – Titrimetry
7.	Calcium	EDTA – Titrimetry
8.	Magnesium	EDTA – Titrimetry
9.	Sodium	Flame photometry
10.	Potassium	Flame photometry
11.	Chloride	Argentometric
12.	Nitrate	Spectrophotometry
13.	Sulphate	Spectrophotometry
14.	Phosphate	Spectrophotometry
15.	Dissolved oxygen	Titrimetry
16.	Fluoride	Fluoride meter

Table 2: Water quality results compared with the standard stipulation in the study area

Parameters	Range in the study area	WHO 1984	BIS (1983)		Desirable limits as per IS : 10500, 1991 & 1993
			Highest Desirable	Maximum Permissible	
Physical					
1. Odour	odorless	Unobject-odour	-	-	Unobjectionable
2. Turbidity(NTU)	-	5	-	-	5
3.EC (micromhos/cm)	90-1400	1400			
4. TDS	256-1324	1000	500	1500	500
Chemical					
1. pH	8.3-8.7	6.5-8.5	7.0-8.5	6.5-9.2	6.5-8.5
2. Alkalinity	70-253	-	-	-	200
3. Hardness	110-410	500	-	300	300
4. calcium	35-155	75	75	200	75
5. Magnesium	40-190	50	30	100	30

6. Sodium	28-140	200	-	-	-
7. Potassium	1.9-9.7	55	-	-	-
8. Chloride	39-405	250	250	1000	250
9. Sulphate	3.0-37	400	150	400	200
10. Bicarbonate	58-340	-	300	600	-
11. Carbonate	15-54	-	-	-	-
12. Nitrate	0.9-5.9	50	-	45	45

Units = mg/l

Table 3: Physico- chemical parameters during pre-monsoon season of Bhadra river near Gondi area

Sites	Temp.	Tur	pH	EC	TDS	TH	Ca	Mg	NO_3	Cl	SO_4
1	35	12	8.3	182	390	120	40	35	1	39	84
2	30	15	8.4	160	360	180	70	60	1.4	58	82
3	32	11	8.2	152	370	260	110	90	2.2	72	90
4	31	10	8	165	350	310	160	140	2.5	90	76
5	33	11	7.7	135	410	220	120	105	3.1	105	65
6	34	16	7.8	175	380	190	95	80	2.9	85	85
7	32	18	8.4	40	420	340	105	90	4.9	205	98
8	31.5	13	8.3	110	355	380	140	130	1.8	30	102
9	30.5	14	8.1	234	510	400	150	125	1.9	305	100
10	32.5	12	7.9	190	460	390	170	145	3.8	295	75

Table 4: Physico- chemical parameters during post-monsoon season of Bhadra river near Gondi area

Sites	Temp.	Tur	pH	EC	TDS	TH	Ca	Mg	NO_3	Cl	SO_4
1	20.5	26	8.3	95	250	115	50	40	1.8	240	96
2	18	27	7.4	190	700	140	45	30	1.9	300	95
3	21	30	7.8	270	690	250	70	60	4.2	242	97
4	19	32	8.0	680	890	290	90	75	0.95	250	87
5	17	29	7.5	850	1200	360	120	95	4.6	245	90
6	16.5	35	7.8	390	900	290	110	90	3.2	192	110
7	20.5	37	7.6	1420	700	390	160	140	5.8	260	116
8	21	38	7.9	1100	1320	420	190	170	5.7	210	89
9	22	22	7.4	1300	800	410	180	150	4.8	270	114
10	17	20	8.1	710	1250	350	85	65	3.6	220	92

Table 5: Occurrence of health disorders in different age groups of peoples in Gondi area

Sl.No	Age groups	Skin rashes	Skin irritation	Diarrhoea	Dysentry	Water borne diseases
1	1-10	-	✓	✓	✓	✓
2	11-20	-	-	✓	✓	✓
3	21-30	-	✓	-	-	-
4	31-40	✓	✓	-	-	-
5	41-50	✓	✓	✓	✓	✓
6	51-60	✓	✓	✓	✓	✓

Figure1: Views of Bhadra river water at Gondi anicut

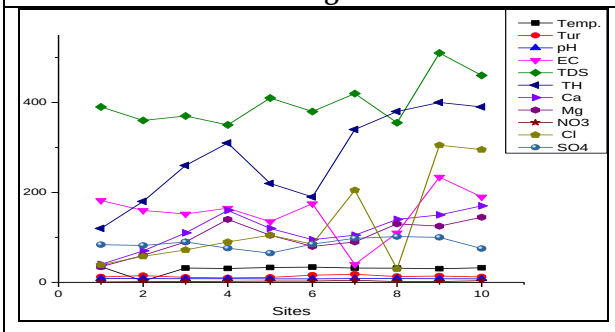


Figure 2. Physcio chemical parameters during pre-monsoon season of water of Bhadra river near Gondi area

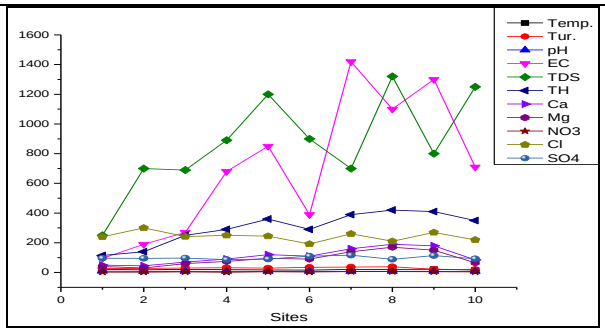


Figure 3. Physcio chemical parameters during post-monsoon season of water of Bhadra river near Gondi area.

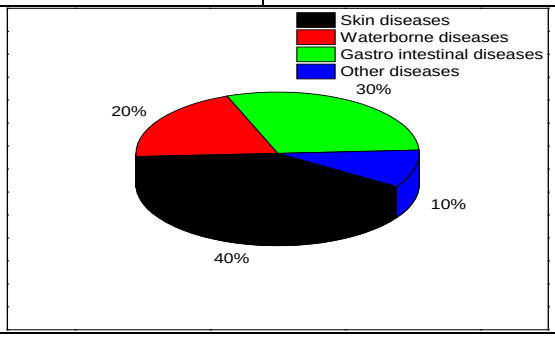


Figure 4. Percentage occurrence of health hazards in peoples at Gondi anicut area

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Incidence of Stroke in Hypertensive Patients Presenting at Tertiary Care Hospital

Abdul Ahad*, Aleem Ur Rahish, Saima Qadir, Amir Zeb Swati, Haroon Ur Rashid and Amin ul Haq

Saidu Medical College, Saidu Teaching Hospital, Swat, Pakistan.

Received: 04 Nov 2020 Revised: 06 Dec 2020 Accepted: 08 Jan 2021

***Address for Correspondence**
Abdul Ahad
Saidu Medical College,
Saidu Teaching Hospital,
Swat, Pakistan.
Email: dr.aahad@yahoo.com

ABSTRACT

Stroke is a frequent medical problem occurring in patients with hypertension and other risk factors. The current study aimed to measure the frequency of hypertension as an imperative risk factor in stroke patients. Patients who clinically presented with features of a stroke at Saidu teaching hospital, swat from 1st September 2019 to 27th February 2020, were included in this study. A total of 150 cases of hemorrhagic stroke and Ischemic stroke cases were included. Subjects with a history of trauma, brain tumors, or severe other diseases were excluded from the study. The study was conducted following the declaration of Helsinki, and informed consent was obtained from each participant. Hypertension was found to be the most common risk factor in the current study. 25.3% showed a history of 4 years of hypertension, while 19% showed a history of 7 years of hypertension. 68% of patients were male, while 32% of patients were female with Peak stroke-prone age was between 45-55 years. Hypertension is the leading risk factor for stroke. It is, therefore, essential to detect and manage hypertension from its onset.

Keywords: Stroke, hypertension

INTRODUCTION

World Health Organization (WHO) defines stroke as a sudden onset of a neurological discrepancy, contributing to developing obstruction or rupturing the cerebral arterial system. It is a significant health issue of Pakistan [1] and global as well, the primary basis of disability while the 2nd leading reason for mortality universally [2], responsible for more than 3 million deaths in developing countries [3]. Both cardiovascular problems and Stroke rates in middle-aged people are five to ten times higher in Pakistan [4]. Higher prevalence of established stroke risk factors or potential nontraditional risk factors such as water pipe smoking, daldaghee or naswaar, and paan chewing are among the major contributor [5-7]. Previous studies have shown that most people aged 18 years or above have either

controlled or uncontrolled hypertension, dyslipidemia, and a history of active smoking [8,9]. Existing treatments for stroke are comparatively ineffective, yet interventions based on risk factors are the real hope to decrease stroke-causing illness and deaths [10]. Randomized, controlled interventional studies have revealed substantial prevention from stroke with the hypertension management alone [11]. Definite risk factors have been continuously identified as significant predictors of stroke outcome, including age, hypertension, alcohol consumption and a trial fibrillation [12]. Hypertension not only leads to the occurrence of stroke but also numerous cardiovascular diseases. Moreover, it has been observed that timely admission to a stroke rehabilitation program results in better outcomes at discharge and reduces the length of stay. On the other hand, it has been witnessed that Primary aspects like patient education, management of bladder and bowel dysfunction, psychological evaluation and management of depression, prevention of falls, mobility aids prescription, vocational rehabilitation, speech therapy, occupational therapy, and community reintegration are neglected most of the time [13-15]. The present study aims to identify the frequency of hypertension as an imperative risk factor in Pakistan's stroke patients.

METHODOLOGY

This study was conducted following the declaration of Helsinki, and informed consent was obtained from each participant. Ethical committee approval was obtained from the study site before the commencement of the study.
A Cross-sectional study was conducted for six months from 1st September 2019 to 27th February 2020 at Saidu teaching hospital, Swat-Pakistan. Subjects with hemorrhagic stroke and ischemic stroke were included in this study with a non-probability consecutive sampling technique. A total of 150 cases of hemorrhagic stroke and Ischemic stroke cases were included. Subjects with a history of trauma, brain tumors or with other serious diseases were excluded from the study. Study-related information was recorded in a specifically designed questionnaire by the researcher, including outcome variables. All the collected data were entered in Microsoft Excel and analyzed on SPSS version 22.0. The data were presented as frequency and percentages. Post-stratification chi-square test was applied, taking p-value < 0.05 as significant.

RESULTS

A total of 150 cases were included in the study, with 68% male and 32% female patients. Total 25.3% showed a history of 4 years of hypertension, while 19% showed a history of 7 years of hypertension. The lowest percentage was 7%, with a history of 8 years of hypertension. Out of 150, 87 cases, i.e. 58% were diagnosed with hemorrhagic stroke, while 63 cases, i.e. 42%, were diagnosed with ischemic stroke. With respect to age, Hemorrhagic stroke was present among 30patients, 55-65 years of age, and ischemic stroke was present among 28 subjects aged between 45-55 years. In comparison, the gender-based distribution showed that 60 males and 27females had Hemorrhagic stroke. There was no significant effect of age or gender on hemorrhagic stroke frequency and ischemic stroke ($p>0.05$). A high frequency of Hemorrhagic and ischemic stroke was present among males compared to females (Figure 1).

DISCUSSION

It is well-known that stroke occurrence, incidence and deaths vary broadly within diverse populations. Several studies have revealed a higher prevalence of stroke in the Asians population. The stroke burden in Asia is expected to rise, both in absolute terms and as a proportion of total disease burden, because of rapid population, advanced age and lifestyle variations [16]. The present study on the swat population showed that the prevalence of stroke was high 68% in males while 32% were female with a higher prevalence of hemorrhagic stroke, i.e 60% in males and 28% in females. Many previous studies showed that the risk of stroke doubles for each successive decade after age 55 years [17, 18]. The accumulative impact of advanced age on the cardiovascular system and the advanced nature of stroke risk factors for an extended time period noticeably raises risks of stroke. Consistent with that, the mean age group of

acquiring stroke in our study was 45-55 years, much more significant than studies in United States [19]. This difference is possible because of better awareness and proper control of risk factors in the United States. Hypertension influences at the slightest 65 million people within the United States and is a significant danger factor for localized cerebral necrosis and intra cerebral bleeding [18, 21]. The risk of stroke rises with elevated blood pressure, and its risk can be reduced to 38% only by managing hypertension [22]. Studies on male subjects subsequently displayed upsurges in ischemic stroke rates at higher total cholesterol levels, particularly for levels above 240 to 270 mg/dl [23, 24]. On the contrary, this study reveals a higher prevalence of hemorrhagic stroke in male subjects, i.e. 60%. This might be because of smoking and diabetes. Smoking increments stroke chance by creating intense impacts on the chance of thrombus within small arteries and persistent impacts related to an expanded burden of atherosclerosis [25]. The higher smoking occurrence in the current study can be because of the patient's lower socio-economic status. They are probably smokers because they assume it a relaxing factor and cannot stop due to no proper awareness. Another study stated that smokers find it hard to stop smoking, and this perception increased the longer they had been a regular smoker [6,25].The current study's outcomes were undoubtedly displaying that hypertension, diabetes mellitus, dyslipidemia, and smoking as the independent risk factors of stroke in Pakistan.

CONFLICTS OF INTEREST

None.

ACKNOWLEDGMENT

The authors would like to acknowledge the Medical Affairs Department of Getz Pharma for their technical support.

FUNDING

None.

REFERENCES

1. Ali S, Akhter H, Altaf KF, Noushad S, Asghar N, Ahmed SS, Ahmed S. Relationship of Physical stress and Hypertension among Cardiovascular Disease Patients. Int. j. endorsing health sci. res. 2016; 4(4): 45-52.
2. World Health Organization. The world health report 2003: shaping the future. World Health Organization; 2003.
3. Murray CJ, Lopez AD. Alternative projections of mortality and disability by cause 1990–2020: Global Burden of Disease Study. The lancet. 1997;349(9064):1498-504.
4. Arif M, Saleem Y, Riaz S, Mahapara, Imran M, Sharjeel A, Khan SA, Khan GU. Young Adults Undergoing Coronary Artery Bypass Grafting (CABG). 2020. Int. j. endorsing health sci. res. 8(1): 41-46.
5. Farooq MU, Majid A, Reeves MJ, Birbeck GL. The epidemiology of stroke in Pakistan: past, present, and future. Int J Stroke 2009; 4:381–389.
6. Azher Z, Ahmed S, Noushad S, Qudsia Z, Yaseen A. The major apprehensions in young chronic cigarette smokers to quit smoking. FUUAST Journal of Biology. 2012;2(2 December):59-63.
7. SherwaniS, Ahmed H, Ahmed S, Naushad S, Bashir A, Ghaffar A. Rising Trends in Water Pipe (SHISHA) Smoking among Male University Students in Karachi-Pakistan. Indian J Hlt. Wellbeing. 2011; 2(4): 728-731.
8. Zulfiqar I, Mushtaq N, Ahmed S, Noushad S, Khan S. Pre-eclampsia-pregnancy induced hypertension; threats and consequences. Int. j. endorsing health sci. res. 2014;2(2):104-106.
9. Zaman R, Anam S, Muneeb M, Ashraff S. Attitude and practice regarding effective blood pressure measurement among health professionals at a tertiary care hospital in Hyderabad - Pakistan. Int. j. endorsing health sci. res. 2019;7(2):101-107.

10. Adams H, Adams R, Del Zoppo G, Goldstein LB. Guidelines for the early management of patients with ischemic stroke: 2005 guidelines update a scientific statement from the Stroke Council of the American Heart Association/American Stroke Association. Stroke. 2005;36(4):916-923.
11. Chobanian AV, Bakris GL, Black HR, Cushman WC, Green LA, Izzo JL, Roccella EJ. The seventh report of the joint national committee on prevention, detection, evaluation, and treatment of high blood pressure: the JNC 7 report. JAMA. 2003; 289(19):2560-2571.
12. Knuiman MW, Vu HT. Risk factors for stroke mortality in men and women: the Busselton Study. J CardiovascularRis. 1996; 3(5):447-452.
13. Rajput AH, Naz H, Iqbal A. Depressive symptoms among patients of Myocardial Infarction. Ann. psychophysiol.2017;4:50-58.
14. Altaf M, Ahmed S. Neurological disturbances caused by nutritional deficit. Ann. psychophysiol. 2016; 3:69-70.
15. Noushad S, Ahmed S, Jaffri H and Sherwani S. Effect of Exercise on Hematological Parameters: A Study on Trained versus Un-Trained Male Subjects. Pak. j. life soc. Sci. 2012; 10(1): 18-21.
16. Taqui A, Kamal AK. Stroke in Asians. Pakistan J Neuro Sci. 2007;2(1):14-17.
17. Jerath NU, Reddy C, Freeman WD, Jerath AU, Brown RD. Gender differences in presenting signs and symptoms of acute ischemic stroke: a population-based study. Gender Medicine. 2011;8(5):312-319.
18. Wolf PA, D'Agostino RB, O'Neal MA, Sytkowski P, Kase CS, Belanger AJ, Kannel WB. Secular trends in stroke incidence and mortality. The Framingham Study. Stroke. 1992;23(11):1551-1555.
19. Basharat RA, Kousuf, M. Frequency of known risk factors for stroke in poor patients admitted to Lahore General Hospital in 2000. Pak J. Med Sci. 2002; 18(4): 280-283.
20. Mehmood T. Moderate intraoperative hypertension as a cause of delayed recovery. Anaesthesia, Pain & Intensive Care. 2000;4(1).
21. Arboix A, Roig H, Rossich R, Martinez EM, Garcia-Eroles L. Differences between hypertensive and non-hypertensive ischemic stroke. European Journal of Neurology. 2004;11(10):687-692.
22. Feigin VL, Krishnamurthi RV, Barker-Collo S, McPherson KM, Barber PA, Parag V, Arroll B, Bennett DA, Tobias M, Jones A, Witt E. 30-year trends in stroke rates and outcome in Auckland, New Zealand (1981-2012): a multi-ethnic population-based series of studies. PloS one. 2015;10(8):e0134609.
23. Iso H, Moriyama Y, Sato S, Kitamura A, Tanigawa T, Yamagishi K, Imano H, Ohira T, Okamura T, Naito Y, Shimamoto T. Serum total homocysteine concentrations and risk of stroke and its subtypes in Japanese. Circulation. 2004;109(22):2766-2772.
24. Leppala JM, Virtamo J, Fogelholm R, Albanes D, Heinonen OP. Different risk factors for different stroke subtypes: association of blood pressure, cholesterol, and antioxidants. Stroke. 1999;30(12):2535-2540.
25. Burns DM. Epidemiology of smoking-induced cardiovascular disease. Progress in cardiovascular diseases. 2003;46(1):11-29.

Table 1: Characteristics of study participants

Variables		Frequency	Percent
Gender	Female	48	32
	Male	102	68
Age Group	45 - 55 years	57	38
	55 - 65 years	54	36
	66 - 75 years	39	26
Diagnosis	Hemorrhagic Stroke	87	58
	Ischemic Stroke	63	42
Hypertension history	1 years of HTN	22	14.7
	2 years of HTN	14	9.3
	3 years of HTN	14	9.3
	4 years of HTN	38	25.3

	5 years of HTN	8	5.3
	7 years of HTN	29	19.3
	8 years of HTN	7	4.7
	9 years of HTN	18	12

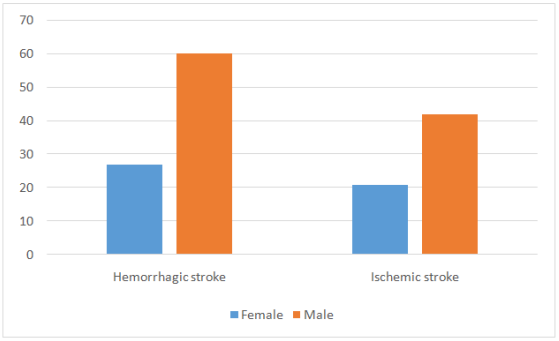


Figure 1: Distribution of hemorrhagic and ischemic stroke in relation to gender distribution

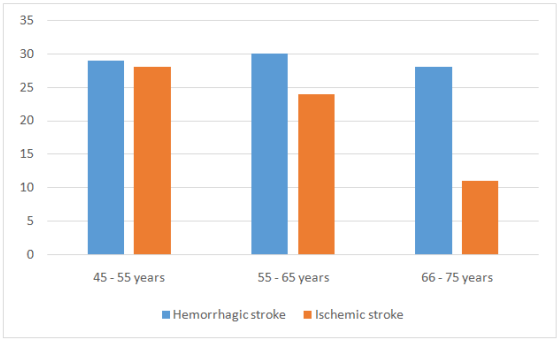


Figure 2: Distribution of hemorrhagic and ischemic stroke in relation to age groups

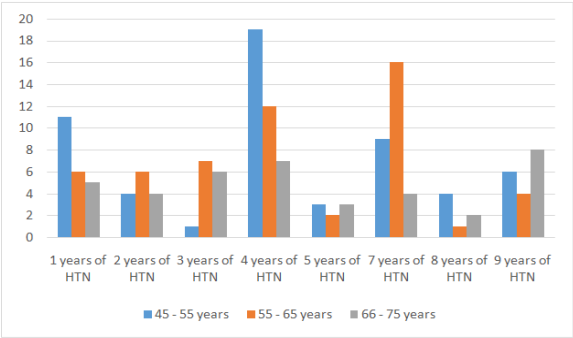


Figure 3: Distribution of hypertension history in relation to age groups

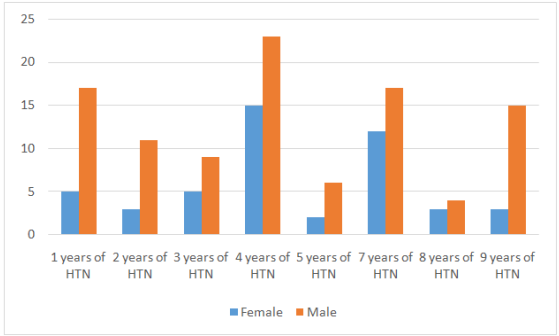


Figure 4: Distribution of hypertension history in relation to gender

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Studies on Medicinal Plants in the Rural Health Care Commonly used by the Tribal Communities in Purulia District, West Bengal, India Based on Indigenous Traditional Knowledge (ITK) System

Nandadulal Sannigrahi [1]* and Amal Kumar Mondal[2]

[1]Research scholar, Plant Taxonomy, Biosystematics and Molecular Taxonomy Laboratory, Department of Botany and Forestry, Vidyasagar University, Midnapore, West Bengal, India.
[1]Associate Professor, Department of Botany, Nistarini College, Purulia, West Bengal, India.
[2]Professor of Botany and Coordinator UGC-DRS-SAPII and DBT-BOOST-WB, Plant Taxonomy, Biosystematics and Molecular Taxonomy Laboratory, West Bengal, India.
Department of Botany and Forestry (UGC-DRS-SAPII and DBT-BOOST-WB Funded), Vidyasagar University, Midnapore, West Bengal, India.

Received: 24 Nov 2020 Revised: 05 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
Nandadulal Sannigrahi
Research Scholar, Plant Taxonomy,
Biosystematics and Molecular Taxonomy Laboratory,
Email: nandadulal2002prl@gmail.com / akmondal@mail.vidysagar.ac.in

ABSTRACT

The preserved distinctive understandings impregnated with cultural experience through man-ecosystem interactions by aboriginal are defined as Indigenous traditional knowledge (ITK). It is a very deep-rooted ancient repository that transmitted verbally and non-documented form since the time immemorial and the systematization and canonization have been done by the so called elite science with the passage of time. This treasure incorporates the wisdom in multiple fields through the millennium of experimentation by trial and error. People close to the nature suffered from discrimination tried to legitimize and fortifying the knowledge under suitable institutional frameworks, culture and practices. Purulia, the one of the most backward district of West Bengal with more than 20 tribal groups enriched with rich biodiversity and cultural heritage has the proud privilege of the ITK midst its bountiful landscape and ecological paradise. .Documentation of this kind of information particularly unsung and untold little bit unexplored will be extremely beneficial in general and different socioeconomic issues like health care , sustainable development in particular in the new millennium in the retrospect of ruthless destruction of natural resources.

Keywords: ITK, Sustainable development, Folks, Motifs, Biodiversity, Ecology, Ethnomedicine, Health care

INTRODUCTION

"When a knowledgeable old person dies, a whole library disappears"- an African proverb goes. Indigenous-an adjectival word means 'belonging to a place, native' (Oxford English Dictionary). The culture and history of a local community is influenced by the indigenous traditional outfits and it is evolved and refined through many years of regular experiments in every sphere of life. Indigenous communities having distinctive modes of encoding the useful data within philosophies of thought and modes of activity linked to particular landscapes are the treasure of the knowledge and this data includes geographical, genealogical, biological and other evidence and this data can draw the relations of human with the flora and fauna, land and water and the supernatural forces existing during the period. With the passage of time, the verbal knowledge remains in non-documented form due to lack of proper ambience but are being transmitted from one generation to the next generation as a weapon of their life style and livelihood. According to Ferrington and Martin (1991), ITK can be defined as a basis of knowledge, beliefs and customs which are internally consistent and logical to those holding them and it has much influence upon the people of aboriginal origin than the people of modern substitute. It is a complex set of integrated express of intellectual, empirical, social and spatial factors that finally shape the human culture.ITK is "unique, traditional , local knowledge existing within and developed around specific condition of women and men indigenous to particular geographical areas"(Gramier, 1998). Basically, ITK is mostly refereed as the knowledge reared by the tribal people as a part of their old age heritage having no authorization. Indigenous people's knowledge can be considered as a subset of what is more broadly referred to as Indigenous knowledge" (Nakatu, 2002). The traditional medicinal system based on herbal remedies has always played a role in the health systems of many developing and under developed countries. The significance of the traditional medicine has also gained vital importance in the developed countries (Rai & Nath, 2003).The most recent definition of ITK has been drafted by Gadgil. "It is a cumulative knowledge and beliefs handed down through generation by the cultural transmission about the relationship of the living beings including human with one another and their environment. It is unique to given culture or society" (Gadgil).

The practice in herbal medicine are continuing until today because of its many biomedical benefits as well as cultural belief in many parts of the world (Savithramma et al, 2016).The term never to be static one but innovation and exploration of the intangible knowledge require door to door Interaction in general and old age people in particular. Traditional Knowledge system (TKS) is the know-how of the people, gathered through day to day activities to overcome the hurdles and tap the potentialities from their immediate neighbourhood. It is mostly evolved in a specific location within certain physical and social-cultural environment , where it reflects people's specific knowledge , understanding as well as observational and experimental information about their dwelling environment , along with skills and technology to design a life style in that specific environmental context(ITK is a community based functional knowledge system, developed, preserved and refined by the different people through the passage of time via continuous interaction, observation , experimentation with their surroundings to address the emerging need of the existing social norms and socioeconomic stricture and beliefs. Such knowledge is developed and passed from one generation to next generation in the form of songs, stories, different cultural values, dance, rituals, healing arts, agricultural practices, traditional laws, motifs that convey both literal and metaphorical truths about these relations. Such knowledge accumulated in course of the life styles is adapted to a local culture and it becomes the integral part of the cultural identities to a particular community for the time being and ultimately become the treasure of knowledge to a nation. Thus indigenous peoples along with their profound understanding of the environment for life styles and sustainability become the input for the conservation and sustainable use global biodiversity. Thus, it is the most useful attribute for the sustenance for continuous support to the existing society as a part of the means of sustainability. In many developing and underdeveloped countries, ITK plays a very deep impact to address the different socioeconomic parameters including the food security and health care attributes. All the knowledge entangled with ITK ultimately flow from practices although the organization and management differs from the different domain of knowledge. But recently, it is very alarming to observe the gradual depletion of this knowledge due to a number of reasons. Firstly the modern innovations of health care practices have been

replacing the old aged values and another important cause of the depletion of ITK is the declining of the local resources due to rapid industrialization and urbanization to address the human greed by the corporate houses for the sake of development of the market economy.

Over the last seventy five years, the indigenous people and their treasure of knowledge has credited recognition at international level due to the continuous expedition by different international bodies, stakeholders, academics and other people who deserves the recognition of this non-documented knowledge (Adrea Zappalagio, Oxford). As an outcome of these endeavors, the various types of treaties, declarations have come to the limelight by the strong argument to constitute a convincing legal frame for the exploration and protection of TK. After the Second World War, this type of movement for mass recognition of TK has become expedited and the UN charter (1945) played a crucial role by emphasizing the human rights and sovereignty of every man and people. The UN Conference on Environment and Development in Rio de Janeiro in 1992 is to be considered as first occasion on which the value of ITK was got the broad worldwide recognition and it encourages to make ITK generally accessible to all subject to the consent of the stake holders of this imponderable knowledge along with their equitable participation. "Traditional knowledge (TK) refers to the knowledge, innovations and practices of indigenous and local communities around the world. Developed from experience gained over the centuries and adapted to the local culture and environment, traditional knowledge is transmitted orally from generation to generation. It tends to be collectively owned and takes the form of stories, songs, folklore, proverbs, cultural values, beliefs, rituals, community laws, local language, and agricultural practices, including the development of plant species and animal breeds. Sometimes it is referred to as an oral traditional for it is practiced, sung, danced, painted, carved, and chanted and performed down through millennium. Traditional knowledge is mainly of a practical nature, particularly in such fields as agriculture, fisheries, health, horticulture, forestry and environmental management in general." (Rio-declaration of CBD, World Summit 1992).

Ethnobotany is the association between plants and people with a specific emphasis on traditional cultures and societies(Mesfin et al, 2013, Gbekley et al, 2017, Amjad et al, 2017, Andrade et al, 2017).Recently, the TKS has claimed recognition in the different international bodies like Intellectual Property Organization (WIPO), the International Labour Organization (ILO), the Food and Agricultural Organization (FAO), World Health Organization (WHO), United Nation Educational, Scientific Organization (UNESCO), United Nation Environment programme (UNEP), United National Developmental Programme (UNDP), United National Commission on Human Right (UNCHR) by taking the effective measures for the validation and efforts for the protection and preservation of this valuable knowledge and to ensure the fair and desired sharing of the stakeholders. Moreover, World Conference on Science (Budapest, June 1999) addressing ITK recommended through 'Science Agenda: Framework for Action'(UNESCO, 2000), that , "modern scientific knowledge and traditional knowledge should be brought closer together in interdisciplinary projects dealing with the links between culture, environment and development in such areas as the conservation of biological diversity, management of natural resources, understanding of natural hazards and mitigation of their impact. Local Communities and other relevant players should be involved in these projects. Individual Scientists and the scientific community have a responsibility to communicate in clear language the scientific explanations of these issues and the ways in which science can play a role in addressing them". Thus, the need of ITK is its usefulness for the sustenance of the community in one hand as well as maintenance of genetic resources for continued survival of the community, the real stake holders of this intangible knowledge. In summary, traditional ecological knowledge include sophisticated site specific and cultural-specific instructions, embedded in the landscape and evidenced in unique skill sets, activities and localized knowledge. If followed properly these instructions and intended to ensure both short term survival and long term human health, community, sustainability, and preservation of unique ecosystems (Apffel-Marglin 2011; Berkes 2012).

India & ITK in Health care practices

India having 5000 years old heritage is a land of diversity as treated as miniature of continent due to its diversity in terms of geological, climate, population, language, culture, religion and other attributes. Besides the whole plethora of knowledge like mathematics, astronomy, Philosophy, grammar, literature, India rears a long sound heritage in the health care practices since the time of Sushruta (800BC) especially in traditional medicinal systems in current global context. Traditional knowledge in health care has emerged as an alternative to modern allopathy due to lot of promising attributes. The Indian subcontinent have 138 crore population, over 54 million (7%) of the tribal population belonging over 698 communities, spreading over 106 different linguistic groups with 227 subsidiary dialects. The tribal and ethnic communities use more than 8000 species of plants and approximately 25000 folk medicine based formulations in the health care practices. More than 1.5 million traditional medical practitioners in India use medicinal plants for preventive, promotional and curative purposes. More than 65% of the total population depends upon traditional medicine to address their medical needs. The communities can utilize the biological resources without disturbing the delicate balance of the nature as worshiping the motto of sustainable development. As far as present plant status is concerned, India has a rich biodiversity resources and the number of documented statistics can speak a lot in this regard. More than 7434 algae, 15447 of Fungi, 2917 of Lichen, 2786 Bryophytes , 1303 Pteridophytes, 82 Gymnosperms along with 22243 angiosperms can enough to speak the diversity which is more than 52216 in comparison of the total expected taxa of 203027 that yet to be explored. The total documented taxa are 20% in comparison to 80% taxa yet to be explored in near future. In addition to that, out of 75000 species of animals, the subcontinent is also have the proud privilege of catering 340 species of mammals, 1200 kind of birds, 420 reptiles, 2000 species of fishes, 4000 mollusc, 50000 species of insects along with other diverse group are the real treasure of the incubation of ITK in this regard to perform different socioeconomic activities. An appreciable amount of these biological species are used by the indigenous population for different value added products that reflect the intimate association of the people with the biological resources since the dawn of the civilization. The urge for the exploration of ITK are as follows:

- To improve the livelihood of the ITK holders and communities,
- To accelerate national economy,
- To conserve and restore environment
- To retard and prevent bio piracy.
- To combat the climate change for the sustainable development to address the call of the time.

The aboriginal people are the real stake holders of the nature as most of the life supporting ingredients are procured by the communities from the surroundings keeping sufficient space for regeneration for the next consecutive years as part of the sustainable development. The indigenous as well as the aboriginal people play a pivotal role in generating knowledge based system of the understanding of the environment. The indigenous as well as aboriginal play a pivotal role in generating knowledge based system of the understanding of the environment devising mechanisms to conserve and sustain their natural resources and to establish community based organization that serve as a forum for identifying the issue along with to deal with them through local-level experimentation, innovation, and exchange of information with other societies (March, 1992).

Survey area

Purulia district belongs to the district of West Bengal; India is one of the most under developed district of this state although it is the part of the grand old rock of the world. The district is symbolized for its bountiful landscape along with large indigenous as well as aboriginal population comprising of more than 20% tribal population. The district is under the latitude of 23°42'-22º43'N and longitude of 86º54'-85º49'E with an area of 6259sq Km under the Achaean Genesis of Chotonagpur plateau. The Tropic of Cancer passes through the north that marks the border area of West Bengal from the state of Jharkhand. The landscapes appeared like an Isosceles triangle with remote fragmented hillock zones it undulating topography. Dense scrub jungles are interspersed with dry deciduous vegetation. The

attitudinal variation is the key features of its topography and it varies from 250-699 mt. Hills and Hillocks are adorned with studs of jungle with south easterly course hill fed streams, temperature runs in between 5.8ºC in winter to extreme high heat of 50°C during summer. Rainfall ranges from 820mm-1800 mm annually but it mainly depends on monsoon. Rainy days are 64-99/ year. High tropical climate is the prevalent feature that makes the area as a treasure of biodiversity. Soil is porous, slightly acidic made up of gravels, sands and laterite. Warm and humid climate is the cause of deciduous vegetation (Haines, 1925). Mean relative humidity is 72%.This district is not only the paradise of biodiversity but also it has diverse heterogeneous population with a wide degree of the representation of the indigenous population. More than 21 tribal groups of population are represented by Bedias, Bedomajhis, Bhumijs(19%), Birhores(1%), Chikbariks, Karmalis, Kormundas, Loharas, Mahalis, Mundas(6%), Oraons, Paharias, Sabars (7%), Santhals (60%) etc and the Santhals occupy the dominant position as far as demographic features of the tribal peoples are concerned. The economy is mainly catalyzed by agriculture, tourism and some industrial settlement consisting of coal, scrap irons and cement industries. A wide variety of the plant species are used by them in order to address their different social-economic needs. The health care system is very poor in the inaccessible areas of this plateau and most of the indigenous people are habituated to use a wide variety of plants as a part of their indigenous traditional knowledge of the health care system.

Almost 95% of the tribal population belongs to the rural areas. The tribal societies of Purulia having distinct characteristics where most of the people are proto-austroloids group with dark skin color, sunken rose and lower forehead. The statistics convey the unstable number of the tribal population as far as the census is concerned and the negative population of the tribal population becomes visible due to migration, sanskritization, conversion into Christine and deserving social status keeping with pace of the urbanization. The ecological degradation of the region of this district where the tribal people becomes the victims of poverty and degradation due to fragile ecological attributes. The reduction of the tribal people in general and the aged people in particular is also a threat to the non-documented, verbal traditional knowledge due to the emerging threats of globalization and the market economy. It is the high time to transform tacit knowledge to explicit knowledge by proper documentation.

METHODOLOGY

Beside the field data being the researchers in this domain, extensive literature review in both peer-reviewed and Grey literature was exercised to extract the information in this particular field. The different plans, programs and national initiatives in the promotion and documentation as far as India's TK & ITK in relation to medicinal plants was thoroughly undergone for comprehensive data exploration. As told earlier regarding the raw ground data collected via primary and secondary sources as deployed by the local informers, the preliminary review started with a Google of the overarching terms like traditional knowledge (TK), indigenous Traditional knowledge (ITK), traditional Indian knowledge (TIK), access and benefit sharing (ABS).A key words list including the vocabulary of this domain like traditional knowledge, Indigenous Traditional Knowledge, Biodiversity etc were prepared. After the in depth preparation of the vocabulary, an extensive literature from January to August 2019 was exercised by using main two search engines like Google and Google scholar. Google Scholar was mainly used for the over view of peer reviewed papers that basically enables to explore the different attributes of Indigenous Traditional Knowledge and traditional knowledge comprising of reports, plans, action plans report on TK practices of the Indian subcontinent. The report of the AYUSH was also considered in this exploration of the relevant data which lies in the inventory of this national organization. The different NGO reports and the plans taken in this regard to cover the TK field was an important attributes in this regard to pulse the 'Magic of Reality' of this most uttered issues .The different stories, myths, religious sermons, festivals, culture and the different local artefact were also considered for the exploration of the traditional knowledge reared by the common people in local levels. The women are the key treasure of the unexplored knowledge in general and medicine particularly gynecological issues are very important. The medicine men like Kobiraj, Baidyya etc are also the treasure of this non-documented knowledge. The comprehensive talks with those people were also exercised to explore the truth behind their so called magic of

medicine. The Self Help group (SHG) are the most important social institution where the women of the marginal class contribute a major share as far as their knowledge and labour are concerned. The interactive session with these SHGs also extend a ray of hope to gather information in this regard. In addition to that, comprehensive questionnaires were also prepared and the extraction of data in this regard has been done with the help of the deployed informers in this regard.

Indigenous Traditional Knowledge & Purulia district

Traditional knowledge is the reflection of community's interest for their survival as well as sustainable development. Traditional knowledge regarding environment comprising of taboos, proverbs, cosmological knowledge, flower motifs, sacred grooves etc provide a lot of conservation ethos for biodiversity preservation and maintenance. Purulia is very rich in biodiversity and the aboriginal peoples cater a heritage of Traditional knowledge (TK) and Indigenous traditional knowledge (ITK) in the diverse fields. This is the culture and heritage of the indigenous people transmitted from generation to generation in verbal as well as non-documented form irrespective of their needs. There are different types of indigenous knowledge reared by the aboriginal people in Purulia district comprising of agriculture, cottage industries, environment and ecology, irrigation practices, harvesting technology along with the medicinal knowledge in the domain of the health care practices. Plants and trees are associated almost in every cases. Besides the below traditional plants (Table 2.) used by the aboriginal people in the primary health care practices, a lot of indigenous knowledge are there in the repository of traditional knowledge as far as agriculture, animal domestication, irrigation, Crop harvest and post harvest practices, for musical instruments, animal domestication and environmental conservation as a part of the sustainable development is concerned in this field.

DISCUSSION

The ITK of the indigenous people is the real repository of the medicinal plants being used by them for their health care systems but the formulations of the medicine generally differ on the basis of the different factors like the tribal groups, nature of the disease and other prerequisites available in the surroundings. The herbal preparations of the active principles are done either mono herbal or poly herbal formulations. In the mono herbal preparation, only single plant along with the particular additives are taken into account but as far as poly herbal preparation, more than one plants along with one or more additives are used depending upon the nature of the chemistry of the active ingredients. For example, In *Aloe vera*, being mono herbal in nature, only water is added as additive for the preparation in poly herbal preparation, more than one plant along with two or more additives are used for the same. More than 20 exotic plants are also used by the indigenous people in this regard but most of the cases in poly herbal preparations. Out of the 200 plants enumerated through in depth review of the existing exercise, a number of the families appeared higher number as per as species used are concerned. The most used family is Fabaceae followed by the other families like Rubiaceae, Asteraceae, Euphorbiaceae, Amaranthace, Combretaceae, and other as far as dicot is concerned whereas Poaceae, Aeraceae, Liliaceae etc in monocot. As far the used part is concerned, the morphological part of the plant specially leaves and stem occupied in significant in position followed by roots and stem. Besides this, a number of preparations are followed, the other used of the plant parts has been observed in this regard. It has also been observed that the aboriginal populations are habituated to use a number of plants as dietary supplement as complementary products. These are the supplements that are intended to supplement the diet and contain contain one or more nutrients include vitamins, amino acids, fibers etc. Some of the common plants used in this regard are *Asperagus racemosus* wild., *Cyperus rotundus*, *Hygrophila spinosa*, *Oxalis corniculata*, *Amaranthus viridis* etc. Besides the usual food plants, the tribal of this area use a number of non-traditional food plants like *Amorphophallus bulbifer*(Roxb.), *Bahuinia purpurea* Linn. of Fabaceae, *Dioscorea alata* Linn. of Dioscoreaceae, *Eleusine coracana* Linn.) Gaertn. of Poaceae, *Panicum miliare* Lam. of Poaceae etc in this regard. As a part of the festivity, number of plants are used in this regard. In a word, the indigenous people use the plenty of biodiversity for their different social-economic attributes since the time immemorial due to their misfortune economic status. The distribution of the top 10 families possessing maximum number of plants as shown in picture2.

CONCLUSION

Tribal people and traditional knowledge are synonymous and the aboriginal people have evolved their unique way of identifying medicinal plants and their traditional uses in health care systems is quite the old one. Demand for medicinal plants is growing all round and it is the right time to utilize our plant wealth and traditional medical knowledge in the service of the humanity as a component of the economic growth of our country.the present study revealed that the traditional medicines are still in common by the tribal people of Purulia district, west Bengal and the accurate knowledge of the plants and their medicinal values are yet to be explored. Hence, a thorough investigation of the plants along with their chemotherapy values is the call of the time. The efficacy and safety of all the reviewed ethnomedicinal plants needs to be evaluated for phyto-chemicals and pharmacological studies especially of those plants which are the components of polyhedral preparations. The exploration of the indigenous traditional knowledge with special reference to the health care practices surely a treasure of knowledge for the formulation of fourth generation medicines as well as the conservation of the genetic resources which are now at the verge of extinction due to the urbanization and ignorance of the urban thinkers.

ACKNOWLEDGEMENT

We wish to express our heartfelt thanks and sincere gratitude to all the leaders and residents of the people of this district for their support. We also extend acknowledgement to all those who contributed by exploring this areas tirelessly. We are also grateful to all those who are directly or indirectly associated to publish this article in virtual domain.

NO CONFLICT OF INTEREST

Authors have any conflict of interest.

REFERENCES

1. Ayanner, M. and Ignacimuthu, S. 2009b. Some less known Ethnomedicinal plants of Tirunelveli hills, Tamilnadu. *Journal of Economic and Taxonomic Botany*, 33 (Suppl.), 73-76.
2. Ayanner, M. and Ignacimuthu, S. 2011. Ethnobotanical survey of medicinal plants commonly used by kanti tribals in Tirunelveli hills of Western Ghats, India. *Journal of Ethnopharmcology*, 134, 851-864.
3. Basu R, Studies on Sacred Groves and taboos in Purulia district of West Bengal, Indian Forester, 126(12): 1309-1318.
4. Basu R, Traditional utilization of Plants in Intestinal, Malarial and Sexual Diseases by Tribals of Purulia. Advances in Plant sciences, 18(10) 133-137.
5. Basu R & Mukherjee PK, Food Plants of the tribe 'Paharias' of Purulia, Advances in plant sciences, 9 (2): 209-210.
6. Bruche Margaret. 2014. *Indigenous Knowledge and Traditional knowledge Encyclopedia of Global archaeology*. New York: Springer.
7. Chakraborty MK, Bhattacharyya A & Pal DC,2003, Ethnomedicinal uses of some exotic plants of Purulia district, West Bengal, India, J Econ Taxon Bot, 27 (3) 559.
8. Chakraborty MK & Bhattacharyay A, Some common Ethnomedicinal uses of various diseases in Purulia district, West Bengal, India, Ind. J. Trad. Knowledge, 9 (1): 77-89.
9. Chakraborty, Nihar Ranjan and Duary, Buddhadeb. 2014. Utilization of Some Weeds as Medicine by the Local People in Birbhum district of West Bengal. *India International Journal of Bioresource and Management*, 5 (1), 148-152.
10. Chopra RN, Chopra IC, Handa KL & Kapur LD, 1958, Indigenous Drugs of India, Academic Publishers, Calcutta.

11. Chopra RN, Chopra IC & Verma VS, 1968, Supplement to Glossary of Indian Medicinal Plants, National Institute of science Communication, New Delhi.
12. Dev, Such. 2006. *A selection of Prime Ayurvedic Plant Drugs*. New Delhi: Anamaya Publishers.
13. De JN, Further Observation of the ethnobotany of purulia district, West Bengal, Indian forester, 95 (8): 551-559.
14. Dey SK, De A, Karmakar S , De P K Chakraborty S, Samanta A & Mukherjee A, Ethnobotanical study in a remote district of West Bengal, India, Pharmbit, 20(2): 91-96.
15. Duary, B., Mukherjee, A. and Bhowmick, M. K. 2015. Phyto-sociological attributes of weed flora in major crops of red and lateritic belt in West Bengal. *Indian journal of Weed Science*, 47 (1), 89-95.
16. Ghosh A,2008, Ethnomedicinal Plants used in West Rarrh region of West Bengal, Natural Product Radiance, 7(5): 461-465.
17. Gogate, V. M. 2000. *Ayurvedic Pharmacology and Therapeutic Uses of Medicinal plants*. Mumbai: Bharatiya Vidya Bhavan
18. Hill, A. F. 1989. *Economic Botany: A Text book of useful plants and plants products*. New York: Mcgraw Hill Book Company,
19. Jain, S. K. 1964. The role of botanist in folklore research. *Folklore*, 5(4), 145-150.
20. Jain. S. K., 2010. *Manual of Ethnobotany*. Jodhpur, India: Scientific publishers.
21. Jain SK & Goel AK, Workshop Exercise-I, Proforma for Field Work. In: Jain, S.K (Editor) A Manual of ethnobotany, Scientific Publishers, Jodhpur,1995: 142-147.
22. Maity A & Manna CK, Some Ethnomedicines used by the santals of Purulia district, West Bengal, In controlling Fertility, 2002, Ethnobotany, 12: 72-76.
23. Martin, G. J. Ethnobotany. 1995. A 'People and Plants'. Conservation Manual *Chapman and Hall*, 268.
24. Maundu, P. 1995. Methodology for collecting and sharing indigenous knowledge: A Case Study. *Indig knowle Dev Monitor,* (3) 3-5.
25. Mishra, A. P., et. al. 2018. Bioactive compounds and health benefits of edible Rumex species, *Cell Mol Biol*, 64(8), 27-34.
26. Pal DC & Jain SK, Tribal Medicine, Nayaprakash Publishers, Kolkata, 1998
27. Pandey MM, Rastogi S & Rawat KS, Indian Traditional Ayurvedic System of Medicine and Nutritional Supplementation, Hindwai Publishing Corporation, 2013, 1-12.
28. Prabhu S and Vijaykumar S. 2016. Ethnobotanical study of Traditionally Used Medicinal plants in malyali ethnic People of Pachaimalai hills, Tamilnadu, India. *Journal of Pharmaceutical and Medical Research,* 2(1) 39-42.
29. Puspagandhan P & Kumar B, Ethnobotany, CBD, WTO and the Biodiversity Act of India, 2005, 17, 2-12.
30. Sur PR, Sen R, Halder AC & Bandopadhyay S, Ethnomedicine in the Ajodhya hills region of the Purulia district, West Bengal, India, J Econ Taxon Bot, Addl ser 10 (1992) 333.
31. Vedavathy. S. 2002. Tribal Medicine-The real medicine. *Indian Journal of traditional knowledge*,1(1), 25-31.
32. WHO. 2018. Traditional Medicine, Fact sheet. Retrieved from http:/*www.who.int/medicentre/factsheets/fs134/en.* (Accessed on: 12.12.2018).

Table: 1 Survey area

Census year	Total Population	Tribal Population	% of tribal Population	Comment
1971	16020875	313793	19.57	Fluctuation of population
1981	1853801	348372	18.79	-
1991	2254577	427766	19.23	+
2001	2536516	463452	18.27	_
2011	2927965	568027	19.4	+

Table: 2. Traditional plants and used

Sr. No.	Botanical name	Local Name	Family	Used part	Therapeutic value
1.	*Acanthus ilicifolius* L.	Hargoza	Acathaceae	Leave	Liver ailments
2.	*Abrus pectatorius* Linn.	Mirubaha	Fabaceae	Root	Fever
3.	*Abutilon indicum* L.		Malvaceae	Leaf & Fruit	Throat pain
4.	*Acacia nilotica* L.	Babla	Fabaceae	Bark	Malaria
5.	*Achyranthus aspera* Linn.	Apang	Amaranthaceae	Root	Healing of wound
6.	*Achyranthus bidentata* Blume	Sitirkad	Amaranthaceae	Whole plant	Boils
7.	*Achyranthus aspera Linn.*	Apang	Amaranthaceae	Leaves	Urinary disorders
8.	*Adansonia digitata*	Gorakh-amli	Bombaceae	Leaves	
9.	*Agave cantula* Roxb.	Konga	Agavaceae	Root	Intestinal worms
10.	*Ageratum conyzoides* L.	Tonka mani	Asteraceae	Whole plant	Skin sores
11.	*Alysicurpus vaginalis*(Linn.) DC	Bir but	Fabaceae	Root	Urine infection
12.	*Allophylus cobbe* (L)Raeusch.	Rakhal phul	Sapindaceae	Leaves	Stomach disorder
13.	*Alternanthera* sessilis(L.) R.Br.ex.DC	Salantisak	Amarathaaceae	Leaves	Asthma
14.	*Alpinia golonga*(L.)Wild.	Kulanjan	Zingiberaceae	Rhizome	Urinary troubles
15.	*Amaranthus spinosus* L.	Katanate	Amaranthaceae	Leaves	Boil & Acne
16.	*Ammania baccifera L.*	Dadmari	Lathyraceae	Leaves	Burning
17.	*Ampleocissus tomentosa* (Roth.) Planch	Datropm bili	Vitaceae	Root	Boil
18.	*Amorphophallus paeoniifolius* (Dennst.) Nicolson.	Ole	Araceae	Stem	Constipation
19.	*Andrgraphis paniculata* Wall ex Nees	Kalmegh	Acanthaceae	Leaves	Liver troubles, fever
20.	*Annona squamosa* L.	Newa	Annonaceae	Root	Abortion
21.	*Ardisia solanacea* Roxb.	Raktaphar	Myrsinaceae/Primulaceae	Stem-bark	Headache
22.	*Argemone mexicana* Linn.	Sialkanta	Papaveraceae	Stem & Seed	Eye & Skin diseases
23.	*Aristolochia indica* L.	Ishermul	Aristolochiaceae	Root	Lukewarm
24.	*Artimisia absinthium* L.	Mastaru	Asteraceae	Whole plant	Liver trouble
25.	*Artocarpus heterophyllus* Lam.	Kathal	Moraceae	Inflorescence	Boil
26.	*Asperagus racemosus*	satmul	Liliaceae	Root	Dysentery
27.	*Astracanthus longifolia (L.)Nees.*	Kulata	Acanthaceae	Leaves	Blood purifier
28.	*Atylosia scarabaeoides* (L) Benth.	Birhara	Fabaceae	Whole plant	Dysentery

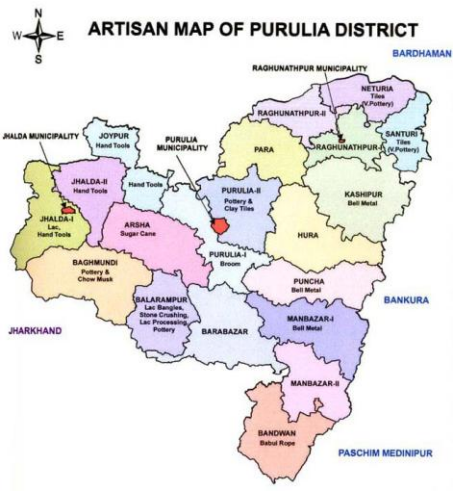
29.	*Averrhoa carambola* L.	Kaamranga	Oxalidaceae	Roots	Ulcer
30.	*Azadirachta indica* A. Juss.	Bokon-dare	Meliaceae	Stem bark	Contraceptive
31.	*Bauhinia purpurea* Linn.	Singara	Fabaceae	Stem	Healing of wounds
32.	*Bacopa monieri (L.) Wettest.*	Bramhi	Scrophulariaceae	Whole plant	Rejuvenation
33.	*Bombyx ceiba* L.	Shimul dare	Bombacaceae	Root	Irregular menstrual cycle
34.	*Barleria lupulina* Lindl.	Bialykaran	Acanthaceae	Leaves	Burning
35.	*Blumea lacera (Bum.F)DC*	Sialmutra	Asteraceae	Leaves	Wounds
36.	*Borreria hispida* (L.) Schum.	madanghanti	Rubiaceae	Leaves	Wounds
37.	*Boerhavia diffusa* L.	Kathsak	Nyctaginaaceae	Leaves	Cut
38.	*Bombyx ceiba* L.	Bakul	Bombacaaceae	Leaves	Mouth ulcer
39.	*Bryenia retusa* (Dennest) Alston	Jirul	Phyllanthaceae	Leaves	Pneumonia
40.	*Bryophyllum pinnatum* (Lam.) Oken.	Marang upu	Crassulaceae	Whole plant	Recent wound
41.	*Buchanania lanzen* Spreng.	Piyal	Anacardiaceae	Root	Urinary problems
42.	*Butea superba* Roxb.	Lata Palash	Fabaceae	Leaf	Stop bleeding
43.	*Byttneria herbacea* Roxb.	Kamraj	Rubiaceae	Leaves	Wounds
44.	*Caesalpinnia bonduc* (L)Roxb.	Nata	Fabaceae	Seed	Malarial fever
45.	*Caesaulia axiilaris* Roxb.	Ote kesam	Asteraceae	Leaves	Skin disease
46	*Cannnabis sativa* L.	Ganja	Cannabaceae	Leaves	Cut
47.	*Calotropis gigantea*	Akanda	Asclepiadaceae	Root & leaves	High fever
48.	*Cardiospermum halicacabum* Linn.	Lataphatki	Sapindaceae	Root	Rheumatism
49.	*Careya arborea* Roxb.	Kumbir	Lecythidaceae	Stem bark	Leucoderma
50.	*Cassia fistula* Linn.	Bahamuru	Fabaceae	Fruits	Constipation
51.	*Cayratia pedata* (Wall.) Gagnep.	Goali-lata	Vitaceae	Stem	Healing bone fracture
52.	*Celastrus paniculatus* Wild.	Munjuri	Celastraceae	Root bark	Chest pain
53.	*Chenopodium album* L.	Bethua	Amaranthaceae	Leaves	Dysentery
54.	*Cipadessa baccifera* (Rth.) Mlq.	Unavaible	Meliaceae	Stem	Sores
55.	*Cissampelos pareira* Linn	Tijumala	Menispermaceae	Root	Stomach pain
56.	*Cissus repanda* Vahl.	Bod nari	Vitaceae	Whole plant	Cuts & Bruises
57.	*Cleistanthus collinus*(Roxb.) Benth	Kargali	Euphorbiaceae/ Phyllanthaceae	Stem bark	Skin disease
58.	*Cleome icosandra* Linn.	Hurhuria	Cappridaceae/ Cleomaceae	Leaves	Haematuria
59.	*Clerodendrum infortunatum* auct. Non Linn C.B. Clarke	Ghato	Verbenaceae	Leaves	Rheumatism

60.	*Coccinia grandis L.*	Kundru	Cucurbitaceae	Fruit	Menstrual disorders
61.	*Combretum pilosum* Roxb.	Sikarbans	Combretaceae	Leaves	Water sores
62.	*Commelina nuduflora* Linn.	Kansira	Commeliniaceae	Whole plant	Skin disease
63.	*Cotula anthemoides* L.	Tar dingla	Asteraceae	Whole plant	Pimples
64.	*Costus speciosus* (Koening) Sm.	Kewa-kanda	Zingiberaceae	Rhizome	Rheumatism
65.	*Crinum asiaticum* Linn.	Baniyaj	Amarylidaceae	Bulbs	Boil
66.	*Crotalaria prostrata* Rott.ex.Wild	Chotojhunjhuni	Fabaceae	Root	Abortion
67.	*Curculigo orchenoides* Gaerta.	Sareng judu	Amaryllidaceae	Tuber	Helminth infection
68.	*Curcuma longa* L.	Halud	Zingiberaceae	Rhizome	Ulcer, cut, Acne
69.	*Cynotis axillaris* Roem and Sch	Tena ara	Commelinaceae	Root	Fever
70.	*Cynodon dactylon* pers.	Dhobigahs	Poaceae	Whole plant	Piles bleeding
71.	*Cyanoglossum glochidiatum* Benth.	Latenga	Boraginaceae	Roots	Wounds
72.	*Cynanthillium cinereum(L.)H.Rob.*	Sahadevi	Asteraceae	Whole plant	Antibiotic
73.	*Cyperus rotundus* L.	Mutha	Cyperaceae	Whole plant	Ulcer
74.	Datura metel Linn.	Dhutra	Solanaceae	Seeds	Leprosy
75.	*Dentella repens (Linn.) J.R.Forst & G.Frost*	Chetrasak	Rubiaceae	Leaf	Sores.
76.	*Desmodium gangeticum*(L.) DC	Titakari	Fabaceae	Root	Abortion
77.	*Desmodium triflorum* (L) DC	Latalati	Fabaceae	Root	Abortion
78.	*Dioscorea oppositifolia* L.	Panialu	Discoreaceae	Tuber	Constipation
79.	*Elephahantropus scaber* L.	Somdulum	Asteraceae	Root	Antidysmenhoric
80.	*Eriodendron anfractuosum* DC.	Halud simul	Bombacaceae	Latex of bark	Leucorrhoea
81.	*Enhydra fluctuans Lour.*	Hincha	Asteraceae	Leaves	Appetite
82.	*Eulophia nuda* Lindl.	Budbari	Orchidaceae	Root	Snake bite
83.	*Evolvulus alsinoides* L.	Dhanka puspi	Convolvulaceae	Whole plant	Memory loss and bronchitis
84.	*Euphorbia hirta* L.	Lalpata	Euphorbiaceae	Aerial part	Measles& Cut
85.	*Euphorbia milli* var. Longifolia D. Moul.	Latjakha	Euphorbiaceae	Leaves	Burning
86.	*Ficus benghalensis* L.	Asasta	Moraceae	Leaves & bark	Pimples
87.	*Ficus hispida* L.f	Dumur	Moraceae	Leaves	Boils
88.	*Gardenia gummifera* L.	Bhuru	Rubiaceae	Leaf	Chronic pain
89.	*Gardenia latifolia* Ait.	Popra	Rubiaceae	Leaves	Water sore

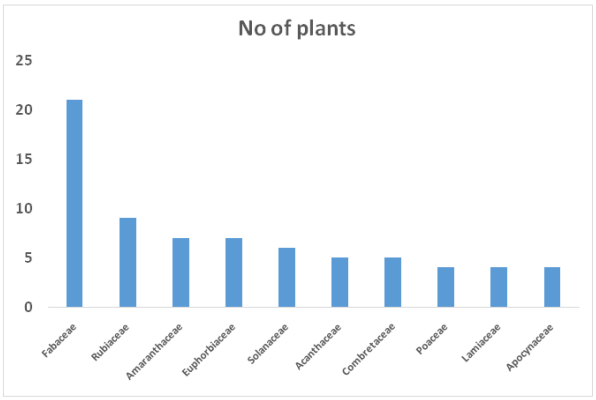
90.	*Glinus oppositifolius Linn.*	Gimashak	Molluginaceae	Leaves	Antimalarial
91.	*Gmelina arborea* Roxb. Ex Sm.	Gamhar	Verbinaceae/Lamiaceae	Stem	Wounds
92.	*Gloriosa superba* L.	Languli lata	Liliaceae	Tuber	Leprosy
93.	*Gomphrena celosoides Mart.*	Chanchi	Amaranthaceae	Whole plant	Antimicrobial
94.	*Helicteres isoara* L.	Kukurbicha	Sterculiaceae	Fruit	Scabies
95.	*Hemidesmus indicus* R. Br.	Analsing	Asclepiadaceae	Root	Snakebite
96.	*Hibiscus sabdariffa* L.	Kudrung	Malvaceae	Leaves	Dyspepsia
97.	*Hygrophila auriculata* Heine	Dhela kanta	Acanthaceae	Roots	Wounds
98.	*Holarrhena pubescens* Wall. Ex G Don	Hat	Apocynaceae	Stem bark	Chronic dysentery
99.	*Holostemma annulare* (Roxb.) K.Schum	Moron ara	Asclepiadaceae	Root	Cough
100.	*Hymenodictyon orixense*(Roxb.) Mabb.	Bhorkud	Rubiaceae	Bark	Malaria
101.	*Hygrophila auriculata* Heine.	Kulekhera	Acanthaceae	Leaves	Anaemia
102.	*Hyptis suaveolens* (L.) Poit.	Ganga tulsi	Lamiaceae	Whole plant	Dermal infections
103.	*Ichnocarpus frutescens* R.Br.	Ishalanguli	Apocynaceae	Root	Syphilis
104.	*Jatropha caracus* Linn.	Bhrenda	Euphorbiaceae	Seed	Bowl problem
105.	*Lemna minor L.*	Taramoni	Araceae	Whole Plant	Fodder
106.	*Leonitis nepetaefolia* R.Br.	Janum dhompo	Lamiaceae	Flower	Breast inflammation
107.	*Leucas aspera(Wild.) Link.*	Halkusa	Lamiaceae	Leaves	Cough & cold
108.	*Lippia alba* (Mill.) N.E.Br.ex. Britton and P.wilson	Laltia	Verbenaceae	Aerial parts	Eczema
109.	*Litsea glutinosa* (Lour.) C.B. Robins	Harila	Lauraceae	Stem bark	Bone fracture
110.	*Lobelia alsinoides Lam.*	Badnali	Campunalaceae	Whole plant	Stomach disorders
111.	*Ludwigia perennis L.*	Bon lobonga	Onagraceae	Fruit	Intestinal disorders
112.	*Madhuka longifolia* (Koening) Macb.	Mahul	sapotaceae	Seed	Rheumatism
113.	*Martynia annua* L.	Bagh nakh	Martyniaceae	Seed oil	Rheumatism & Scabies
114.	*Mellotus philippensis* Muell Arg.	Rora	Euphorbiaceae	Root	Birth pain
115.	*Mellilotus indica Mill.*	Bon methi	Fabaceae	Leaves	Antioxidant
116.	*Melothria perpusilla* (Blume) Cogn.	Birkudri	Cucurbitaceae	root	Syphilis
117.	*Martynia annua* Linn.	Gaymukhi	Martyniacea	seed	Body sores
118.	*Mimosa rubicaulis* Lamk.	Sega jamun	Fabaceae	Root	Vomiting
119.	*Mimosa pudica Linn.*	Lajjabati	Fabaceae	root	Leprosy

120.	*Mimusops elengi* L.	Bohur	Sapotaceae	Stem bark	Toothache
121.	*Moringa olifera* Lam.	Munga-sag	Moringaceae	Stem bark	Anti-inflammatory
122.	*Mucuna pruriens* (l.) DC	Alkushi	Fabaceae	Roots	Abortion
123.	*Nycanthus arbor-tristis* L.	Saparom	Oleaceae	Leaf juice	Cough & cold
124.	*Opuntia dilleni* (Kar Gawl.) Haw	Nagphena	Cactaceae	Leaves	Skin irritations
125.	*Oxalis corniculata* L.	Amboti	Oxalidaceae	Whole plant	Antidote to poison of Dhutra seeds
126.	*Pavetta indica* Linn.	Budhi ghasse	Rubiaceae	Root	Headache
127.	*Peperomia pellucida* L.	NA	Peperomiaceae	Leaves	Fever
128.	*Peucedanum nagpurense* (C.B.Ci.) Prain.	Oponum	Apiaceae	Root	Intestinal disorders
129.	*Phoenix acaulis* Roxb.	Ban khajur	Arecaceae	Pith	Stomachache
130.	*Physalis minima Linn.*	bontepri	solanaceae	Fruit	Analgesic
131.	*Plumeria rubra* L.(Cult.)	Gulunch	Apocynaceae	Leaves	Indigestion
132.	*Polyygonum plebecium* R.Br.	Muni ara	Polygonaceae	Whole plant	Diarrhoea
133.	*Polygala arvensis* Wild.	Rali	Polygalaceae	Root	stomachache
134.	*Portulaca oleracea L.*	Nunishak	Potulacaceae	Whole plant	Dysentery
135.	*Pterocarpus marsupium* Roxb.	Pesar	Fabaceae	Stem bark	Dysentery
136.	*Pterospermum acerifolium* Willd.	Muskundu-daru	Sterculiaceae	petals	Indigestion
137.	*Pueraria tuberosa*(Roxb.ex.Wild.)DC	Patal dingla	Fabaceae	Tuber	Syphilis
138.	*Randia dumetorum*(Retz.) Poir.	Barkamahia	Rubiaceae	Bark	Fever
139.	*Ricinus communis* L.	Jara	Euphorbiaceae	Leaves	Eczema
140.	*Ruellia suffruticosa* Roxb.	Chaulia	Acanthaceae	Root	Venereal disease
141.	*Schrebera swietenioides* Roxb.	Eksira	Oleaceae	Root	Body sores
142.	*Schleichera oleosa* (Lour.) Oken.	Pusar	Sapindaceae	Seed oil	Scabies
143.	*Scilla indica* Baker non-Roxb.	Lakrapiyaj	Liliaceae	Bulb	Headache
144.	*Scoparia dulcis* Linn.	Jastimodhu	Scrophulariaceae	Whole plant	Gout
145.	*Semicarpus anacardium* L.f	Sosobili	Anacardiaceae	Seed oil	Rheumatism
146.	*Senna alata (L.) Roxb.*	dadrumardan	Fabaceae	Leaves	Anti fungal
147.	*Shorea robusta* Gaertn. f	Sakhu	Dipterocarpaceae	Resin	Chest pain
148.	*Sida cordata*(bum.f) Borss. waalk	Barial	Malvaceae	Fruits	Skin disease
149.	*Sida rhombodifloia* L.	Bagjati	Malvaceae	Root	Abortion

150.	*Smilax zeylanica* L.	Ramdatan	Smilaceae	Roots	Boils & eczema
151.	*Solanum xanthocarpum* S & W	Gothbengan	Solanaceae	Fruit	Cough
152.	Solanum nigram *Linn.*	Kakmachi	Solanaceae	Fruits & Leaves	Psoriasis
153.	*Solanum surattense* Burm. f	Rambaigan	Solanaceae	Root	Teeth caries
154.	*Solanum sisymbrifolium* Lamk.	Got-begun	solanaceae	Fruit	Cough
155.	*Spermacoce hispida* Linn.	Satgithea	Rubiaceae	leaves	wounds
156.	*Sphaeranthus indicus* L.	Murmuri	Asteraceae	Leaf juice	Elephantiasis
157.	*Spilanthes paniculata* Wall. Ex DC	Raipuru	Asteraceae	Leaves	Boils
158.	*Symplocos racemosa* Roxb.	Titikar	Symplocaceae	Stem bark	Inducing abortion
159.	*Syzygium Cerasoideum* (Roxb.) Chatterjee & kanjilal f	Katijamum	Myrtaceae	Fruits	Rheumatism
160.	*Tamarindus indicus* L.	Jojos	Fabaceae	Fruit	Antidote of poison
161.	*Tephrosia purpurea(Linn.) Pers.*	Bon nil	Fabaceae	Leaves	Diarrhoea
162.	*Terminalia arjuna* (Roxb.) Wight & Arn.	Arjun	Combretaceae	Stem bark	Anaemia in women
163.	*Terminalia bellerica* Roxb.	Bahera	Combretaceae	Stem bark	Cold
164.	*Terminalia chebula* Retz.	Haritaki	Combretaceae	fruits	Intestinal problem
165.	*Thysanolaena agrostis* Nees.	Phulijharu	Poaceae	Root	Fever
166.	*Tinospora cordifolia* (Wold.) Miers ex Hooj. F & thoms.	Latgulanj	Menispermaceae	Stem	Venereal disease
167.	*Tridex procumbens* Linn.	Dochanti	Asteraceae	Leaves	Liver disorders
168.	*Uraria picta* Desv.	Ishwarjata	Fabaceae	Root paste	Antidote of snakebite
169.	*Urena lobata Linn.*	Bon-ochra	Malvaceae	Leaves	Antifungul
170.	*Vitex negundo Linn.*	Begna	Lamiaceae	Leaves	Expectorant
171.	*Vitex peduncularis* Wall. ex Schauer	Bhadu	Verbenaceae	Leaves	Eye
172.	*Ventilago denticulata* Wild.	Bonga-sarjom	Rhamnaceae	Root paste	Antiabortion
173.	*Vernonia anthelmintica* Diluton.	Bonjira	Asteraceae	Whole plant	Acidity
174.	*Vetiveria zizanioides* (L.) Nash.	Siromu	Poaceae	Root juice	Control vomiting
175.	*Vitex negundo* L.	Sinduhari	Verbenaceae	Leaf	Rheumatism
176.	*Woodfordia fruticosa* Kurz.	Dahiphul	Lathyraceae	Leaf infusion	Nausea & cough
177.	*Ziziphus oenoplia* Mill.	Siakul	Rhmnaceae	Root	Anti-helminthic
178.	*Zingiber officinale* Rosc.	Adi	Zingiberaceae	Rhizome	Cough



Picture. 1. Location of the study area of Purulia district(Downloaded from Google).



Picture: 2. Maximum number of top 10 families along with plant numbers.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Floristic Diversity of Flood Affected Kunthipuzha Riparian Basin-Thathengalam, Palakkad, District, Kerala

Rasmi A R[1]*, Suresh V[1], John Tom[2], Navya T[2], M. Midhun[2], Irfana MA[2], Athira MP[2], Athira PR[2], Divya PK[2], Anju PK[2] and O. Ashfak Ahammed[2]

[1]Assistant Professor, Department of Botany, Government Victoria College, Palakkad, Kerala, India.
[2]Post Graduate and Research Department of Botany, Government Victoria College, Palakkad, Kerala, India.

Received: 08 Nov 2020 Revised: 10 Dec 2020 Accepted: 13 Jan 2021

***Address for Correspondence**
Rasmi A R
Assistant Professor,
Department of Botany,
Government Victoria College,
Palakkad, Kerala, India
Email : : rasmibotany@gmail.com

ABSTRACT

Riparian zones are considered ecotones, disturbance like a flood, in such areas, drastically affects species diversity. Thus, enumeration of the plant diversity in flood-affected riparian area has immense value in making biodiversity conservation strategies for such an area. The present study analyzed the floristic element of the Kunthipuzha river basin, near Thathengalam, Palakkad district, Kerala, India, which was drastically affected by the flood in 2018. The study observed several endemic, vulnerable, endangered species even after the flood in this area which is presently covered with sand and pebbles for about 500m. Now this area is known as "Thathengalam beach" and became converted into a tourist spot. The present inquiry summarizes the angiosperm diversity in the riparian system of the Kunthipuzha river basin near Thathengalam along with its percentage endemism and biological invasion. The evaluation of the angiosperm diversity of this area revealed the presence of 142 species belonging to 63 families with a higher elemental contribution by Fabaceae followed by Euphorbiaceae and Asteraceae. The riparian flora of this river basin embodies three characteristic riparian taxa together with 23 wetland species.

Keywords: Riparian flora, Kunthipuzha, Palakkad, flood, flora, Thathengalam

INTRODUCTION

The word 'Riparian' originated from the Latin word 'Ripa' which means the bank of a river, pond, or lake of the surrounding landscape [1,2]. Riparian ecosystems are located next to streams, rivers, lakes, wetlands, and have a direct influence on aquatic and wildlife habitat. It is also known as gallery forests and streamside forests [3]. Riparian

systems are the three-dimensional ecotones of interaction including terrestrial and aquatic ecosystems that extend down into the groundwater, up above the canopy, outward across the floodplain, up the near-slopes that drain to the water, laterally into the terrestrial ecosystem and along the watercourse [4]. They form a complex assemblage of organisms and their environment existing adjacent to and near flowing water [5]. As ecotones, they encompass sharp gradients of environmental factors, ecological processes and plant communities. The riparian ecosystem provides a corridor for the movement of biota and serves many important roles for humans [6]. However, there is a lacuna in the riparian floristic research in India except for a few ecological studies [7,8,9,10,11,12,13,14,15,16]. As per these reports, the Western Ghats river ecotones comprise an amalgamation of evergreen, semi-evergreen, deciduous, riparian, wetland, and mangrove components with high species diversity and endemism. Moreover, the vegetation in the riparian area has characteristics of both aquatic and upland habitats. Plants of the riparian forest have numerous morphological and physiological adaptations that suit them for life in high-energy and wet environments [17,18]. The diversity of angiosperm in the riparian system along Thuppanad river, Southern Western Ghats, India, along with their phytogeographical affinities, percentage endemism, morphological adaptations, and biological invasions. Their inquiry revealed the presence of 270 species belonging to 70 families [19]. The study on riparian flora of the Pamba River recorded 545 angiosperms in 119 families, 3 gymnosperms in 3 families,31 pteridophytes in 14 families. The project concluded by saying that this riparian flora is highly endemic, endangered, and species-rich[12].The diversity studies on herbaceous riparian flora in the lower stretch of the Bharathapuzha river, Kerala recorded collected 176 angiosperms belonging to 63 families [20].

Disturbances have large effects on species diversity in any community [21]. It has been reported that the riparian ecosystems are the most species-rich, productive, and sensitive to anthropogenic interference resulting in disturbance to adapted species communities [22,23].From the literature, it is clear that there is a lacuna in the studies on the flood-affected riparian zone of Kerala. In 2018, the whole state was affected by a severe flood, resulting in severe destruction. At that time, the river basin of the famous river of Kerala, Kunthipuzhanear Thathengalam in Mannarkkad taluk of Kerala drastically affected and floodwater which brought lot of pebbles and in this created a feeling of “beach”. Kunthipuzhariver originates from the Silent Valley National Park as a tributary of the river Thuthapuzha. Due to the flood, the river Kunthi bifurcated to about a half kilometer and this area was underwater for almost two weeks. Till now, no diversity analysis has been conducted in riparian zones of Kunthipuzha at Thathengalam, Mannarkkad after the flood. This research aimed to study the flora of flood-affected riparian zone in Thathengalam, Mannarkkad by enumerating the plant diversity.

MATERIALS AND METHODS

Study area

The present investigation focuses on the floral diversity of the riparian system of selected sites of Kunthipuzha basin such as Thathengalamin Mannarkkad of Palakkad district, Kerala, India (Fig 1).The research approach involves a taxonomic/ vegetational survey of the lower stretch of Kunthipuzha river. This site is located between 11°01'54" N latitude and 76°26'47" E longitude. The survey was conducted by collection of plants from the study area during different climatic periods from September 2018 to March 2019. Repeated visits were made for getting specimens with both the vegetative phase and reproductive phase of each plant. After observation, habit, structure of the leaf, the colour of the flower and other morphological features were noted. The specimens were identified using relevant literature and enumerated as per APG IV [24]. The nomenclature validation has been done with IPNI (www.ipni.org) and the plant list (www.theplantlist.org) [25]. RET taxa assessment was based on IUCN (2018). Wetland elements were also designated [26].

RESULT

Study area

From the site study, it was revealed that Kunthipuzha river had changed its actual flow direction for almost 500m in this area. A huge amount of pebbles, sand, and silt got deposited on the banks of the river and the river bed became almost dry. Now, this area looks like a mini beach (Plate1).Water washed the fertile topsoil of the nearby cultivated land thereby making it unfit for agricultural purposes. The flood and bifurcation of the river cause several damages to the flora and fauna of this particular area.

Floristic Analysis

Analysis of riparian flora of Kunthipuzha at Thathengalam basin revealed the presence of 152 plant species. Among them, 142 were angiosperms, belonging to 63 families, followed by 9 pteridophytes of 6 families and 1 gymnosperm from the family Cycadaceae. The 142 angiosperm species were belongs to 130 genera. Among the angiosperm's taxa recorded, 118 belongs to dicotyledons and 24 belongs to monocots. The vegetation mainly consists of herbaceous members (67), followed by trees (30), shrubs (26), and climbers (19). The present study recorded the dominance of families Fabaceae species (13) followed by Euphorbiaceae and Asteraceae which recorded ten species each. The results are represented in Table:1 After analysis with the IUCN red list 2018 it was found that 6 species were recorded as endemic (4 endemic to Southern Western Ghats, 1 each endemic to peninsular India and Kerala). Based on the data on *bsienvis.nic.in* (2017)[27], 26 were invasive alien species among the collected plant species. In these collected plants *Heliotropium keralensis* Sivarajan & Manilal has reported as endemic to Kerala. *Hopea ponga* (Dennst.) Mabb, *Impatiens gardneriana* Wight, *Naregamia alata* Wight & Arn, *Torenia bicolor* Dalz. were reported as endemic to Southern Western Ghats. *Chionanthus Mala-elenji* (Dennst) P.S. Green and *Spermacoce hispida* L. were endemic to Peninsular India. Among this, *Hopea ponga* is coming under endangered and *Torenia bicolor* is coming under Least concern. *Syzygium caryophyllatum* collected from this area also has been reported as endangered. The riparian flora of this river basin embodies three characteristic riparian taxa such as *Pandanus furcatus* Roxb., *Homonoia riparia* Lour. *Persicaria barbata* (L.) H. Hara., together with 23 wetland species. Among the identified alien species, *Chromolaena odorata* (L.) R.M.King & H. Roband *Mikania micrantha* Kunth. were dominant. Highly invasive species like *Lantana camara* was also present. The IUCN categorized important plants enlisted in Table 1 are presented in Plate 2. Invasive alien species are given in Table 2.

Analysis of Human intervention

Thousands of people visiting the site here to capture the visuals that make the place a tourist spot. It pollutes the area by the accumulation of plastic wastes, food wastes, burning of wastes, etc. This may lead to the destruction of many species. For example, *Impatiens gardneriana* Wight is a species endemic to Southern Western Ghats which was collected from the study site in the early collection period however, in the latter surveys didn't see this plant in that area. According to the IUCN red list (2016) category, *I.gardneriana* was considered as vulnerable. Another important reason that affects the flora of this region is overgrazing. Local peoples graze their cattle on the river bank. Overgrazing also disturbs the balance of the riparian ecosystem. As the survey recorded 2 endangered,1 vulnerable,28 least concern, and 6 endemic species, this area is found to be rich in plant diversity. So, overgrazing became a chance to affect these RET taxas and may get extinct or become vulnerable in near future. The riparian forests of Thathengalam have diverse flora with high species richness. Flood undulation and anthropogenic disturbances like sand mining, cultivation, waste disposal in the lower stretch of Kuthipuzha river is a great threat. The vegetation profile of lower stretches of the Kuthipuzha river indicated that the number of herb species is high probably due to the disturbance in the region and primary wetland nature of the stretch. Climbers and shrubs were observed in low numbers due to the seasonal clearings in this area. Due to increased flooding, there was an overall decline in riparian species richness, while riparian plant biomass increased.

DISCUSSION

Riparian zones are one of the most diverse and productive ecosystems[28].They are considered as potentially threatened and endangered ecosystems in the world due to abusive and intensive land use[29,30].This may lead to the extinction of many species and thus disturbs the balance of the ecosystem. As the place became a tourist spot, anthropogenic activities have polluted the area by the accumulation of plastic wastes, food wastes, burning of wastes, etc. The riparian buffer zone and flood plain areas were dominated by agricultural practices on both sides which disturb the ecological condition of the river [11].The impact of anthropogenic disturbances on forest structure and plant diversity in the riparian forest in the Cauvery wildlife sanctuary, Karnataka, and concluded that species-rich areas in riparian forests are under threat [31]. Marginal vegetation of river zones in Thamirabarani river of Kanyakumari district, Tamil Nadu, recorded 720 species of angiosperms belonging to 449 genera under 126 families [32]. The study on the riparian buffer zone of Chandni Nalla, India assessed that the zone had less vegetation along the stream banks ·Riparian vegetation of river Manuni in the Western Himalayan region of India enumerated 55 genera of trees, 34 genera of shrubs, 64 genera of herbs, and 12 genera of climbers [34]. In Kerala, studies on the riparian flora recorded different species present under several hierarchical categories [7,34,19,35,36,20,37,38]. Increased flooding resulted in the arrival of more seeds of additional species to the riparian zone, thereby potentially facilitating the shifts in riparian plant species composition [39]. In Thathengalam also the flood destroyed riparian forest due to bifurcation of the river and uprooting of trees.

CONCLUSION

Riparian zones are rich with species diversity. Trees can hold fertile topsoil and can reduce the effect of flood. A riparian forest provides food and fodder for local peoples. Since it is an ecotone, many important floras and faunas can be found. But at present, the riparian zones are encroached by humans for their various needs like agriculture, construction, grazing animals, mining, etc. This can cause an imbalance in the steady ecosystem. All the activities of humans in one way or another affect their stability. If these undesirable activities continue, many endemic and RET species will soon get extinct. So, the government should take necessary action to protect the riparian zones. Protection of these areas should be prioritized in policy because anthropogenic disturbance has led to decreasing riparian forest species diversity and structure. This study also revealed that the species-rich riparian area of Kunthipuzha basin like Thathengalam is under threat. The phytosociological results indicate the need for the adoption of a strong ecosystem management strategy, particularly for rare, endemic flora.

REFERENCES

1. Tabacchi AMP, Tabacchi E, Naiman RJ, Deferrari C, Decamps H. Invasibility of species-rich communities in riparian zones. Conserv. Biol. 1996; 10(2):598-607.
2. Junk WJ, Piedade MTF. Plant life in the flood plain with special reference to herbaceous plants. In: The central Amazon flood plain, Springer, Berlin, Heidelberg; 1997. p. 147-185.
3. Brinson MM. Riverine forests. In: Lugo AE, Brinson MM, Brown SL (Eds.) Forested wetlands. Ecosystems of the world, Elsevier, Amsterdam, 1990. p. 15:87-141.
4. Ilhardt BL, Verry ES, Palik BJ. Defining riparian areas. Chapter 2. In: Riparian Forest Management. Forestry and the riparian zone, Orono, Maine2000;7-14.
5. Lowrance R, Leonard R, Sheridan J. Managing riparian ecosystems to control non - point pollution. J. Soil Water Conserv.1985;40(1): 87-91.
6. Kemper NP. RVI: Riparian Vegetation Index. Draft report to the Water Research Commission.WRC Project No. K5/850, 1999.
7. Bachan AKH. Riparian vegetation along the middle and lower zones of the Chalakudy river. Kerala (India): Limnological Association of Kerala, Irinjalakuda,2003;58–63.

8. Sreedharan TP. A study on the Status of Valapattanam river with special reference to its ecology and socio-cultural aspects, Project report. The Kerala Research Programme on Local level Development, CDS, Thiruvananthapuram, 2005.
9. Sunil C, Somashekar RK, Nagaraja BC. Riparian vegetation assessment of Cauvery river basin of South India. Environment monitoring Assessment 2010; 170:545-555.
10. Nandhakumar MK, Ramya MK, Shinila P, Divyalakshmi P. Phytosociological analysis of riparian tree species of Alakyam stream; Pariyaram, Kerala, India. Int. J. Environ. Sci. 2012;(2) :1896-1903.
11. Vyas V, Kumar A, Wani GS, Parashar V. Status of riparian buffer zone and floodplain areas of River Narmada, India. Int. J. Environ. Sci. 2012;3(1):659-674.
12. Paul J. Taxonomy, distribution and ecology of the riparian flora of Pamba river Kerala. PhD Thesis. Mahatma Gandhi University, 2013.
13. Vincy MV, Brilliant R, Paul J, Pradeep kumar AP. Comparison of riparian species diversity between the main river channel and sub watersheds of Meenachil river basin, Kerala, Southern India. Braz. J. Bot. 2014;38(1):81-98.
14. Bhaskar A, Karthick NM. Riparian forests for healthy rivers. Curr. Sci. India 2015;108 (10):1788-1789.
15. Sankhwal AO, Shah S D, Gavali, DJ, Dudani SN. Riparian Flora of Mahi River, Gujarat. *Biolife*, 2015;3 (4):820-826.
16. Shah DR, Dudani SN, Sankhwal AO, Gavali DJ. Riparian vegetation of Mini River in Vadodara, Gujarat. Int. J. Environ. Sci.2015;6(2):307-317.
17. Barker JR, Ringold PL, Ballman M. Patterns of tree dominance in coniferous riparian forests. Forest Ecology and Management, 2002;166 (1-3):311-329.
18. Merritt DM, Scott ML, Poff NL, Auble GT, Lytle DA. Theory, methods and tools for determining environmental flows for riparian vegetation: riparian vegetation-flow response guilds. Freshwater Biol., 2010;55:206–225.
19. Jisha K, Maya C. Diversity analysis of Angiosperms in Riparian system along Thuppanad River, Southern Western Ghats, Kerala, India. Int. J. Adv. Res. 2018;6(9):531-539.
20. Cherullipadi LB, Paul J. Diversity of herbaceous riparian flora in the lower stretch of Bharathappuzha river, Kerala. South Indian J. Biol. Sci. 2016;2(1): 191-197.
21. Buckling A, Kassen R, Bell G, Rainey PB. Disturbance and diversity in experimental microcosms. Nature 2000;408 961-964.
22. Malanson GP. Riparian landscapes. Cambridge University Press,1993
23. Cowell MC, Dyer JM. Vegetation development in a modified riparian environment: Human imprints on an Allegheny River wilderness. Ann. Assoc. Am. Geogr. 2004;92(2):189-202.
24. Chase MW, Christenhusz MJM, Fay MF, Byng JW, Judd WS., Soltis DE, Stevens PF. An update of the Angiosperm Phylogeny Group classification for the orders and families of flowering plants: APG IV. Botanical Journal of the Linnean Society 2016;181(1):1-20.
25. The Plant List: Version 1.1. Published on the Internet, http://www.theplantlist.org, 2013
26. Cook, C. D. K. Aquatic and Wetland Plants of India, Oxford University press, New York.1996
27. Bsienvis.nic.in. (2017): Invasive Alien species. http:// www. bsienvis. nic. In /Database/ Invasive_Alien_species_15896. aspx. Accessed 4 Sep., 2017.
28. Lovett S, Price P. *Principles for riparian lands management*. Canberra (ACT): Land and Water Australia,2007.
29. Gopal B. Wetlands: management and conservation in India. Water Qual. Bull. 1988;13:3–6, 29–30.
30. Natta AK, Sinsin B, van der MaesenJose LG. Riparian forests, a unique but endangered ecosystem in Benin. *Notulae Florae Beninensis*2002;124(1):55–69.
31. Sunil C, Somashekar RK, Nagaraja BC. Diversity and composition of riparian vegetation across forest and agroecosystem landscapes of river Cauvery, Southern India. Trop. Ecol. 2016;57(2):343-354.
32. Felix AJS, Paul MZ, Jeeva S, Sukumaran S. Riparian flora of Thamiraparani River in Kanyakumari district, Tamilnadu, India. Int. J. Sci. Res. Mod. Edu.2017;2(1):72-90.
33. War SA, Kumar A. Vyas V. Assessment of riparian buffer zone of Chandni Nalla-A stream in Narmada Basin, India. Adv. Appl. Sci. Res.2014;5(2):102-110.
34. Sharma NK, Gusain OP. A Survey of Riparian Vegetation of River Manuni in Western Himalayan Region of India, Int. J. Curr. Res. Biosci. Plant Biol. 2015;2(5): 141-147.

35. Jisha K, Shanid M, Nair MC. Angiosperm Diversity and Phytogeographical Affinities of Riparian Vegetation along Kanjirapuzha Basin, Kerala, India.Trends in Biosciences2018;11 (7):1102-1107.
36. Manoj K, Nandakumar MK, Remya MP, Shinila K, Lakshmi DP. Phytosociological analysis of riparian tree species of Alakyam stream; Pariyaram, Kerala, India. Int. J. Environ. Sci. 2012;2(4):1895-1903.
37. Sherina S, Jagatheskumar S, Balasubramaniam V. Systematic studies on The Riparian Flora of Sokanasini River, Chittur Taluk, Palakkad District, Kerala, Int. J. Rec. Adv. Mult. Res.2018;5(7):3997-4001.
38. Balan L, Paul J. Diversity of herbaceous riparian flora in the lower stretch of Bharathapuzha river, Kerala. South Indian J. Biol. Sci. 2016;2(1), 191-197.
39. Garssen AG, Baattrup-Pedersen A, Riis T, Raven BM, Hoffman CC, Verhoeven JTA, Soons MB. Effects of increased flooding on riparian vegetation: Field experiments simulating climate change along five European lowland streams. Glob. Chang.Biol. 2017;23(8):3052-3063.

Table 1: Names and families of Angiosperm plants collected

Sl. No.	Botanical Name	Family	True Riparian	Wetland	Endemic	RET
1.	*Colocasia esculenta* (L.) Schott	Araceae		*		LC
2.	*Cryptocoryne retrospiralis* (Roxb.) Kunth	Araceae		*		LC
3.	*Pandanus furcatus* Roxb.	Pandanaceae	*			
4.	*Gloriosa superba* L.	Liliaceae				LC
5.	*Caryota urens* L.	Arecaceae				LC
6.	*Murdannia spirata*(L.) G. Bruckn.	Commelinaceae		*		LC
7.	*Monochoria vaginalis* (Burm. f) C.Presl	Pontederiaceae				LC
8.	*Kyllinga nemoralis*(J.R.Forst.) Dandy ex Hutch.& Dalziel	Cyperaceae		*		LC
9.	*Cyperus digitatus*Roxb.	Cyperaceae		*		LC
10.	*Eragrostis tenella*(L.) P.Beauv.exRoem &Schult	Poaceae		*		
11.	*Saccharum spontaneum* L.	Poaceae		*		LC
12.	*Cayratia pedata* (Lam.) Gagnep.	Vitaceae				VU
13.	*Trema orientalis* (L.) Blume	Ulmaceae				LC
14.	*Homonoia riparia* Lour.	Euphorbiaceae	*			LC
15.	*Ludwigia hyssopifolia* (G.Don) Exell	Onagraceae		*		LC
16.	*Ludwigia octovalvis* (Jacq.) P.H.Raven	Onagraceae				LC
17.	*Syzygium caryophyllatum* (L.) Alston	Myrtaceae				EN
18.	*Naregamia alata* Wight & Arn	Meliaceae			Endemic to South western Ghats	
19.	*Hopea ponga* (Dennst.) Mabb	Dipterocarpaceae			Endemic to Southern Western Ghats	EN

20.	*Persicaria barbata* (L.) H.Hara	Polygonaceae	*			LC
21.	*Alternanthera sessilis* (L.)R.Br.ex DC.	Amaranthaceae		*		LC
22.	*Glinus oppositifolius* (L.) Aug. DC.	Molluginaceae		*		
23.	*Impatiens gardneriana* Wight	Balsaminaceae			Endemic to South Western Ghats	
24.	*Oldenlandia corymbosa* L.	Rubiaceae		*		
25.	*Oldenlandia herbacea* (L.) Roxb.	Rubiaceae		*		
26.	*Canscora diffua* (Vahl) R.Br. ex Roem. & Schult	Gentianaceae		*		
27.	*Alstonia scholaris* (L.) R. Br.	Apocynaceae				LC
28.	*Heliotropium keralense* Sivarajan & Manilal	Boraginaceae		*	Endemic to Kerala	
29.	*Ipomoea hederifolia* L.	Convolvulaceae		*		
30.	*Hydrolea zeylanica*(L.) Vahl	Hydrophyllaceae		*		LC
31.	*Physalis angulata* L.	Solanaceae				LC
32.	*Capsicum annuum* L.	Solanaceae				LC
33.	*Chionanthus mala-elengi* (Dennst.) P.S.Green	Oleaceae			Endemic to peninsular India	
34.	*Torenia bicolour* Dalz.	Scrophulariaceae		*	Endemic to Southern Western Ghats	LC
35.	*Lindernia antipoda* (L.) Alston	Linderniaceae		*		LC
36.	*Lindernia rotundifolia* (L.) Alston	Linderniaceae		*		LC
37.	*Lindernia crustacea* (L.) F.Muell.	Linderniaceae		*		LC
38.	*Lindernia caespitosa* (Blume) Panigrahi	Linderniaceae		*		
39.	*Acmellapaniculata* (Wall.ex DC.) R.K. Jansen	Asteraceae		*		LC
40.	*Ageratum conyzoides* (L.) L.	Asteraceae				LC
41.	*Spilanthes radicans* (Jacq) synonym	Asteraceae		*		

EN – Endangered, LC – Least concern, VU-Vulnerable

Table 2: Invasive Alien species* recorded from Thathengalam

SL.NO	Botanical name	Family
1	*Cleome viscosa* L.	Cleomaceae
2	*Urena lobata* L.	Malvaceae
3	*Sida acuta Burm.*f	Malvaceae
4	*Melochia corchorifolia* L.	Malvaceae

5	*Triumfetta rhomboidea* jacq	Malvaceae
6	*Senna tora*(L.)Roxb	Fabaceae
7	*Mimosa pudica* L.	Fabaceae
8	*Ludwigia octovalvis*(Jacq.)P.H.Raven	Onagraceae
9	*Passiflora foetida*L.	Passifloraceae
10	*Ageratum conyzoides*(L.)L	Asteraceae
11	*Chromolaena odorata* (L.)R.M.King & H.Rob	Asteraceae
12	*Emilia sonchifolia*(L.)DC.ex DC	Asteraceae
13	*Tridax procumbens*(L.)	Asteraceae
14	*Synedrella nodiflora*(L.)Gaertn	Asteraceae
15	*Spilanthes radicans*(Jacq)	Asteraceae
16	*Mikania micrantha*Kunth	Asteraceae
17	*Asclepias curassavica* L.	Apocynaceae
18	*Ipomoea hederifolia* L.	Convolvulaceae
19	*Ipomoea pes-tigridis* L.	Convolvulaceae
20	*Solanum torvum*Sw	Solanaceae
21	*Physalis angulate* L.	Solanaceae
22	*Lantana camara* L	Verbenaceae
23	*Hyptis suaveolens*(L.)Polt	Lamiaceae
24	*Peperomia pellucida* (L.) Kunta	Piperaceae
25	*Monochoria vaginalis* (Burm.f) C.Presl	Pontederiaceae
26	*Saccharum spontaneum* L.	Poaceae

*** Invasive Alien species according to *bsienvis.nic.in* (2017)**

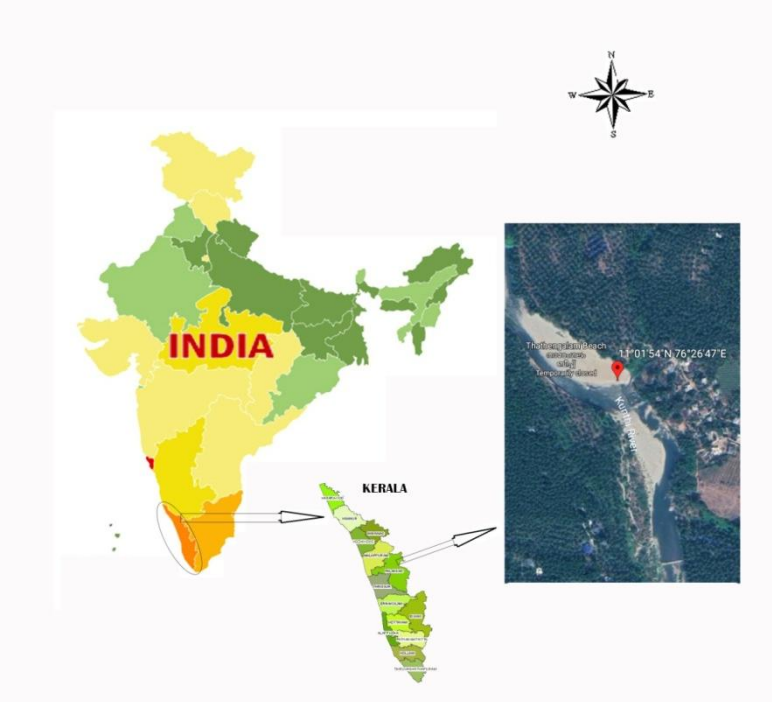


Figure 1: Study area

Plate 1: Flood affected basin of Kunthipuzha at Thathengalam

Plate 2: Important plants collected from the study area: **A.** *Hopea ponga*(Dennst.)Mabb; **B.** *Syzygium caryophyllatum* (L.)Alston; **C.** *Impatiens gardneriana* Wight; **D.** *Chionanthus Mala-elenji* (Dennst)P.S.Green; **E.** *Heliotropium keralense* Sivarajan & Manilal; **F.** *Torenia bicolor* Dalz.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Mathematics of Systems Dynamics

R.Sivaraman*

Associate Professor, Department of Mathematics, D. G. Vaishnav College, Chennai, Tamil Nadu, India.

Received: 05 Dec 2020 Revised: 15 Dec 2020 Accepted: 22 Dec 2020

***Address for Correspondence**
R.Sivaraman
Associate Professor,
Department of Mathematics,
D. G. Vaishnav College,
Chennai, Tamil Nadu, India.
Email: rsivaraman1729@yahoo.co.in

ABSTRACT

Systems thinking is a method of thought as opposed to an actual modeling technique. It is a form of qualitative modeling that focuses on viewing the system as a whole, instead of a collection of individual parts. The goal of systems thinking is to describe how the various parts of a system interact qualitatively. The process of describing and applying systems thinking scientifically is called as Systems Dynamics, In this paper, I will introduce the basic concepts of Systems Dynamics and apply it to medical systems.

Keywords: Systems Dynamics, Stock, Flow, Differential Equation, Fundamental Theorem of Calculus.

INTRODUCTION

Systems thinking models are best visualized as a collection of boxes connected by arrows and influence signs. Each box represents a part of the system, and each arrow and influence sign combination represents how that box impacts another box in the system. A positive sign means that an increase in the box from which the arrow leaves causes an increase in the box into which the arrow arrives. A negative sign means that an increase in the box from which the arrow leaves causes a decrease in the box into which the arrow arrives. Systems thinking models can be created and viewed in many other ways than the influence diagrams described above. As one of the major goals of any systems thinking model is to clarify interactions between various parts of a system, any visual or descriptive model that accomplishes this suffices. If the model does not accomplish this, one should "rethink" the system. In this paper, I will describe a model using Systems Dynamics which will be suitable for addressing medical systems.

System Dynamics

Once the systems thinking approach is employed and an influence diagram is created, a modeler often wishes to quantify his or her model in order to be able to make predictions regarding policy changes and future workloads. To do this, we often turn to system dynamics. System Dynamics models are defined by their use of stocks and flows to

describe feedback loops and complex systems; therefore in order to understand system dynamics, I must begin with the definitions of stocks and flows.

Stock

The term 'stock' is derived from the business concept, which refers to the value of an asset at a balance date. In a more general sense a stock is better described as an entity that is accumulated over time by inflows and/or depleted over time by outflows. In medical systems, the entity in question is often patients, so a stock might measure the number of patients in a hospital emergency department, the number of patients infected with a specific disease, or the number of patients who require home-care nursing. Returning to systems thinking, stocks measure the contents of the boxes in an influence diagram.

Flow

The term 'flow' is also derived from concepts in economics and refers to the total value of changes to a stock during a given period. Thus in medical systems, flows could represent the number of patients entering and exiting a hospital emergency department, the number of patients becoming infected or recovering from a specific disease, or the number of patients who degrade to the point where they need homecare nursing minus the number of patients who improve (or degrade) to the point where they no longer need home-care nursing. Returning to systems thinking, in a system dynamics model of an influence diagram, flows measure the amount of influence an arrow has on a given box. Of course stocks and flows can measure objects other than patients. In fact, one of the strengths of systems modeling is that stocks and flows can measure anything that is quantifiable. The number of beds in a hospital, the number of washrooms in use at a given time, and the number of staffed ambulances sitting idle at a given time could all be modeled using stocks and flows. In short, if you can measure it, you can model it.

Describing the Model

With stocks and flows defined and an influence diagram created, the next step in creating a system dynamics model is to determine the equations that govern each stock. These equations generally depend on the time t and the state of the system $S(t)$ at that time. To describe a full system dynamics model, we consider the situation as depicted in Figure 1. In Figure 1, let the function $N(t)$ represent the number of new medicines introduced during the month t. This function could be created by the user to test certain scenarios or it could be determined using historical data to predict future trends. The function $T(t)$ represent the number of hours of staff training provided in month t. Like $N(t)$, this number is chosen by the user to test various training strategies or it is set based on historical training strategies. Let the function $M(t)$ represent the number of medicines employed at the hospital during month t. Let the function $E(t)$ represents the number of medicinal errors in a given month t.

Since the change in the number of medicines employed per month is the number of new medicines introduced, we have $\frac{d}{dt}\left(M(t)\right) = N(t)$ **.....(1)**

This quantity is increased as new medicines arrive and decreased as staff training takes effect. For the sake of example, we assume both of these effects are linear. That is, doubling the time spent training per month doubles the effect of training on the number of errors. Though this assumption is somewhat unrealistic, it makes the mathematics modeling achievable without use of a computer. Additionally, as long as the number of drugs introduced and the amount of training done do not fluctuate too much, a linear approximation is reasonably accurate. This leads to the differential equation $\frac{d}{dt}\left(E(t)\right) = \alpha N(t) - \beta T(t)$ **.....(2)** where α, β are positive real numbers.

Finally, the function $L(t)$ is the total number of lives saved from the beginning of the model formulation until month t. Each month, L increases with the number of medicinal options available and decreases by the number of medicinal errors. For the sake of example, assume each medicinal option has the capacity to save γ lives.

With these assumptions, we get $\frac{d}{dt}\left(L(t)\right)=\gamma M(t)-E(t)$. .....**(3)**

Solution for the Proposed Model

Under the possible assumptions I have created three first order differential equations given by equations (1), (2) and (3). Making use of these three equations and fundamental theorem of calculus (since the functions involved are continuous), I will obtain the solution for the number of lives that can be saved in the period of time $[0,t]$.

Now differentiating (3), and using the rate changes of $M(t)$ and $E(t)$ from (1) and (2) respectively, we get

$$\frac{d^2}{dt^2}\left(L(t)\right)=\gamma\frac{d}{dt}\left(M(t)\right)-\frac{d}{dt}\left(E(t)\right)=\gamma N(t)-\left(\alpha N(t)-\beta T(t)\right)=\left(\gamma-\alpha\right)N(t)+\beta T(t)$$

$$\frac{d^2}{dt^2}\left(L(t)\right)=\left(\gamma-\alpha\right)N(t)+\beta T(t) \quad \text{.....(4)}$$

Notice that (4) is a second order differential equation with constant coefficients. Using Fundamental Theorem of Calculus we can integrate twice on the left hand side of (4) to extract $L(t)$. Doing this process over the interval $[0,t]$ we get $L(t)=\int_0^t\int_0^t\left[\left(\gamma-\alpha\right)N(t)+\beta T(t)\right]dt_1\,dt_2$

Therefore, $L(t)=\left(\gamma-\alpha\right)\int_0^t\int_0^t N(t)\,dt_1\,dt_2+\beta\int_0^t\int_0^t T(t)\,dt_1\,dt_2$ .....(5)

If we know $N(t),T(t)$ then from (5), we can determine the number of lives that can be saved until given period of time. Thus (5), provides the required solution to the proposed system dynamics model.

CONCLUSION

After introducing the basic ideas of systems thinking and systems dynamics, I had tried to describe a systems dynamics model with respect to medical scenario, in which we wish to know how many lives that we can save up to given period of time. With this objective, I had described a simple mathematical model using first order differential equations in section 3. In section 4, I had solved the model by obtaining a closed expression for number of lives that can be saved in the period $[0,t]$ by means of double integrals in equation (5). If $N(t),T(t)$ are nice functions like polynomials, rational expressions or even in exponential order, then we can easily integrate them using standard techniques of integration and obtain $L(t)$. But, in case if $N(t),T(t)$ are non-standard functions which are not readily integrable, then we may sought numerical integration methods to obtain the require solution.

REFERENCES

1. Thomas Rohleder, Diane Bischak, and Leland Baskin, Modeling patient service centers with simulation and system dynamics, Health Care Management Science, 10(1):1–12, 2007.
2. Geoff Royston, Ayesha Dost, Jeremy Townshend, and Howard Turner, Using system dynamics to help develop and implement policies and programmes in health care in England, System Dynamics Review, 15(3):293–313, 1999.
3. Brian Skyrms and Robin Pemantle, A dynamic model of social network formation. Proceedings of the National Academy of Sciences, 97:9340–9346, 2000.
4. Bernice A. Pescosolido. Beyond rational choice: The social dynamics of how people seek help, American Journal of Sociology, 97:1096–1138, 1992.
5. R. Sivaraman, Application of Queues to Allocation of Hospital Beds, Indian Journal of Natural Sciences, Volume 10, Issue 60, June 2020, 20876 – 20871.
6. R. Sivaraman, Modeling Health Care Facilities, Strad Research, Volume 7, Issue 7, (2020), 45 – 49.

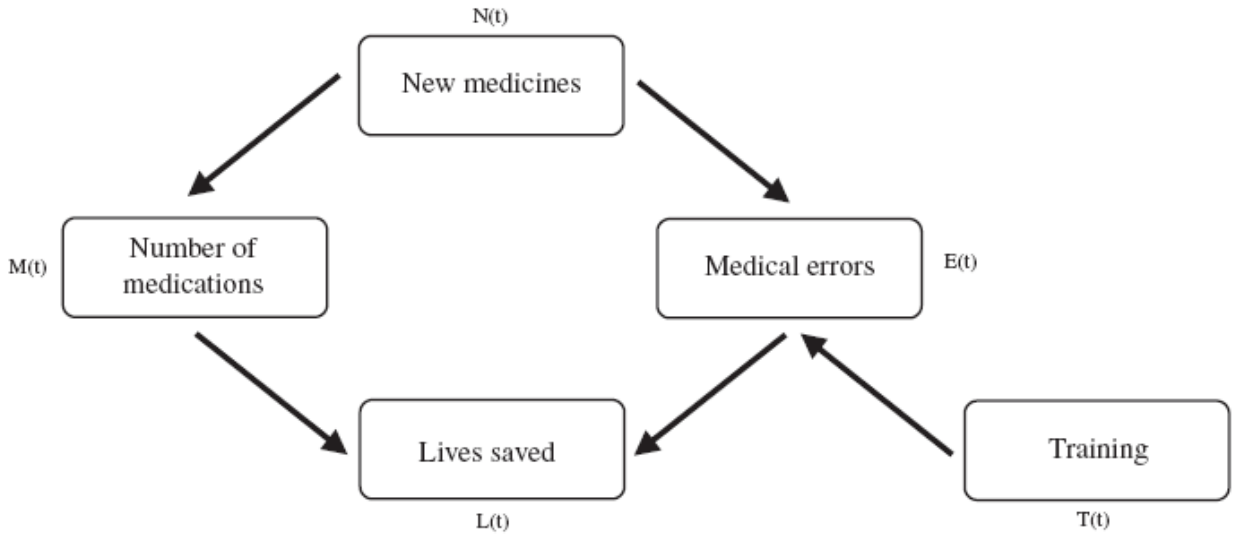


Figure 1: Different States in a Systems Dynamics Model

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Energy Efficient Routing Technique for Cloud Outsourcing of Picture Archiving Communication System

J. K. Jeevitha[1]*, G. Athisha[2], R. Subhashini[3] and B.Keerthi Samhitha[4]

[1]Assistant Professor, Department of Information Technology, PSNA College of Engineering and Technology, Tamil Nadu, India.
[2]Professor and Head, Department of Electronics and Communication Engineering, PSNA College of Engineering and Technology, Tamil Nadu, India.
[3]Professor, School of Computing, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.
[4]Assistant Professor, School of Computing, Sathyabama Institute of Science and Technology, Chennai, Tamil Nadu, India.

Received: 17 Nov 2020 Revised: 19 Dec 2020 Accepted: 21 Jan 2021

***Address for Correspondence**
J. K. Jeevitha
Assistant Professor,
Department of Information Technology,
PSNA College of Engineering and Technology,
Tamil Nadu, India.

ABSTRACT

Distributed computing is ongoing worldview that offers the different sorts of administrations, for example, Software administration, Platform Service and Infrastructure Service, used to convey the equipment and programming administrations through the web. The cloud innovation added one new help that is known as clinical imaging stockpiling. Picture Archive Communication System (PACS) have been utilizing the cloud based web innovations for data stockpiling, data circulation and data recovery. As of late medical clinics and medical care foundations are finding their capacity in the outside of organization. Energy effectiveness and defer decrease are the significant intricacy in geo circulated data enter correspondence. To improve the energy proficiency of the clinical imaging network framework in the cloud based circulated data enter, a steering strategy and reserve substitution method were created. The proposed reserve framework utilized article parting procedure and it utilized Adaptive Cache Replacement (ACR) calculation. The store substitution strategy used to lessen the entrance delay in the clinical imaging dispersed organization.

Keywords: Cloud Computing, Cache System, Digital Imaging and Communication in medicine (DICOM), picture archive and communication system (PACS).

INTRODUCTION

Medical imaging is the one of the best tool for medical practice; it provides efficient diagnosis support and treatment support. It used to create new images and the implementation of Picture Archiving and Communication System (PACS). PACS in charge for picture acquisition, storage, display and medical object or data sharing among the interconnected network .The cloud computing offers various types of on-demand services, these are computed, storage and databases. The major problem arises from storage service, because of storing the large volume of medical data those consequences in the Big Data problem. The cloud computing provides their services in the flexible manner, it supports scalability, maintenance is very simple, enhance the high reliability and very secure [1] [2]. This facility is additionally being investigated for outsourcing of medicinal imaging services; with two principles utilize cases [3]:

- PACS chronicle outsourcing. In-house PACS arrangements have high support costs, infrastructure versatility is generally restricted and throughout the years it effectively winds up old.
- Inter-institutional work processes and sharing of medicinal imaging. For example, the cloud is incredible for instantiating a teleradiology platform a services.

One of the major disadvantages of PACS services with cloud is access latency [4], an especially basic worry in medicinal imaging situations since remote access over the Internet is impressively slower than Intranet associations. In addition, a few examinations can add up to a couple of gigabytes of information, which additionally worsen this issue [5]. Cloud computing had introduced one model, that was called as "pay per use". This technique used in industry and enterprises and telemedicine (healthcare) also. Now the cloud computing designated with PACS, this new idea is to perform PACS outsourcing global access of the data. PACS with Cloud computing have integrated with Digital Imaging Communication in Medicine (DICOM). DICOM routers allow the two types of facilities (services): DICOM storage and retrieval process. DICOM router is commonly used for evaluating and taking the action of first stage diagnosis with radiology department and PACS [6]. The PACS and DICOM were used to reduce the access latency. Cache replacement policies improves fatness of access and improves the cache hit rate and also it reduces the power consumption in the cache proxy servers. The energy consumption, bandwidth and computational power are significantly reduced by a high cache hit ratio [7]. So this article tries to reduce the energy efficiency in DICOM routers cache system in cloud computing through the high hit cache proportion. Here we used Adaptive Cache replacement algorithm were used. The proposed algorithm's performance compared with LRU, LFU and LRFU.

Related Works

Picture Archiving Communication System

The author concentrated on availability of medical information and simplifies the activity of telemedicine with the platform of PACS with DICOM. In this paper recommended an architecture, which is composed of three main components: 1. collaborative, 2. Medical Management information system (MMIS) and 3. Web client application. MMIS act as a gateway for the communication of PACS. In the experimental results consider the parameters are server response time, downtime and search response time. Down time and search response time calculated based on cache hit and miss ratio [8]. The expanding fuse of current medicinal imaging hardware requires the task of frameworks that store, transmit and show pictures, PACS (Picture Archiving and Communication System) through advanced systems to give wellbeing administrations higher quality. The main aim of the paper is to avoid the network congestion, during the transformation of medical images in DICOM network. This paper developed and implemented a network of medical images by free software and its similarity with its own information system of a RIS (Radiology Information System). For that development, it used LAN and WAN through WLAN [9].For the improvement in the client side environment of the web based DICOM users, this paper proposed caching and pre fetching method to reduce the data access latency. An essential conclusion turns out from its utilization in a genuine case situation – the Portuguese program for breast cancer sample screening. The usage of the pre fetching and

storing module in the Web DICOM watcher has fundamentally enhanced the update work process and expanded the profitability of the inside [10].

Cache replacement

The web caching is one of the best powerful resolutions for developing the internet system performance. An In Web reserving, the prominent web protests that liable to be gone by soon are put away in positions nearer to the client like customer machine or intermediary server. Thus the way caching reduces the network traffic, congestion, bandwidth and delay on the internet [11]. The Author proposed least recently used (LRU) replacement algorithm in DRAM/PCM, which is especially in the big data, distributed, cluster and cloud computing. LRU reduced the energy consumption by 9.6% [12]. This author used least error rate (LER) algorithm for reducing the error rate in spin transfer torque RAMs (STT-RAMs). This article's experiment compares the LER algorithm with LRU. The proposed one improves 3.6% performance, compare LRU. And LER also reduces the energy consumption level and reduce the system overhead [13]. Least recently used (LRU) cache eviction method used in the DICOM based routing platform. The result of the LRU method throws out the objects or studies, which are slightly used. They chose the LRU algorithm with splitting techniques [14][15]. The author implemented the caching system in the cloud storage gateway for improving the fast access in the remote medical picture archives. Here they were used LRU policy for object replacement. They followed long term and short term prefetching scheme [16]. In this paper is concentrated on the important parameter (i.e.) power efficiency and energy efficiency in PACS with cloud computing. Thus the way reduces the access delay of the object, this work implements Adaptive Cache Replacement (ACR) algorithm.

Proposed work

Distributed Cache System

This paper is concentrated for rising data accessibility and to minimize the medical imaging retrieval's delay in cloud computing, for example, an outsourced territorial Picture archive Communication System (PACS) chronicle. To accomplish this objective, a consistent cache framework for our DICOM energy efficient routing was produced.In cloud computing PACS system, the proposed work should be carrying the four fundamental highlights: 1.request (query), retrieval, population and depopulation. Here the first task is query service. There are a few issues identified with advancement of the cache system to help medicinal imaging situations. The retrieval, storing, and exchange of restorative imaging contemplates must manage tremendous measures of data, yet additionally with its heterogeneity. Diverse modalities deliver information with particular attributes, for example, image matrix, number of frames, ands normal size of the picture [17]. A few modalities, such as computed tomography (CT), may create a large number of image documents per contemplate up to 1 GB. Different modalities, as cardiac US, can create a few cine-circle records with several Megabytes. Caching immense documents is an intricate issue, regarding storage area, as well as delay in data transfer. The system accepted to deal with this issue, i.e., expanding the storage capacity in router's cache and cut off the delay, depends on part DICOM objects. Along these lines, each document is legitimately split into pieces of a predefined chunk size. Besides, the caching system might not have all investigation pieces and it is conceivable to execute remote retrieval procedures of just particular pieces of an investigation. Also, if the data is accessible in more than one chronicle, the requester router can recover chunks from various data sources such as supplier routers, expanding the performance of system and in addition enhance the energy efficiency in some system conditions. Those methods will be talked about further in this paper.

The proposed cache system consists of four types of components, which is explained. The components are the following:

1. Cache storage management
2. Cache metadata management
3. Cache service layer
4. Design of cache system interface

The architecture of cache system was implemented with the Java programming language. We exploited of a few tools like Java Simple Plug-in Framework (JSPF), dcm4che, MapDB and Data Base for Object (DB4O) (see Fig. 1).The store framework was produced in Java. We exploited of a few instruments like Java Simple Plugin Framework (JSPF), dcm4che, MapDB and DB4O (see Fig. 1). JSPF was utilized to detect, enroll, stack, and execute the modules, for example, the knowledge module that gives calculations to deal with the reserve. Thus, the dcm4che system is in charge of parsing and building DICOM correspondence messages. The responsibility of cache meta data management module is used for indexing meta data related objects that are stored in the cache system that means pictures can be identified with summary, arrangement of series and so on.

This technique empowers quick inquiry replying, since it abstains from examining put away DICOM objects each time a query must be handled. This database depends on data base (DB4O) and an Application Programming Interface (API) for question databases. Cache storage management module consists of two components: cache perseverance and large storage management. The cache perseverance is considered as low level components in the system. This perseverance is functioning by way of key objects and binary data. It generates some of basics functions such as put, get, remove and contain. These functions are used to handle in the perseverance medium. It utilizes MapDB and relating key objects. The large memory manager creates DICOM objects (picture) abstraction in the top of cache perseverance. For the key conversion, it uses service layer to convert the objects to keys, retrieval or earlier storage. Theoretically the large memory management triggers the events through cache external interface plug-ins.

Cache service layer

The main intension of the service layer is to provide external functionalities to another module. It consists of two modules: one is cache key translator and another one is cache external interface plug-ins. Object key translation is conducted by the cache key translator in centralized manner. For the key translation this module used hash function for the translation objects into key. Along these lines, the large memory management module does not have to manage object metadata, but rather just with the apparently homogeneous key created by the cache key translator for distinguishing proof of each piece of data. While explaining the necessities of the proposed cache framework design, we saw that diverse requesters would expect distinctive practices from their cache framework. These practices are related with various populace and depopulation approaches. Also, alongside various approaches comes the need to oversee metadata related with accumulated objects. In this way, rather than building a static module for these arrangements, our design consolidates a module framework where store framework requesters can supply their own particular approach usage module. . In the event that vital, they can take activities on the cache framework utilizing people in general cache framework interface, (for example, evacuating an object or cache one)[3][19].

Design of cache system interface

Cache system architecture act as interface used to connect all the modules of system. There are two primary explanations behind packaging all detail in a remarkable interface. This is mainly used for storing and retrieving[19].

Plug-ins

The necessary feature of the implemented framework is that can change its capacity depends on the cache prediction module. So here this paper used plug-ins architecture. There are two sorts of modules: the large memory management and the information (data) collector. The framework requires one memory management plug-ins. In any case, concerning information gatherer modules, they are discretionary and the framework bolsters in excess of one module of this kind. 1) Repository Management Plug-ins: By definition, a cache framework is a transitory stockpiling territory with restricted limit. The plug-ins is used to managing the repository of cache, improving the availability of data (information) in cache. There are two principle activities in the memory administration process: store populace and eviction. At long last, this module must enlist data in a database about the pictures, studies and arrangement put away in the cache. 2) Data Collector Plug-ins: Distinct populace and expulsion techniques need to

gather particular measures to find which studies will be disposed of or pre fetched. In this way, it was important to have an adaptable information authority system. The information gatherer modules are in charge of gathering the measures required by the archive administration module. For example, contemplating client designs expects access to the client's demand information. Moreover, now and then there are other outside information sources that could have imperative data for the cache framework [10][19].

Image routing in medical cloud with caching management

Already this project built a DICOM routing technique in the cloud environment among the remote location through the internet using the communication protocol of Hyper Text transfer protocol [6], which offers DICOM request processing or retrieval and data storage service .This method established storage integration and the setting up of teleradiology instructions for exchanging summary or study of patients. The proposed architecture of the DICOM router unit, it is kept inside the DICOM island, that means DICOM network doesn't have connectivity with any other DICOM networks. In every home PACS the routers are considered as DICOM hubs (nodes). The DICOM hubs offered various services, which is distributed by the island (other routers). Thusly, administrations gave DICOM applications inside the islands will at that point be available from outside applications, through the router. The interchanges between router is helped out through the connect transfer which can be situated on a cloud service provider [3][10][19]. The DICOM routing platform encourages the interoperability among the remote imaging storages. Doctors or any technicians can do their extra work at the home environment. Doctors can work at home, as though they were in the human services organization, without changing their work process. Be that as it may, the arrangement has a few issues in regards to access to medicinal pictures in situations with restricted transmission capacity, which is particularly basic while recovering extensive size investigations. Next this paper is going to combined new cache system with routing technology.

Study retrieval process entirely explained by using the Fig.2. Study can be retrieved from archive outsourced hosted by physician any where It outlines the exchange of an investigation made out of six pieces of information. To exploit reserved items, the C-MOVE command work process is done as takes after.

1. The requester send request(C-MOVE Request) to the router, then router receives the request that will be forwarded to the local routers cache. Whether the investigation or study is present in the store, the router starts to send the content or study to the requester (client). If the local caching system satisfied the client demand, the demand is regularly throw to the bridge relay, in light of the fact that the document may have gotten new pictures or arrangement (Step 1). Alongside this message, a "CR" is additionally transmitted containing the articles put away in the router's nearby cache (hit-list). In the illustration, the hospital A router, hold chunks 1,2 and 3 in its nearby cache , that is the "CR"[3][10][19].
2. The C-Move command control collected by the bridge and shortens the work list. The CR received by the router that supports (prop ups) the destination of AETitle. The end router is used for tracking the response. The destination router is the out sourced location router.
3. In the step 2 the C-Move command's request props up to the PACS Storage System by the remote router. The cache storage system automatically receives the request. In the event that the archive is inaccessible, the set FS will be exhaust.
4. A list of DICOM objects (blocks) are generated by the out sourced location router according to the bridge response. The DICOM study or objects are resides in the archive system, that is known as shorten (prune) work list. In the step 3 specifies the transfer work list such as UL study 4,5, and 6. The 4,5 and 6 return back to the router of hospital A(step 4).
5. For the requested result conclusion, hospital A router combines the response from its cache and got the new chucks in step 5. In step 6, At last the Hospital A router transmitting the objects (or chunks) by using the network.

CR→Content of the Hospital A router.
OL→Content of the out sourced location router.
FS→Full study of objects that is resides in the PACS.
UL→Upload list that is known as response, out sourced location router send blocks to Hospital A router.

Cache replacement algorithm's performance based on the physician study retrieval process on cloud data store. In our experimental results calculates the standards through our cache size range. The most well-known measurements are cache hit proportion (CHP)(1), or the proportion of store hits to all solicitations; cache byte hit proportion (CBHP)(2), or the proportion of bytes came back from the cache to all bytes asked for; and Cache delay proportion (CDP)(3), or the defer experienced by a client for objects recovered from the reserve verses that accomplished if no objects were cached. Measures take from [18].

$$\boldsymbol{CHP} = \frac{\sum_{i \in Ro} \boldsymbol{hp_i}}{\sum_{i \in Ro} \boldsymbol{fr_i}} \quad \textbf{(1)}$$

$$\boldsymbol{CBHP} = \frac{\sum_{i \in Ro} \boldsymbol{hp_i} . \boldsymbol{sz_i}}{\sum_{i \in Ro} \boldsymbol{sz_i} . \boldsymbol{fr_i}} \quad \textbf{(2)}$$

$$\boldsymbol{CDP} = \frac{\sum_{i \in Ro} \boldsymbol{hp_i} . \boldsymbol{de_i}}{\sum_{i \in Ro} \boldsymbol{de_i} . \boldsymbol{fr_i}} \quad \textbf{(3)}$$

Where

$sz_{i \to Object\ (study\ )size}$

$hp_{i \to}$Total number of Cache hit proportion for study

$de_{i \to}$ Normal retrieval delay of the server for object i

$fr_{i \to}$ Total number of requests for objects i;

$Ro_{i \to}$ Total number of requests for objects i;

Clearly the tradeoff between the energy cost and access delay is an inflexible one, we can diminish the uplink and download messages or enhance the access delay to diminish the energy cost.

The maximization of cache hit ratio and minimization of cache miss ratio improves the efficiency of cache, which leads to a improve cache hit ratio, reduce the delay and improve the resource utilization. A hit proportion of 90% and higher implies that a large portion of the solicitations are fulfilled by the cache. An incentive underneath 80% on static records shows wasteful storing because of poor setup. Energy consumption, bandwidth and power of computation are greatly reduced by high cache hit ratio.

$$\boldsymbol{CHR} = [[\boldsymbol{CH}/(\boldsymbol{CH} + \boldsymbol{CM})] * \boldsymbol{100}\%] \quad \textbf{(4)}$$

Where:

CHR→Cache Hit Ratio

CH→Cache Hits

CM→Cache Misses.

The energy inverse proportion to the cache hit ratio/proportion. So the energy calculated by the cache hit ratio. If the hit ratio increased automatically the energy consumption of server or cache system is decreased. Total power consumption of the cache server calculated by using maximum and idle power consumption and the utilization of the server. Energy consumption through the time, object retrieval and total power consumption of the server.

$$P = \frac{kc}{hp} \quad (5)$$

$$P = K.P_{max} + (1 - K).P_{max}.U \quad (6)$$

$$K = \frac{P_{idlie}}{P_{max}} \quad (6)$$

$$E = Time * NOR * P \quad (7)$$

Where
P→Power Consumption in cache systems or servers
kc →Constant
hp →Hit proportion
P_{max} →Power consumption at the maximum level in the study/object retrieval
P_{idle} →Power consumption at the idle period of the cache server
U →Utilization of the cache server
E →Energy Consumption in Cache server
$Time$ →Object (study) retrieval time
$NOR \rightarrow Number\ of\ Object\ Retrieval$

The main function of the adaptive cache replacement (ACR) algorithm is used to monitor the recently used pages and frequently used pages. The implementation process is very easy in the adaptive cache replacement algorithm. The algorithm executes the request as per the time constrain. This request is basically autonomous of the cache size. When contrasted with LRU it has an execution pick up as for hit proportion for variable store sizes. It is a low-overhead calculation that reacts online to changing access designs. In this paper uses a Adaptive cache replacement (ACR) algorithm in the routing scheme, this cache eviction algorithm merge with DICOM object dividing technique. This algorithm ejects objects (studies) with reference of age, object captured date and recently and frequently utilized information or object. This splitting technique improves to save the cache storage space by storing the incomplete studies. In this manner, we don't generally expel finish objects from cache, however, we continuously ejects bits of a studies considering the measure of storage space accessible in cache and the need of the examination. This ACR algorithm improves the cache hit ratio and cache storage efficiency. The two factors are plays major role in the power and energy consumption. If the hit ratio is increased then automatically the cache power consumption and storage space is reduced.

RESULT AND DISCUSSION

To assess the performance of proposed cache system with the parameter of delay, cache hits and misses proportion, power consumption and energy efficiency. The DICOM router platform with caching system implemented in the model of client server architecture by the java based simulation. In this experiment creates client and server for sharing the PACS with the help of cloud repository. The client and servers are located in the different location. The each location has its own DICOM routers with the implementation of ACR algorithm. PACS studies or objects transmitted between the client and server. During the transmission period we calculated the cache hit and miss proportion and delay. Power and energy consumption are calculated through the delay and hit ratio. The ACR algorithm compared with the various types of algorithm such as LRU, LFU and LRFU. The results were considered the parameter of Hit proportion, miss proportion, delay and energy.

In this Figure 3 illustrates hit rate comparison of algorithms. In our experiment ACR achieved the high hit rate value, compare to LRU, LFU and LRFU. Likewise Figure 4 shows the miss rate that is inverse of the hit ratio, while the hit value increased then automatically misses value decreased. In this Figure 5 explains the energy consumption of the

cache server. Energy consumption and delay also the inverse of the hit ratio, then the ACR algorithm enhance the high hit value, so it reduces Energy consumption and delay of the cache server. In this figure 6 demonstrates the overall performance of the algorithms. In this graph shows that ACR algorithm is one of the best algorithm, compare to the remaining three algorithms (LRU, LFU and LRFU). ACR algorithm provided the better results for improving the energy in medical image routing in cloud data center. In this action had done greatly with the help of DICOM router. DICOM router is one of best approach for the delay reduction compare to VPN and mail system, it improves the fastness in the retrieval of medical objects (study).

CONCLUSION

The proposed caching and routing mechanism increased the energy efficiency, reduce the access latency and improves the data availability in the cloud based PACS surroundings. DICOM image splitting techniques improves performance of cache system. The splitting techniques majorly used for perfecting and eviction in the cache. The ACR algorithm improves cache hit ratio, reduces the cache miss ratio and delay. The reduction of delay improves the power and energy efficiency in cache system. In the experimental results compared proposed ACR replacement algorithm compared with least recently used (LRU), least frequently used (LFU) and least recently and frequently used (LRFU) algorithm. The results provided the fruitful result for ACR, compare to other three algorithms.

REFERENCES

1. Carlos Viana-Ferreira, António Guerra, João F. Silva, Sérgio Matos, and Carlos Costa," An Intelligent Cloud Storage Gateway for Medical Imaging", arXiv:1708.06334v1 [cs.DC] 27 Jul 2017
2. S.-J. Weng, D. Gotcher, H.-H. Wu, Y.-Y. Xu, C.-W. Yang, and L.- S. Lai, "Cloud image data center for healthcare network in taiwan," Journal of Medical Systems, vol. 40, no. 4, p. 89, 2016.
3.] L. A. B. Silva, C. Costa, and J. L. Oliveira, "A pacs archive architecture supported on cloud services," International journal of computer assisted radiology and surgery, vol. 7, no. 3, pp. 349–358, 2012.
4. J. Philbin, F. Prior, and P. Nagy, "Will the next generation of pacs be sitting on a cloud?," Journal of digital imaging, vol. 24, no. 2, pp. 179–183, 2011
5. L. A. B. Silva, S. Campos, C. Costa, and J. L. Oliveira, "Sensor based architecture for medical imaging workflow analysis," Journal of Medical Systems, vol. 38, no. 8, p. 63, 2014.
6. Philbin J, Prior F, Nagy P: Will the Next Generation of PACS Be Sitting on a Cloud? Journal of Digital Imaging:1-5.
7. https://blog.stackpath.com/glossary/cache-hit-ratio/
8. Eriksson J. Melício Monteiro; Carlos Costa; José L. Oliveira, University of Aveiro, DETI / IEETA, Aveiro, PortugaL," A Cloud Architecture for Teleradiology-as-a-Service*" Original Articles,2017.
9. L. R. Alvarez and R. C. Vargas Solis," DICOM RIS/PACS Telemedicine Network Implementation using Free Open Source Software", IEEE LATIN AMERICA TRANSACTIONS, VOL. 11, NO. 1, FEB. 2013.
10. Eriksson Monteiro, Carlos Costa, José Luís Oliveira," Caching and Prefetching Images in a Web-based DICOM Viewer", 2016 IEEE 29th International Symposium on Computer-Based Medical Systems.
11. Noriaki Kamiyama, Yuusuke Nakano, and Kohei Shiomoto," Cache Replacement Based on Distance to Origin Servers", IEEE TRANSACTIONS ON NETWORK AND SERVICE MANAGEMENT, VOL.13, NO. 4, DECEMBER 2016.
12. Gangyong Jia, Guangjie Han, Jinfang Jiang and Li Liu," Dynamic Adaptive Replacement Policy in Shared Last-Level Cache of DRAM/PCM Hybrid Memory for Big Data Storage", IEEE TRANSACTIONS ON INDUSTRIAL INFORMATICS, VOL. 13, NO. 4, AUGUST 2017
13. Amir Mahdi Hosseini Monazzah, Student Member, Hamed Farbeh, and Seyed Ghassem Miremadi," LER: Least-Error-Rate Replacement Algorithm for Emerging STT-RAM Caches", IEEE TRANSACTIONS ON DEVICE AND MATERIALS RELIABILITY, VOL. 16, NO. 2, JUNE 2016.

14. W. Ali, S. M. Shamsuddin, and A. S. Ismail, "A survey of web caching and prefetching," Int. J. Adv. Soft Comput. Appl., vol. 3, pp. 18–44, 2011.
15. Tiago Marques Godinho, Carlos Viana-Ferreira, Lu´ıs A. Basti˜ao Silva, and Carlos Costa," A Routing Mechanism for Cloud Outsourcing of Medical Imaging Repositories", IEEE JOURNAL OF BIOMEDICAL AND HEALTH INFORMATICS, VOL.20, NO. 1, JANUARY 2016.
16. Carlos Viana-Ferreira, António Guerra, João F. Silva, Sérgio Matos, and Carlos Costa," An Intelligent Cloud Storage Gateway for Medical Imaging", arXiv:1708.06334v1 [cs.DC] 27 Jul 2017.
17. S. Langer, "Issues surrounding PACS archiving to external, third-party DICOM archives," J. Digital Imag.,vol. 22, pp. 48–52, 2009.
18. Sherif Elfayoumy, Sherif Elfayoumy" Adaptive Cache Replacement: A Novel Approach", International Journal of Advanced Computer Science and Applications, Vol. 5, No. 7, 2014.
19. Tiago Marques Godinho, Carlos Viana-Ferreira, Lu´ıs A. Basti˜ao Silva, and Carlos Costa," A Routing Mechanism for Cloud Outsourcing of Medical Imaging Repositories" IEEE JOURNAL OF BIOMEDICAL AND HEALTH INFORMATICS, VOL. 20, NO. 1, JANUARY 2016 367.
20. ACR-NEMA, "Digital imaging and communications in medicine (DICOM) part 18: Web access to DICOM persistent objects (WADO)," ed. Rosslyn, VA: Nat. Elect. Manuf. Assoc., 2011.

Table 1. DICOM Commands

Command Name	Descriptions
C-STORE	The most essential DICOM task, also called "DICOM Push" permits a SCU to send a Composite Instance to a SCP. For example, it is utilized to send pictures from a methodology to PACS or make the conveyance system for C-MOVE.
C-FIND	At first utilized as a major aspect of the Query/Retrieve benefit, however in this manner re-utilized as a part of the Modality_Worklist and General_Purpose_Worklist benefits, this is an exceptionally basic activity somewhat much the same as a SQL inquiry.
C-MOVE	This request for the C-MOVE SCP to go about as a C-STORE SCU and to duplicate composite_instances to an asked for Application Entity Title(AETitle) which could possibly be the first C-MOVE SCU .
C-GET	C-GET is similar to the command of C-Move,however it is used for minor Assocoation, the request of Instances are passed over through the original association.
C-ECHO	This command is used for "DICOM Ping".

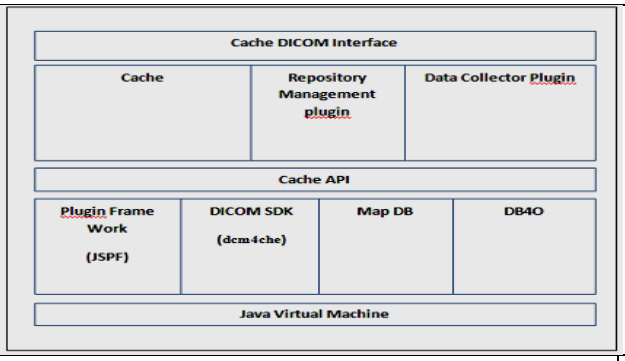


Figure 1: Cache System component and Software tools

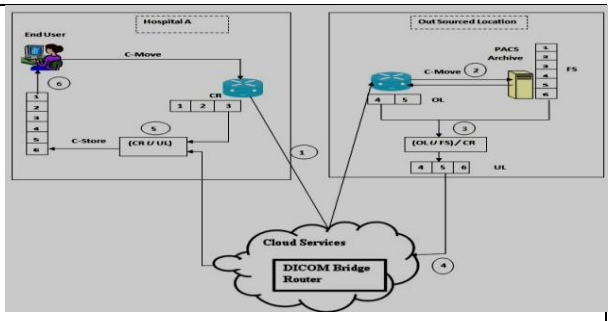


Figure 2: Study retrieval process

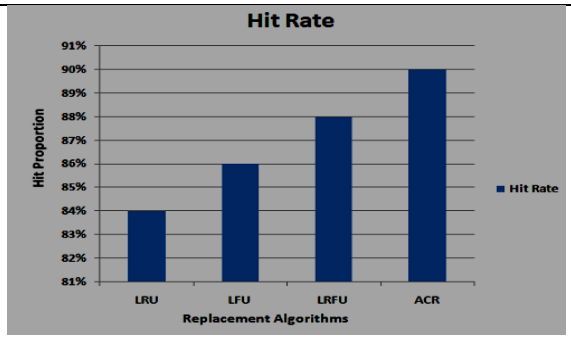


Figure 3: Hit ratio comparison

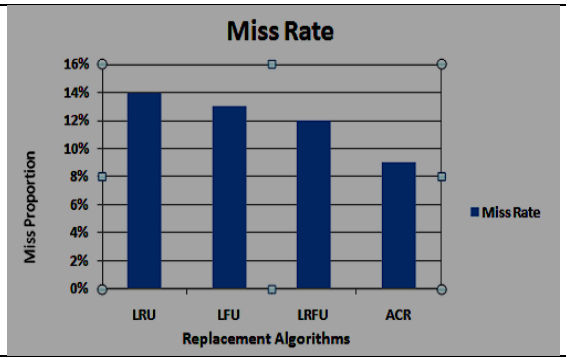


Figure 4: Miss Ratio comparison

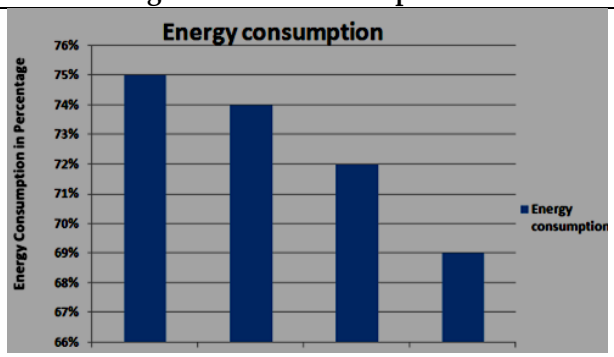


Figure 5: Energy Consumption Comparison

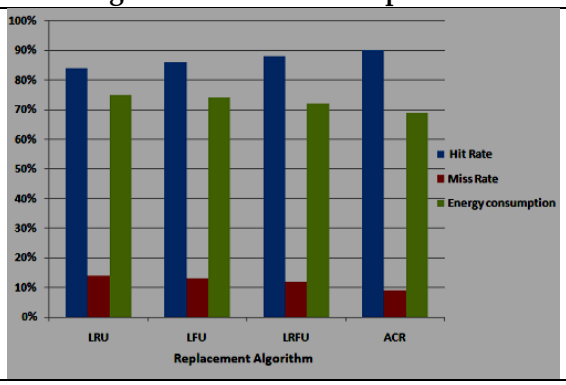


Figure 6: Over all comparison

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

The Frequency of Dyslipidemia in Stroke Patients at the Community Teaching Hospital of Swat, Pakistan

Abdul Ahad*, Aleem Ur Rahish, Saima Qadir, Haroon Ur Rashid, Amin ul Haq and Amir Zeb Swati

Saidu Medical College, Saidu Teaching Hospital, Swat, Pakistan.

Received: 02 Dec 2020 Revised: 27 Dec 2020 Accepted: 08 Jan 2021

***Address for Correspondence**
Abdul Ahad
Saidu Medical College,
Saidu Teaching Hospital,
Swat, Pakistan
Email : dr.aahad@yahoo.com

ABSTRACT

Stroke is one of the most common presentations to the Neurology wards and Emergency departments. The present study aimed to determine the frequency among patients with hemorrhagic stroke visiting Saidu teaching hospital. A Cross-sectional study was conducted for six months from 1st September 2019 to 27th February 2020 at Swat, Pakistan. 157 Subjects with hemorrhagic stroke and ischemic stroke were included in this study. 54.8% of our study population were males, and the mean age was 54.4 ± 7.1 years. 63.1% of subjects were with ischemic stroke and 36.9% with hemorrhagic stroke. The frequency of ischemic stroke was prominent in comparison with hemorrhagic stroke. Dyslipidemias were observed in 49 cases (31.2%) out of 157 cases.

Keywords: Ischemic stroke, Hemorrhagic stroke, Dyslipidemia.

INTRODUCTION

World Health Organization (WHO) report 2002, stated 785,122 mortalities because of stroke in Pakistan. For 2020, the WHO also evaluates and predicts stroke as the second leading cause of death in developed and developing countries [1]. Among the most well-known introductions to the Emergency divisions, Neurology wards and facilities can be profoundly morbid and even deadly and rely on the involved site and the harm's degree. A clot or embolus that interrupts blood supply resulting in ischemic conditions or any damage to vessels causing hemorrhage leads to decreased nutrient supplies to the brain cells, resulting in stroke [2-4]. Usually, it is a sudden and progressive occurrence. The signs and side effects incorporate discontinuance of any cranial nerve or loss of motion of one side of the body or appendage. Hypertension, Diabetes Mellitus, deranged lipid profile, cardiac diseases, alcohol use, obesity, depression and lack of exercise are some of the major risk factors for stroke [5-9]. This event can be investigated by CT (Computed tomography), but MRI (Magnetic Resonance Imaging) might be required, particularly

in ischemic stroke. The occurrence is typically irreversible because of the lack of brain cells' regeneration capability; thus, prior advances should have been taken for essential counteraction of the disease. Within various parts of Pakistan, prior studies have already been done on stroke risk's contributing factors. The contribution of various risk factors for stroke might be diverse in various ethnicities. Since stroke is devastation, and the prognosis's treatment effect is restricted, the possibility to control the illness lies in its primary prevention. Dyslipidemia has been linked as a unique self-determining risk factor that leads to ischemic stroke. However, its effect in hemorrhagic stroke is uncertain, and adjustable outcomes have grown the consideration to compute the burden, with the intention that it is a significant factor. It must be considered accordingly in order to reduce morbidity [10]. The present study's objective was to determine the frequency of dyslipidemias among patients with hemorrhagic stroke and Ischemic stroke.

METHODOLOGY

A Cross-sectional study was conducted for six months from 1st September 2019 to 27th February 2020 at Saidu teaching hospital, Swat, Pakistan. Subjects with hemorrhagic stroke and ischemic stroke were included in this study with a non-probability consecutive sampling technique. A total of 157 cases of hemorrhagic stroke and Ischemic stroke cases were included. Subjects with a history of trauma, brain tumors, or severe other disease were excluded from the study. The study was conducted following the declaration of Helsinki, and informed consent was obtained from each participant. Ethical committee approval was obtained from the study site before the commencement of the study. Study-related information was recorded in a specifically designed questionnaire by the researcher, including the outcome variable. All the collected data were entered in Microsoft Excel and analyzed on SPSS version 22.0.The data were presented as frequency and percentages. The post-stratification chi-square test was applied, taking p-value <0.05 as significant.

RESULTS

In the present study, 54.8% of our study population were males, and the mean age was 54.4 ± 7.1 years. 63.1% of subjects were with ischemic stroke and 36.9% with hemorrhagic stroke. Dyslipidemias were seen in 31.2 % of cases (Table 1). With respect to age, dyslipidemia was present among 27 subjects with 40-55 years of age and 22 subjects aged between 56-70 years (Figure 1). While the gender based distribution showed that 23 females and 26 males had dyslipidemia (Figure 2). There was no significant effect of age or gender on the frequency of dyslipidemia ($p>0.05$). A high frequency of ischemic stroke was present among both males and females than hemorrhagic stroke (Figure 3) and both age groups (Figure 4).

DISCUSSION

Globally, stroke is the leading source of morbidity and the second topsource of mortality. There are various leading irreversible and reversible contributing factors, out of which dyslipidemia is the most probable regulating risk factor [11,12]. In the current study, dyslipidemia was seen in 31.2% of cases presented with a higher percentage of ischemic stroke. There were variable outcomes in the past for this specific situation. Bilic et al., in his study, found a relatively higher prevalence with 20.5% of the cases [13].Whereas in another research done by Fawi G and colleagues, dyslipidemias were observed in 29.5% cases [14]. In this study, out of the nine cases that developed hyperlipidemia in patients with hemorrhagic stroke, the maximum was in the group of elevated cholesterol levels found in five cases. These outcomes were consistent with the examinations done by Khan et al. and Eapen et al., who explore delevated cholesterols levels in patients with hemorrhagic stroke, i.e. 11% and 17% [15,16]. However, on the contrary, one study done by Khan NA explored a high number of cases with reduced HDL, i.e. 29.4% [17]. This, again affirmed by the details that lower HDL cholesterol level, which is settling lipid and maintaining a strategic distance from adverse impact, can incline to hemorrhagic stroke. This was additionally indicated that the higher the BMI and

higher were the odds of dyslipidemia, and the wide range of various investigations discovered this distinction as factually huge. Explores have demonstrated that dyslipidemias are more common in guys than in females. This was also observed by the examinations in the past where male sexual orientation was more connected with dyslipidemia. This can be because of the higher number of heavy drinkers, higher frequency of HTN, and a more significant amount of smoking propensities that can affect the lipid profiles.

CONCLUSION

The frequency of ischemic stroke was prominent in comparison with hemorrhagic stroke among study subjects. Dyslipidemias were observed in 49 cases (31.2%) out of 157 cases. A higher prevalence of dyslipidemia is alarming and requires more attention of the physician on various regulating risk factors.

CONFLICTS OF INTEREST

The Author(s) have no conflicts of interest.

ACKNOWLEDGMENT

The authors would like to acknowledge the Medical Affairs Department of Getz Pharma for their technical support.

FUNDING

None.

REFERENCES

1. Judith M, George AM. The atlas of heart diseases and stroke. Geneva: WHO and CDC; 2004
2. Kandhro AH. Interplay Between Cholesterol, SREBPs, MicroRNA-33 in Dyslipidemia. Int. j. endorsing health sci. res.2016; 4(2): 05-10.
3. Zeeshan M, Imran M, Jabeen H, Begum S, Ahmed N, Qasim R. Prevalence of metabolic syndrome among obese diabetic subjects. Int. j. endorsing health sci. res.2015; 3(4):23-28.
4. Hazrat H, Ahmed S. Maternal Obesity and Gestational Diabetes Mellitus: The Pathological Programming. Int. j. endorsing health sci. res.2017; 5(4): 33-36.
5. Ahmed S, Noushad S. Pathophysiology & Management of Ischemic and Neuropathic Pain.Int. j. endorsing health sci. res.2014; 2(1): 01-04.
6. Jovanović DR, Beslać-Bumbasirević L, Raicević R, Zidverć-Trajković J, Ercegovac MD. Etiology of ischemic stroke among young adults of Serbia. Vojnosanit Pregl. 2008;65:803-809
7. Altaf M, Ahmed S. Neurological Disturbances Caused by Nutritional Deficit. APP. 2016; 3: 69-70.
8. Rajput AH, Naz H, Iqbal A. Depressive symptoms among patients of Myocardial Infarction. APP. 2017; 4: 50-58.
9. Shamoon N, Sadaf A, Hiba J and Sikandar KS. Effect of Exercise on Hematological Parameters: A Study on Trained versus Un-Trained Male Subjects. Pak. j. life soc. Sci. 2012; 10(1): 18-21
10. Shah MA, Khan M,Latif M. Frequency of Hyperlipidemia in Patients Presenting with Ischemic Stroke. PJMHS. 2012; 6(2): 423-427.
11. Malik MA, Hussain A, Mustafa J, Nasim N, Asghar S. Frequency of Dyslipidemia in Hemorrhagic Stroke. Annals of Punjab Medical College. 2019;13(3):229-231.
12. Sherlock PL. Stroke in Developing Countries: Epidemiology, Impact and Policy Implications development policy review. Reser rev. 2010;28(6):693-709.

13. Bilic I, Diamanja G, Lusic I, Matijaca M, Caljkusic K. Risk factors and outcome differences between ischemic and hemorrhagic stroke. Acta Clin Croat. 2009;48(4):399-403.
14. Fawi G, Rahman A,Hassen A,et al. Southern Egypt stroke study. Egypt J Neurol Psychiat Neurosurg. 2005;42(1):255-69.
15. Khan H. Study of the risk factors in patients with cerebersovascular stroke at Peshawar hospital. Pak J Med Sci. 2004;18(4):583-91.
16. Eapan RP, Parikh JH, Patel NT. A study of clinical profile and risk factors for cerebrovascular stroke. Guj Med J. 2009;64(2):47-54.
17. Khan MN, Khan HD, Ahmad M. Umar M. Serum total and HDL-Cholesterol in ischemic and hemorrhagic stroke. Ann. Pak Inst Med. Sci. 2014;10(1)22-6

Table 1: Characteristics of study participants

Variables		Frequency	%
Gender	Female	71	45.2
	Male	86	54.8
Agegroup	40 - 55 years	89	56.7
	56 - 70 years	68	43.3
Diagnosis	Hemorrhagic stroke	58	36.9
	Ischemic stroke	99	63.1
Dyslipidemia	Yes	49	31.2
	No	108	68.8

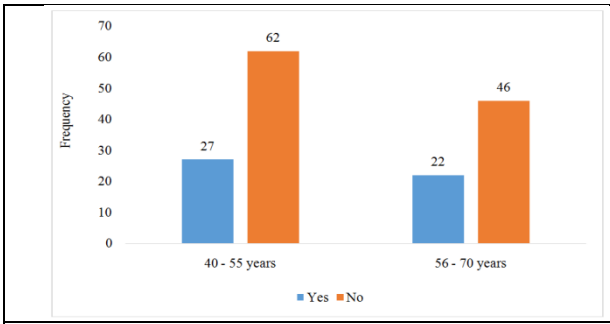


Figure 1: Distribution of Dyslipidemia in relation to age groups

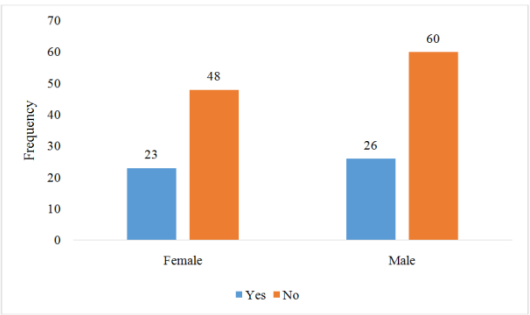


Figure 2: Distribution of Dyslipidemia in relation to gender distribution

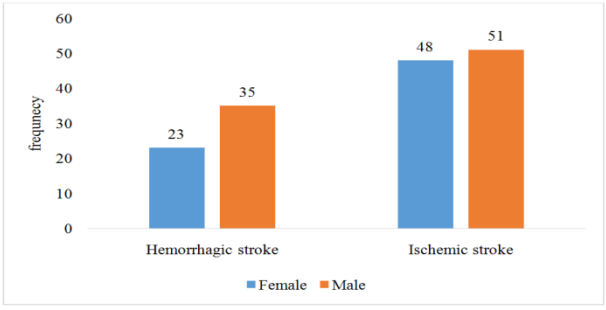


Figure 3: Distribution of Ischemic stroke and Hemorrhagic stroke cases in relation to gender distribution

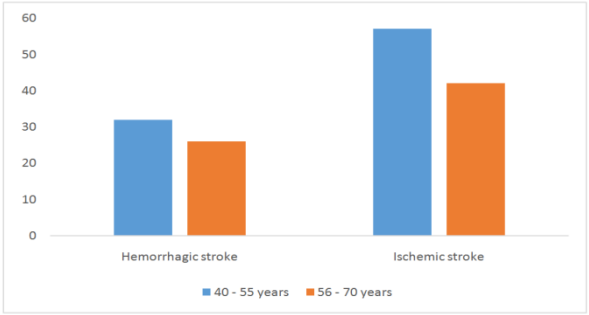


Figure 4: Distribution of Ischemic stroke and Hemorrhagic stroke cases in relation to age groups

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluation of *In vitro* Anti-diabetic Activity of Ethyl acetate Extract of *Notonia grandiflora* Wall.

Meera paul[1]*, R. Sanilkumar[2] and Sabu M.C[1]

[1]Mookambika College of Pharmaceutical Sciences and Research, Muvattupuzha, Kerala, India.
[2]Department of Pharmacy, Annamalai University, Annamalai Nagar,Tamil Nadu, India

Received: 17 Nov 2020 Revised: 19 Dec 2020 Accepted: 21 Jan 2021

***Address for Correspondence**
Meera paul
Mookambika College of Pharmaceutical Sciences and Research,
Muvattupuzha, Kerala, India.
Email : meera5paul@gmail.com

ABSTRACT

The aim of this work was to evaluate the antidiabetic activity and mechanism of action of the ethylacetate extract of the aerial parts of *Notonia grandiflora* Wall. The effects of the plant extract on glucose utilization in HepG2 cells and L6 myoblasts were investigated using cell culture procedures. The inhibitory effects of the extract on the activities of different enzymes such as alpha-amylase and alpha-glucosidase were evaluated. The possibility of the extract for cytotoxicity was evaluated using MTT assay in HepG2 cells. The IC50 values of ethylacetate extract was found to be 232µg/mL and 531µg/mL respectively for alpha amylase and alpha glucosidase inhibition. *In vitro* glucose uptake assay on cultured HepG2 and L6 cell lines showed that the percentage of glucose uptake increased with increase in concentration of sample. The results of the study therefore clearly indicate the potential of this extract to manage diabetes.

Keywords: *Notonia grandiflora*, alpha-amylase, alpha-glucosidase, glucose uptake, diabetes

INTRODUCTION

Diabetes mellitus is a serious complex multifactorial disorder characterized by hyperglycemia and glucose intolerance, either due to the relative deficiency in insulin secretion or reduced effectiveness of insulin's action to enhance glucose uptake [1]. If left untreated, can progress to severe complications. These complications include hyperlipidemia, oxidative stress, and enzymatic glycation of protein [2]. Majority of diabetes medications have imperfect glycemic control and have side effects including weight gain, nausea or vomitting, diarrhoea, and lactic acidosis, reflecting the limitations of current pharmacotherapy [3]. The prevalence of type 2 DM is associated with increased glucose concentrations and this is often due to postprandial glucose concentrations. The increase of blood glucose after a meal is caused by hydrolysis of starch by pancreatic α-amylase as well as uptake of glucose by intestinal α-glucosidase and therefore, an effective strategy for type 2 DM management would be strong inhibition of

pancreatic α-amylase and intestinal α-glucosidase [4]. Plant polyphenols and flavonoids have been reported to possess inhibitory effect on α-amylase and α-glucosidase as well as increasing glucose uptake into skeletal muscle and adipocytes. Plant derived compounds have also shown beneficiary effect on glucose uptake in the liver and this is vital as the organ is an important regulator of plasma glucose level and plays a critical role in glucose metabolism and regulation [5]. The WHO has emphasized on the use of ethnomedicines for the management of diabetes. According to the reports, more than 1200 plants have been traditionally used for their hypoglycemic effects, out of which 800 plants have been scientifically reported to possess antidiabetic potential [6]. Natural products have additional advantages of safety, affordable cost, and ease of availability along with their multi-targeting ability due to presence of diverse chemical compounds which may provide synergistic actions against diabetes. However, the majority of these traditional plants have not been scientifically validated for their efficacy in the management of diabetes [7]. *Notonia grandiflora* (Asteraceae) is commonly known as "Common Fleshy Ragweed" in English and as MuyalKathilai in Tamil. It is generally found on bare, exposed slopes and rocks of deciduous forests from plains to 1400m [8]. The plant has been found to possess pharmacological activities as analgesic and antinociceptive, anti-inflammatory[9], antimicrobial, antibacterial, antifungal, and antipyretic [10]. It is used traditionally in the treatment of joints pains, ear ache [11], to get relief from gastric complaint [12], for pimples and as a remedy for hydrophobia, used in the treatment of urinary disorders, infection, stones, diuretic, oedema [13], for scabies and skin eruptions [14], wounds including sores and ulcers and in the treatment of scorpion bite [15].No scientific research has reported the efficacy of this plant with regard to diabetes mellitus. Therefore, the objective of the present study is to investigate the *in vitro* antidiabetic activity of ethylacetate extract of *Notonia grandiflora* aerial parts on glucose uptake in HepG2 cells. In addition, the *in vitro* inhibitory effect ofthe extract on α-amylase and α-glucosidase will be studied.

MATERIALS AND METHODS

Plant material and extraction

The fresh aerial parts (stems and leaves) of *Notonia grandiflora* were collected from Tirunelveli district of Tamil Nadu, India and authenticated by Dr. V. Chelladurai, Research Officer (Botany), Central Council of Research in Ayurveda and Siddha, Government Siddha Medical College, Palayamkottai, Tamilnadu. The shade dried *Notonia grandiflora* aerial parts were powdered mechanically and stored in an air tight container. The extraction was carried out in a Soxhlet extractor for 8 hours, sequentially with hexane, ethyl acetate, ethanol and water. The extract was concentrated by a rotary evaporator under 40°C and low pressure and finally dried to a constant weight. Dried extracts were kept at 20^0C in air tight containers until further test were carried out [16].

In vitro methods employed in antidiabetic studies

In vitro α amylase inhibitory assay [17].

Different concentrations of *Notonia grandiflora* ethylacetate extract (62.5µg/mL -1000µg/mL) from a stock concentration of 10mg/mL was made up to to 1000µl using 25mM phosphate buffer pH 6.9, containing 25µl of porcine α amylase at a concentration of 0.5 mg/ml were incubated at 25° for 10 min. After pre incubation, 0.5% starch solution (25µl) in 25mM phosphate buffer pH 6.9 was added. The above reaction mixtures were then incubated at 25°C for 10 min. 50µl of 96mM 3, 5-dinitrosalicylic acid colour reagent was added to stop the reaction. The micro plate was then incubated for 5 min in a boiling water bath and cooled to room temperature. Measured absorbance using a micro plate reader at 540nm. Acarbose, a well-known anti-diabetic medication, was used as a positive control. The percentage inhibition was calculated as follows

$$\% \text{ inhibition} = \frac{\text{control} - \text{test}}{\text{control}} \times 100$$

Control inhibitions denotes 100% enzyme activity and were performed in a similar way by replacing extracts with vehicle.

Statistical analysis

All the experiments were done in triplicate. Values are expressed as mean ± SEM.

α-Glucosidase inhibition assay [18]

Alpha-glucosidase activity was measured by the determination of reducing sugar arise from hydrolysis of sucrose by alpha-glucosidase enzyme. The effects of samples were assayed according to the method Matsui et al., with slight modifications. Acarbose drug (10 mg/mL DMSO) was used as reference. Different concentrations of sample such as 125µg/mL-2000µg/mL from the stock solution of 10mg/mL were taken and were incubated for 5 min before initiating the reaction with substrates sucrose (37 mM) in a final reaction mixture of (1 mL)0.1 M phosphate buffer pH 7.2. The reaction mixture was incubated for 20 and 30 min at 37°C and the reaction was stopped by incubating in a boiling water bath for 2 min. A tube with phosphate buffer and enzyme was kept as control. The tubes were added with 250 µL of glucose reagent and incubated for 10 min followed by measuring absorbance at 510 nm using a microplate reader (Erba, Lisascan).

The percentage inhibition was calculated as follow % inhibition $= \frac{\text{control}-\text{test}}{\text{control}} \times 100$

Statistical analysis

All the experiments were done in triplicate. Values are expressed as mean ± SEM.

Glucose Utilization in L6 Myoblast [19]

Maintenance of L6 cell lines

L6 (rat myoblast cell line) was purchased from NCCS Pune was maintained in Dulbecco's modified eagle's media (Sigma Aldrich, USA) supplemented with 10% FBS (Invitrogen) and grown to confluency at 37°C in 5 % CO2 in a humidified atmosphere in a CO2 incubator (NBS, EPPENDORF, GERMANY).

Procedure

The cells were trypsinized using 500µl of 0.025% Trypsin in Phosphate-buffered saline/ 0.5mM EDTA solution (Invitrogen) for 2 minutes and passaged to T flasks in complete aseptic conditions. The cells were then subcultured to a 24 well plate. After attaining 80% confluency, cells were maintained in DMEM without glucose for 24 hours. Extracts were added to grown cells at a final volume of 25 µg/mL, 50 µg/mL and 100 µg/mL from the stock solution and incubated for 24 hours in DMEM containing 300mM glucose and an untreated control with high glucose was also maintained. After incubation cells were isolated by centrifugation at 6000 rpm for 10 minutes. Supernatant was discarded and added 200µl of cell lysis buffer (1MTris Hcl, 0.25M EDTA, 2M Nacl, 0.5% Triton). The incubation was done for 30 minutes at 4°C and estimated the glucose uptake using high sensitivity glucose oxidase kit method. All experiments were repeated in triplicates and mean value was used for calculations.

Calculation: Total Glucose in mg/dL = $\frac{\text{Absorbance of Test}}{\text{Absobance of Standard}}$ X 100

% Glucose uptake = $\frac{\text{OD of Test} - \text{OD of Control}}{\text{OD of Test}}$ X 100

Measurement of glucose uptake in HepG2 cells [20,21]

HepG2 hepatic cells was maintained in Dulbecco's modified eagle's media supplemented with 10% FBS and grown to confluency at 37°c in 5 % CO_2 in a humidified atmosphere in a CO_2 incubator. The cells were trypsinized (500µl of 0.025% Trypsin in PBS/ 0.5mM EDTA solution for 2 minutes and passaged to T flasks in complete aseptic conditions, and were then sub cultured to a 24 well plate. After attaining 80% confluency, cells were kept in DMEM without glucose for 24 hours. Ethylacetate extract of *Notonia grandiflora* were added to grown cells at a final concentration of

25 µg, 50 µg and 100 µg from a stock of 1mg/ml and incubated for 24 hours in DMEM containing 300mM glucose. An untreated control with high glucose was also maintained. After incubation cells were isolated by spinning at 6000 rpm for 10 minutes. Supernatant was discarded and 200µl of cell lysis buffer (1MTris HCl,0.25M EDTA, 2M NaCl, 0.5% Triton) was added. The incubation was done for 30 minutes at 4°C and the glucose content was estimated using glucose kit method (Erba Mannheim, Germany) and the absorbance was read at 505nm (Agilent, USA). All experiments were repeated in triplicates and mean value was used for calculations. Increase in glucose uptake = (As-Ac)/As × 100 Where As is the absorbance of sample and Ac is the absorbance of control

RESULTS AND DISCUSSION

α-amylase inhibitory activity of *Notonia grandiflora*

The enzyme *α* amylase begins the process of starch digestion and breaks them into small pieces with two or three glucose units. Bythe *α*-amylase inhibitory activity the rate of digestion of carbohydrate and the consequent absorption of glucose is reduced [22]. In-vitro, *α*-amylase inhibitory activity of *Notonia grandiflora* was tested. Concentrations of the range 62.5,125, 250, 500, 1000 µg/mLof the of ethylacetate extract of aerial parts of *Notonia grandiflora* was tested on the amylases. The result of experiment showed that, there was a dose-dependent increase in percentage inhibitory activity against *α* -amylase enzyme. The ethylacetate extract showed a significant 50% *α*-amylase inhibitory activity at a concentration of 232µg/mL and the results are comparable with that of the standard acarbose which showed 50% inhibition at a concentration of 189 µg/ml.(Fig. 1). From the results, it is evident that the ethylacetate extract of aerial parts of *Notonia grandiflora* had potent *α*-amylase inhibitory activity comparable to the commercial antidiabetic drug acarbose which could be attributed to the presence of polyphenols (65.41±0.21 (mg GAE/g)) and flavonoids (73.9±0.17mg QE/g) because polyphenols are not only capable of reducing oxidative stress but also of inhibiting carbohydrate hydrolyzing enzymes by binding with proteins. The results are in accordance with the previous study wherein, there is a positive relationship between the total polyphenol and flavonoid content and the ability to inhibit intestinal *α*-glucosidase and pancreatic *α*-amylase. Some phenolic compounds in sweet potato, strawberry, raspbery, olive oil, pears, coca and lentils are stated to be effective human *α*-amylase inhibitors [23] Flavonoids and anthocyanins are also reported to have inhibitory activity against *α*-amylase[23,24] The *α*-amylase inhibitory action of *Notonia grandiflora* might play a role in diabetic treatment.

α-Glucosidase inhibitory activities

α-glucosidase is one of the glucosidases located in the intestinal brush border and is an important enzyme of carbohydrate metabolism [25]. *α*-glucosidase inhibitory activity blocks the actions of *α*- glucosidase enzyme in the small intestine which is involved in the conversion of oligosaccharide and disaccharide to monosaccharide, essential for gastro intestinal absorption. Hence, the inhibition of *α*-glucosidase is one of the important approaches in oral antidiabetic medication. Reducing postprandial glucose level by delaying glucose absorption after meal is the prominent benefit from *α*-glucosidase inhibitor [26].The in-vitro *α*-glucosidase inhibitory activity of ethylacetate extract of aerial parts of *Notonia grandiflora* was tested. Varying concentration of the extract 125, 250, 500, 1000 and 2000µg/ mL were taken and assayed for the *α*-glucosidase inhibitory activity. The extracts exhibited a dose dependent increase in percentage inhibitory activity against alpha glucosidase. The ethylacetate extract showed a significant 50% *α*-glucosidase inhibitory activity at a concentration of 531µg/mL and the results are comparable with that of the standard acarbose which showed 50% inhibition at a concentration of 239 µg/ml. (Fig. 2). From the result, it is evident that ethylacetate extract of aerial parts of *Notonia grandiflora* has significant *α*-glucosidase inhibitory activity comparable to the commercial drug acarbose. This could be justified by the nature of some extract constituents (phenols, flavonoids saponins, steroids, alkaloids, terpenoids) present in the extract could be responsible as being effective inhibitors of *α*-amylase and *α*-glucosidase [27].From the results, it can be concluded that ethylacetate extract of aerial parts of *Notonia grandiflora* can be excellent choice of drug with *α*-glucosidase inhibitory activity and can thus reduce the rate of digestion and absorption of carbohydrates.

Glucose Utilization in L6 Myoblast

Diabetes mellitus is associated with insulin deficiency and decreased glucose utilization in skeletal muscles. Glucose uptake could be enhanced through muscle contraction as well as electrical stimulation, and eventually cause the reduction of blood sugar level (preventing from type-II diabetes [28]. So, any impairment in this glucose uptake by the cells, will result in hyperglycaemia, is the basis for measuring the glucose uptake by different cells. The L6 skeletal muscle cell line has been widely used to explore the mechanism of insulin and exercise stimulated glucose transport [29].The results representing the anti-diabetic activity of ethylacetate extract of *N.grandiflora* are presented in Fig. 3. The results showed that the percentage of glucose uptake increased with increase in concentration of sample. *N. grandiflora* exhibited a percentage glucose uptake of 55.56% at a concentration of 100 µg/mL and the standard pioglitazone exhibited 74.64 % glucose uptake at similar concentration level. These results are indicating the capability of plant extract to stimulate the glucose uptake in the absence of insulin.

Glucose Utilization in HepG2.

Cytotoxicity: The *in vitro* cytotoxic activity of the extract of *N. grandiflora* was measured by the MTT assay against the HepG2 liver cell line at various concentrations. The cytotoxicity result revealed that *N. grandiflora* extract displayed low level of toxicity to HepG2 cells at all the doses tested in a dose-dependent manner (Fig. 4.). However, at the highest dosage (100 µg/ml) tested, the extract showed less than 50% cell death, whereas the IC50 value (concentration that can cause 50 % cell death) was calculated to be249.025 µg/mL (Calculated using ED50 PLUS V1.0 Software)

Glucose Utilization in HepG2:An impaired hepatic glucose utilisation is a common feature in diabetic patients, which contributes to diabetic postprandial hyperglycaemia. Therefore, compounds that stimulate hepatic glucose consumption may have potential anti-diabetic properties [30].The results obtained for glucose uptake in HepG2 cells in the presence of the *N. grandiflora* extract at 25,50 and 100 µg/ml are presented in Fig.5.Metformin was used as the standard for comparison. The result obtained in this study on glucose uptake using HepG2 cells demonstrated that ethylacetate extract of *N.grandiflora* showed a concentration dependent increase in glucose uptake when compared with standard. This suggests that the extract of *N.grandiflora*, therefore, mimics metformin by increasing glucose uptake in the liver. Metformin is a biguanide and it exerts its hypoglycemic effect through activation of the AMP-activated protein kinase (AMPK) in the liver, which in turn may lead to various pharmacologic effects, including inhibition of glucose, lipid synthesis, and also improved hepatic sensitivity to insulin [30].The presence of phytochemicals compounds such as phenols, terpenoids flavonoids, and flavanols has been reported to suppress glucose release from the liver and also enhances glucose uptake in hepatic thereby regulating intracellular signalling pathway. Therefore, the glucose uptake observed in HepG2 cells for this ethylacetate extract might be due to their phytochemical constituents previously reported. On this basis, a mechanism of action of *N. grandiflora* may be hypothesized which could be linked to activation of the insulin signalling cascade, resulting in stimulation of GLUT 2 that facilitates the translocation of glucose into the cell [31].

CONCLUSION

The present study indicates that ethylacetate extract of *N. grandiflora* possess significant antihyperglycaemic activity, with its probable mechanisms of action including stimulation of glucose uptake via insulin-dependent pathways in skeletal muscle and/or insulin-independent pathways in hepatocytes, as well as the inhibition of intestinal alpha amylase and alpha glucosidase in preventing rise in postprandial glucose level. We can therefore conclude from this study that the presence of the phytochemicals in these plants might be the reason for this anti- diabetic activity and that the plant may essentially contain herbal bioactive compounds which demand further structural elucidation and characterization methodologies to identify the bioactive constituents. Further *ex vivo* and *in vivo* investigations should be done for confirming the anti- diabetic activity of these plants. The plant extracts understudy can serve as therapeutic agents and can be used as latent source of novel bioactive compounds for treating Diabetes mellitus type 2.

REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care. 2009;32 Suppl 1:S62-S67. doi:10.2337/dc09-S062.
2. Lyons TJ, Jenkins AJ. Glycation, oxidation, and lipoxidation in the development of the complications of diabetes: a carbonyl stress hypothesis. Diabetes Rev (Alex). 1997;5(4):365-391.
3. Tsang MW. The management of type 2 diabetic patients with hypoglycaemic agents. ISRN Endocrinol. 2012:478120. doi:10.5402/2012/478120.
4. Oboh G, Akinyemi AJ, Ademiluyi AO. Inhibition of α-amylase and α-glucosidase activities by ethanolic extract of *Telfairia occidentalis* (fluted pumpkin) leaf. Asian Pac J Trop Biomed. 2012;2(9):733-738. doi:10.1016/S2221-1691(12)60219-6.
5. Khamis Masitah, Talib Fazilah *et al*. *In vitro* α-amylase and α-glucosidase inhibition and increased glucose uptake of *Morinda citrifolia* fruit and scopoletin. Research J. Pharm. and Tech. 2015; 8 (2):189-193.
6. Pandey A*et al*. Alternative therapies useful in the management of diabetes: A systematic review. J Pharm Bioall Sci 2011.;3:504-12.
7. Asif Muhammad *et al*. Antidiabetic activity of aqueous extract of *Sigesbeckia orientalis* (St. Paul's Wort) in alloxan-induced diabetes model. Braz. J. Pharm. Sci.São Paulo. 2019; vol.55:e18408.
8. Khare CP. Indian medicinal plants: An illustrated dictionary. Allahabad (India); Lalit Mohan Basu, 2007.
9. Bose N.J.J. and Mehalingam P. Antinociceptive and Anti-Inflammatory activities of leaf extracts of ethnomedicinal plant. Acta Hortic. 2014; 1023:117-22.
10. Vanijajiva O, Pornpongrungrueng P, WitthayaPongamornkul W. *Kleinia grandiflora* (Asteraceae: Senecioneae), a species and genus newly discovered in Thailand. Phytotaxa 2014; 159 (1): 017–022.
11. Basha M.G *et al*. Diversity of ethnomedicinal plants used by Malayali tribals in Yelagiri hills of Eastern ghats, Tamilnadu, India. Asian J. Plant Sci. Res. 2013;3(6):29-45.
12. Ayyanar, M and Ignacimuthu S. Traditional Knowledge of Kanitribals in Kouthalai of Tirunelveli hills, Tamil Nadu, India. Journal Ethno-pharmacology.2005; 102:246-255.
13. Sharma A.J, Naresh C. Pharmacognostical Studies of *Bryophyllum pinnatum* (Lam.) Kurz. Phcog J. 2014; 6:20-6.
14. Ganesan K, Xu B. Ethnobotanical studies on folkloric medicinal plants in Nainamalai, Namakkal District, Tamil Nadu, India. Trends Phytochem. Res. 2017;1(3): 153-68.
15. Maharajan M.J, Janakiraman N. Floristic Diversity and Medicinal importance of South Vagaikulam region Tirunelveli, Tamil Nadu, South India. JMHE.2015;1:125-29.
16. ManokariS.L, Meenu N.C. A Study on the Extraction Process of *Wrightia Tinctoria* and Evaluation of Its Antimicrobial Activity. IJRASET. 2017; 5(9).
17. Prasad S B, Srinivasan K K.*In vitro* alpha amylase inhibitory and antioxidant activity studies on the root of *Chonemorpha fragrans* (Moon) Alston.World J Pharm Sci 2015; 3(11): 2202-2206.
18. Brabha M J, Malbi M A. Antidiabetic activity of chelating tris n-methylethylenediamine iron complex. Drug Invention Today, 2020; 14 (1):121-126.
19. S. B. Rajashree*et. al.* Phytochemical screening and *in vitro* antidiabetic activity of *Averrhoa carambola* linn. leaf extracts. Ind Res J Pharm & Sci.2018; June.: 5 (2);1470-1478
20. J.R. Abima Shazhni *et al*. Insights of antidiabetic, anti-inflammatory and hepatoprotective properties of antimicrobial secondary metabolites of corm extract from *Caladium X hortulanum*. Saudi J Biol Sci.2018;25:1755–1761.
21. Siju EN. *et al*. *In vitro* antioxidant and antidiabetic activity of hydroalcoholic extract of *Plumeria pudica* Jacq. IJP.2017; 8(1): 31-40.
22. MalathiV *et al*. Anti diabetic activity by the *in vitro* alpha amylase and alpha-glucosidase inhibitory activity of *Catharanthus roseus*. The bioscan.2010: 5(4) : 655-659.

23. Matsui T *et al*. α-Glucosidase Inhibitory Action of Natural Acylated Anthocyanins.1. Survey of Natural Pigments with Potent Inhibitory Activity. J. Agric. Food Chem. 2001;49:1948-1951.
24. BanerjeeA *et al*. *In Vitro* Antidiabetic and Anti-oxidant Activities of Methanol Extract of *Tinospora Sinensis*.J ApplBiol Biotechnol.2017; 5 (03):061-067.
25. Lankatillake *et al*. Understanding glycaemic control and current approaches for screening antidiabetic natural products from evidence-based medicinal plants. Plant Methods.2019;15:105.
26. Doan *et al*. Antidiabetic activity, glucose uptake stimulation and α-glucosidase inhibitory effect of *Chrysophyllum caimito* L. stem bark extract. BMC Complementary and Alternative Medicine. 2018; 18:267.
27. Matsui T *et al*. α-Glucosidase Inhibitory Action of Natural Acylated Anthocyanins. 2. α-Glucosidase Inhibition by Isolated Acylated Anthocyanins.J. Agric. Food Chem. 2001;49: 1952-1956.
28. Kumar N*et al*. Enhancement of glucose uptake in skeletal muscle L6 cells and insulin secretion in pancreatic hamster-insulinoma-transfected cells by application of non-thermal plasma jet. Applied Physics Letters.2013;103(20), 2037011-4.
29. Richter E A,Hargreaves M. Exercise, glut4, and skeletal muscle glucose uptake. Physiol Rev.2013; 93: 993–1017.
30. Sagbo I J *et al*.*In Vitro* Antidiabetic Activity and Mechanism of Action of *Brachylaena elliptica* (Thunb.) DC. Evidence-Based Complementary and Alternative Medicine. 2018.
31. Odeyemi S, Dewar J.*In Vitro* Antidiabetic Activity A_ecting Glucose Uptake in HepG2 Cells Following Their Exposure to Extracts of *Lauridia tetragona* (L.f.) R.H. Archer. Processes. 2020;8: 33.

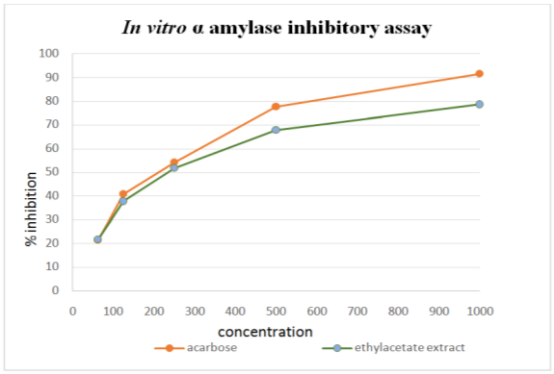


Figure 1: % inhibition of alpha amylase enzyme by ethylacetate extract of *Notonia grandiflora* and reference alpha amylase inhibitor, Acarbose

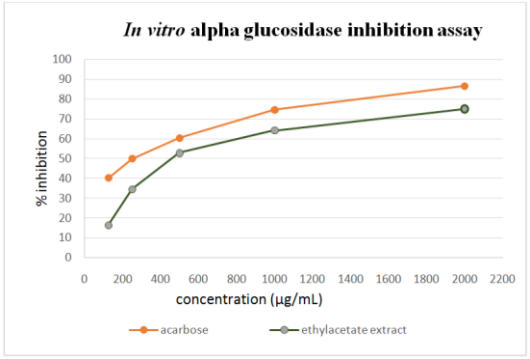


Figure 2: % inhibition of alpha glucosidase enzyme by by ethylacetate extract of *Notonia grandiflora* and reference alpha amylase inhibitor, Acarbose

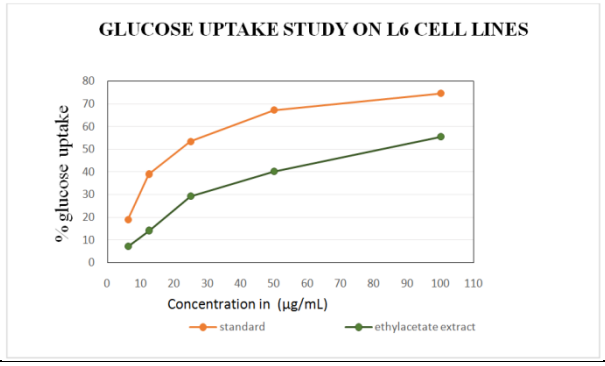


Figure 3: Glucose uptake study of ethylacetate extract of *N.grandiflora* and standard

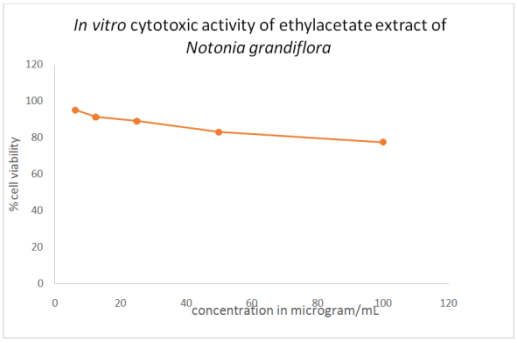


Fig. 4*: In vitro* cytotoxic activity of ethylacetate extract of *Notonia grandiflora*

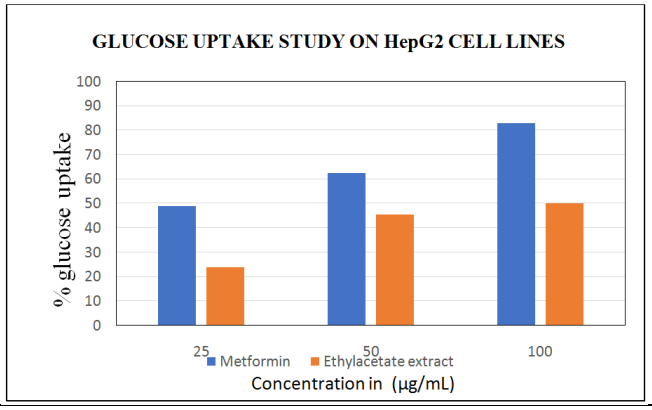


Figure 5: Effect of *N.grandiflora* extract and metformin on glucose utilization in HepG2 hepatocytes.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

A Study on using of Biofertilizer Ingredient and its Health Issues for the Younger Generation in Tamil Nadu

M. Rajamanickam[1] and M. Sakthivel[2]*

Assistant Professor in MBA, K. S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal, Tamil Nadu, India.
Ph.D Research Scholar (Full time), Department of Management Studies, K. S. Rangasamy College of Arts and Science (Autonomous), Tiruchengode, Namakkal, Tamil Nadu, India.

Received: 16 Nov 2020 Revised: 18 Dec 2020 Accepted: 20 Jan 2021

***Address for Correspondence**
M. Sakthivel
Ph.D Research Scholar (Full time),
Department of Management Studies,
K. S. Rangasamy College of Arts and Science (Autonomous),
Tiruchengode, Namakkal, Tamil Nadu, India.
Email: svel70743@gmail.com

ABSTRACT

In the present article an attempt has been made to study on using of bio fertilizer ingredient and its health issues for the younger generation in Tamil nadu. This study of research revealed how the younger generation people are affected using of biofertilizer and how to protect the health issues. In these days people are highly affected for the health immunity due to using of bio chemical fertilizer products and its causes more diseases affect easily with low immunity people. The main aim of the study is using of biofertilizer ingredient and it causes of health issues of the younger generation. For this purpose 600 youngsters are taken as respondents from the study based on the convenience sampling methods. The youngster plays a vital role for every food habit especially using of biofertilizers survival with health issues for the people in Tamilnadu. So the researcher concluded that youngster perception influence to organic products and awareness of health issues using of biofertilizers.

Keywords: Biofertilizer, younger generation, health, immunity, organic products.

INTRODUCTION

The protocols of agriculture have been radically changed in the last two decades in India. The Innovations in agricultural systems have been introduced to maximize the potential and opportunity of farmers to produce adequate supply and accessibility of safe, nutritious and high quality of food to world population. Plants nutrients are crucial for the production of crops and healthy food for the world's ever increasing population and also protect the environment. Soil management strategies are mostly dependents on inorganic chemical-based fertilizers, which

cause a serious threat to human health and also the environment. Bio-fertilizer has been notorious as an alternative for increasing soil fertility and crop production in sustainable growth of farming. Nevertheless the using of chemical fertilizer increases the plant growth and vigour, hence meets the food security of the world, but the plants grow good manner but not good plant characters such as, good root system, shoot system, nutritional characters and also will not get time to grow and mature properly but not a healthy. Chemically twisted plant will accumulate in the human body, toxic chemicals, which are very unsafe. The harmful effect of the chemical fertilizers will itself start from the industrialized of these chemicals, whose products and by products are some toxic chemicals or gases like NH4, CO2, and CH4 etc. which will cause pollution and human health immunity.

Need and Importance of the Study

The main aim for the study has been attempt the using biofertilizer ingredients farming and its health issues for the younger generation. In the recent days using biofertilizers in the agricultural land and it also causes the pollution and affecting health issues for especially for less immunity people. Even today the farmers are not much aware of using the biofertilizer and to protect the crops. While using non biofertilizer a crop does not differentiate whether a nutrient molecule is offered from chemical fertilizer or from compost prepared by the farmer. Most of us believe that one would need large quantity of farm-yard manure (FYM) or compost for growing crops, if we are not using chemical fertilizers. This belief is due to the fact that we measure value of the FYM or compost as a source of nutrients (NPK) a crop needs. This is mis-leading because this perspective ignores the fact that there are different types of agriculturally beneficial microorganisms in nature (available in plenty in compost) with ability to facilitate crop nutrition and even protection.

Scope of the Study

The scope of biofertilizer farming in Tamilnadu has been enormously increasing. This study is help for the new researches made in the field of agriculture sector. It has facilitated the farmers with new measures for more production eliminating the activity of chemical fertilizers. New techniques which are being innovated are entirely related to soil health during organic farming. Apart from these reasons, the various new diseases arising out of artificial production of fruits and vegetables have clearly set the minds of people for a shift to organic farming.

Statement of the Problem

The statement of the problem in this study is the introduction of high yielding varieties and widespread use of chemical fertilizers and pesticides helped in increasing the yield of major crops in India, which resulted in the increase of farmer's income. As the availability of land is decreasing day by day, application of fertilizers and pesticides has become essential to sustain the productivity of major crops to meet the food grain demand. The indiscriminate use of pesticides in intensively cropped areas has led to the destruction of beneficial organisms, outbreak of secondary pests, pesticide resistance, and problem of residues and toxic hazards which in turn has disturbed the ecological balance. Toxic residues of the agricultural chemicals are entering in the human diet, which is of major concern. In India, the average dietary intake of pesticide residue is 362.5 mg per day per person for vegetarians and 356.5 mg per day per person for non-vegetarians.

Objectives of the Study

- To determine the using of biofertilizer and it's affecting health issues for the youngster in Tamilnadu.
- To analysis the opinion towards bio chemical fertilizer products.

Period of the Study

The research period was three months from February to March 2020.

METHODOLOGY

Sample Frame

The research has been conduct in entire districts of Tamilnadu. The respondents are taken from the using of bio fertilizer ingredient and its health issues for the younger generation. 600 respondents are selected on convenient sampling method due to population is large size.

Hypothesis of the Study

- There is no significant difference in the means of using of biofertilizer and it's affecting health issues with respect to the selected independent variables, statistically significant.
- There is no significant difference in the mean score of opinion towards youngster's opinion towards bio chemical fertilizer products between the different categories of selected independent variables.

Data Collection Method

Primary data evaluated by structured questionnaire. This research was travel out fully in survey method through questionnaires.

Statistical Tools

The primary data were received and analyzed. A pilot study was operated with the questionnaires' review for the items analysis. The validity and reliability of the questionnaires were evaluated. The outputs of scale were examined working out by the Cronbach's Alpha (Table.1).

METHODS AND ANALYSIS

ANOVA

Gender and Using of Biofertilizer and its Affecting Health Issues

The difference of using of biofertilizer and its affecting health issues with respect to their gender, an analysis has been made and discussed in the following table 2. It is observed that the mean score and standard deviation of the male younger respondents are 3.15 and 0.85 respectively. On the other hand, female younger respondents got the mean score and standard deviation values as 3.01 and 0.82 respectively. It is found that among the two categories of gender, male younger respondents are having maximum level of using of biofertilizer and it's affecting health issues. The relationship between the gender of the respondents and using of biofertilizer and its affecting health issues is analyzed by making null hypothesis through ANOVAanalysis.

H0:There is no difference in the means of using of biofertilizer and it's affecting health issues with respect to their gender, statistically significant.

From the table 3 analysis, it is revealed that the 'p' value is greater than 0.05 and so null hypothesis is accepted. The 'F' test analysis resulted that there is no significant Difference in means of of using of biofertilizer and it's affecting health issues with respect to different category of gender of the youngster.

4)

5) Marital Status and using of Biofertilizer and It's Affecting Health Issues

The difference of of using of biofertilizer and it's affecting health issues with respect to their marital status; an analysis has been made and discussed in the following table 4. It is explored from the table 4 that the mean score and standard deviation of unmarried youngster are 3.05 and 0.91 respectively. On the other hand, married youngster

attained the mean score and standard deviation values as 3.21 and 0.63 respectively. It is found that among the two categories of marital status, marred youngster are having maximum level of using of biofertilizer and it's affecting health issues. The relationship between marital status of the respondents and using of biofertilizer and it's affecting health **issues** are tested by developing a null hypothesis through ANOVAanalysis.

H0:There is no difference in the means of using of biofertilizer and it's affecting health issues with respect to their marital status, statistically significant**.** It is determined that the null hypothesis is rejected in means of 'p' value is lesser than 0.05 (Table 5). From the 'F' test, it is showed that there is a significant difference in means of using of biofertilizer and it's affecting health issues with respect to different category of marital status of the youngster.

Age and using of Biofertilizer and Its Affecting Health Issues

The following table 6 discusses that the difference of using of biofertilizer and it's affecting health issues with respect to their age. From the analysis, it is divulged that the mean score and standard deviation of below 25 years old people are 3.13 and 0.66 respectively. The 25-35 years old people got the mean score and standard deviation values as 2.98 and 0.96 respectively. The mean score and standard deviation of 35-45 years old people are 3.12 and 0.85 respectively. Further, the belong to above 45 years of age group people attained the mean score and standard deviation values as 3.23 and 0.77 respectively. It is found that among the four categories of age, people who belong to above 45 years old are having maximum level of using of biofertilizer and it's affecting health issues The relationship between age of the respondents and of using of biofertilizer and it's affecting health issues are analyzed by framing null hypothesis through ANOVA analysis.

H0: There is no difference in means of of using of biofertilizer and it's affecting health issues with respect to their age, statistically significant.

It is surmised that the 'p' value is greater than 0.05 thus null hypotheses is accepted (Table 7). The 'F' test results indicated that there is no significant difference in means of using of biofertilizer and it's affecting health issues with respect to different category of age of the people.

KRUSKAL WALLIS H TEST

Marital status and youngster's opinion towards bio chemical fertilizer products

The relationship between the marital status of the respondents and their opinion towards bio chemical fertilizer products has been analyzed by framing a hypothesis with the help of 'Kruskal Wallis H' test.

H0 :There is no significant difference in the mean score of opinion towards bio chemical fertilizer products between the different categories of marital status.

From the table 8, it is observed that the mean rank score of opinion towards bio chemical fertilizer products among the unmarried as 302.94 and married having the mean rank score of 299.44 on opinion towards bio chemical fertilizer products. From the KW H test, it is found those unmarried are having the maximum level of opinion towards customer preference on retail store. Also, the null hypothesis is accepted as the 'p' value is greater than 0.05. Hence, there is no significant difference found in the mean score of opinion towards bio chemical fertilizer products between the different marital statuses of the sample respondents.

6)

Age and Youngster's Opinion towards Bio Chemical Fertilizer Products

The relationship between the age of the respondents and their opinion towards bio chemical fertilizer products is analyzed by developing hypothesis with the help of 'Kruskal Wallis H' test.

H0 :There is no significant difference in the mean score of opinion towards bio chemical fertilizer products between the different categories of age.

From the table 9, it is measured that the mean rank score of opinion towards towards bio chemical fertilizer products among below 25 years aged youngsters as 308.57 and the age of 25-35 years old having the mean rank score of 290.09 on opinion towards bio chemical fertilizer products. The mean rank score of opinion towards towards bio chemical fertilizer products among 35-45 years aged people as 303.57 and the age of above 45 years old have the mean rank score of 301.21 on opinion towards bio chemical fertilizer products. From the KW H test, it is observed that youngsters belonging to below 25 years are having the maximum level opinion towards bio chemical fertilizer products. The null hypothesis is accepted according to the 'p' value which was greater than 0.05. `Hence, there is no significant difference found in the mean score of opinion towards bio chemical fertilizer products between the different age group of the sample respondents.

Educational Qualification and Youngster's Opinion towards Bio Chemical Fertilizer Products

The relationship between the educational qualification of the respondents and their opinion towards bio chemical fertilizer products has been analyzed by making a hypothesis with the help of 'Kruskal Wallis H' test.

H_0: There is no significant difference in the mean score of opinion towards bio chemical fertilizer products between the different categories of educational qualification.

From the table 10, it is evaluated that the mean rank score of opinion towards bio chemical fertilizer products among the youngster having up to H.Sc. qualification as 273.29 and the educated up to diploma have the mean rank score of 285.24 on opinion towards bio chemical fertilizer products. The mean rank score of opinion towards bio chemical fertilizer products among graduates was as 322.50 and the qualified with a professional degree was having the mean rank score of 352.71 on opinion towards bio chemical fertilizer products. The mean rank score of opinion towards bio chemical fertilizer products among have other educational qualifications was 280.90. It is found from the KW H test that the qualified with a professional degree are having the maximum level opinion towards bio chemical fertilizer products. In addition, the null hypothesis is rejected since the 'p' value is lesser than 0.05. Hence, there is a significant difference in the mean score of opinion towards bio chemical fertilizer products between the different educational qualifications of the sample respondents.

RESULT AND DISCUSSION

ANOVA

- It found that there is no difference in the means of using of biofertilizer and it's affecting health issues with respect to their gender, statistically significant.
- There is no difference in the means of using of biofertilizer and it's affecting health issues with respect to their marital status, statistically significant.
- There is no difference in means of of using of biofertilizer and it's affecting health issues with respect to their age, statistically significant.

KRUSKAL WALLIS H TEST

- Also, the null hypothesis is accepted as the 'p' value is greater than 0.05. Hence, there is no significant difference found in the mean score of opinion towards bio chemical fertilizer products between the different marital statuses of the sample respondents.
- The null hypothesis is accepted according to the 'p' value which was greater than 0.05. `Hence, there is no significant difference found in the mean score of opinion towards bio chemical fertilizer products between the different age group of the sample respondents.

The result and discussion of this study using of biofertilizer its affects the more diseases for younger generation. The main aim for the study has been attempt the using biofertilizer ingredients farming and its health issues for the younger generation. In the recent days using biofertilizers in the agricultural land and it also causes the pollution

and affecting health issues for especially for less immunity people. Even today the farmers are not much aware of using the biofertilizer and to protect the crops.

REFERENCES

1. Altuntas. (2018). A comparative study on the effects of different conventional, organic and bio-fertilizers on broccoli yield and quality. applied ecology and environmental research, 16 (2), 1595-1608.
2. Carrapico, F. (2000). Azolla as a biofertiliser in africa. a challenge for the future. Revista de Ciencias Agrarias, 23 ((3-4)), 120-138.
3. Diacono, M. (2019). Recycling AgriculturalWastes and By-products in Organic Farming: Biofertilizer Production, Yield Performance and Carbon Footprint Analysis. Sustainability, 11 (3824), 1-17.
4. Javaid, A. (2011). Effects of biofertilizers combined with different soil amendments on potted rice plants. chilean journal of agricultural research, 71 (1), 157-163.
5. Kumar, K. (2013). Bio-Fertilizers for Organic Agriculture. Popular Kheti, 1 (4), 91-96.
6. Mahdi, S. S. (2010). Bio-fertilizers in organic agriculture. Journal of Phytology, 2 (10), 42-54.
7. Mishra, P. (2014). Rejuvenation of Biofertilizer for Sustainable Agriculture and Economic Development. The Journal of Sustainable Development, 11 (1), 41–61.
8. Onyimba. (2018). A review: Biofertilizer - A key player in enhancing soil fertility and crop productivity. Microbiol Biotechnol Rep, 2 (1), 22-28.
9. Poonia, S. (2011). Rhizobium: A Natural Biofertilizer. International Journal of Engineering and Management Research, 1 (1), 36-38.
10. Rajvir, S. (2013). Role of Bio-Fertilizer in Organic Agriculture: A Review. Research Journal of Recent Sciences, 2, 39-41.

Table 1. Cronbach's Alpha

1.	**Using of biofertilizer and its affecting health issues**	**0.086**

Table 2. Gender and using of biofertilizer and its affecting health issues

S. No.	Gender	Mean Score	SD	Min	Max
1	Male	3.15	0.85	1.00	4.75
2	Female	3.01	0.82	1.00	4.88

Table 3. Gender and using of biofertilizer and its affecting health issues (ANOVA)

	Sum of Squares	DF	Mean Square	F	'p' Value
Between Groups	2.355	1	2.355	3.354	0.068^{NS}
Within Groups	419.895	598	0.702		
Total	422.250	599			

Note: NS – Not Significant.

Table 4. Marital status and using of biofertilizer and its affecting health issues

S. No	Marital Status	Mean Score	SD	Min	Max
1	Unmarried	3.05	0.91	1.00	4.88
2	Married	3.21	0.63	2.00	4.50

Table. 5. Marital status and using of biofertilizer and its affecting health issues (ANOVA)

	Sum of Squares	DF	Mean Square	F	'p' Value
Between Groups	3.371	1	3.371	4.813	0.029**
Within Groups	418.879	598	0.700		
Total	422.250	599			

Note: ** – Significant at 5% level.

Table 6. Age and using of biofertilizer and its affecting health issues

S. No.	Age	Mean Score	SD	Min	Max
1	Below 25	3.13	0.66	1.25	4.50
2	25-35	2.98	0.96	1.00	4.50
3	35-45	3.12	0.85	1.00	4.88
4	Above 45	3.23	0.77	1.75	4.50

Table 7. Age and using of biofertilizer and its affecting health issues (ANOVA)

	Sum of Squares	DF	Mean Square	F	'p' Value
Between Groups	4.287	3	1.429	2.038	0.107^{NS}
Within Groups	417.963	596	0.701		
Total	422.250	599			

Note: NS – Not Significant.

Table 8. Marital status and youngster's opinion towards bio chemical fertilizer products

S. No.	Marital Status	Number of Respondents	Mean Rank	Kruskal-Wallis H	'p' Value
1.	Unmarried	182	302.94	0.052	0.820^{NS}
2.	Married	418	299.44		
	Total	**600**			

Note: NS – Not Significant

Table 9. Age and youngster's opinion towards bio chemical fertilizer products

S.No.	Age	Number of Respondents	Mean Rank	Kruskal-Wallis H	'p' Value
1.	Below 25	121	308.57	0.923	0.820^{NS}
2.	25-35	164	290.09		
3.	35-45	219	303.52		
4.	Above 45	96	301.21		
	Total	**600**			

Note: NS – Not Significant

Table 10. Educational qualification and youngster's opinion towards bio chemical fertilizer products

S.No.	Educational Qualification	Number of Respondents	Mean Rank	Kruskal-Wallis H	'p' Value
1.	Upto H.Sc.	115	273.29	12.394	0.015**
2.	Diploma	90	285.24		
3.	Graduate	227	322.50		
4.	Professional	129	352.71		
5.	Others	39	280.90		
	Total	600			

Note: ** – Significant at 5% level

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antibacterial and Anticancer Activity of Crude Secondary Metabolites of Antagonistic Bacterial Strain *Bacillus cereus*-PA4 Isolated from Marine Sediment

Vignesh Nagooran and Sivasubramani Kandasamy*

Department of Microbiology, Faculty of Science, Annamalai University, Annamalainagar, Chidambaram, Tamil Nadu, India.

Received: 01 Nov 2020 Revised: 02 Dec 2020 Accepted: 18 Dec 2020

***Address for Correspondence**
Sivasubramani Kandasamy
Assistant Professor,
Department of Microbiology,
Faculty of Science, Annamalai University,
Annamalainagar, Chidambaram,
Tamil Nadu, India.
Email: sivasubramanik@gmail.com

ABSTRACT

Marine bacterial metabolites are an ideal source of various antimicrobial and anticancer compounds. In this study, we isolated antagonistic marine bacterial strain *Bacillus cereus*-PA4 from marine soil samples and investigated the antimicrobial, antioxidant and anticancer activity of crude secondary metabolites of PA4. The effective antagonistic bacterial isolate PA4 was identified by 16S rRNA sequencing analysis. The secondary metabolites from strain PA4 were extracted by centrifugation using ethanol. The separated crude secondary metabolite extract (CSME) of PA4 was tested for antibacterial activity against bacterial pathogens by disc diffusion method. The anticancer activity of CSME of PA4 against human oral squamous carcinoma KB cell line was analyzed by MTT assay. The obtained results showed that the CSME of PA4 exhibited effective antimicrobial activity against selected gram-positive and gram-negative bacterial pathogens. Further, it showed effective DPPH radical scavenging activity in concentration established way. Moreover, the CSME of PA4 possesses effective anticancer activity against both tested cancer cell lines with the IC50 value of 87.32 µg/mL. Therefore, the secondary metabolites of strain *B. cereus* PA4 could be used for effective antibacterial and anticancer compounds after the further clinical trial.

Keywords: *Bacillus cereus*, Antimicrobial, Oral squamous cancer, KB cells.

INTRODUCTION

Marine microbe is an ideal source for various active metabolic compounds. Marine Bacteria play a very important role as new and rich sources of biologically active substances. In recent decades, the number of secondary metabolites from marine bacteria and fungi has been steadily increasing [1,2]reflecting the growing interest of academic and industrial groups. Several potential compounds are isolated and identified from the marine environment and the number of the isolated compound now reached more than 10,000 with hundreds of new novel compounds [3].Compared with terrestrial microbes, the compound present in secondary metabolites of by marine organisms have more novel and exclusive structures owing to the complex living circumstance and diversity of species, and the bioactivities are much stronger [4,5]. Among diverse marine bacterial species, the *Bacillus*sp belongs to a phylogenetically different group of bacteria. They are pervasive in the alkaline environment and capable to tolerate adverse conditions such as high temperature, pressure, salinity, and pH[6]. Generally, *Bacillus* strains need more nutrition and space for growing than their competitive organisms. Due to the diluting effect of the ocean drives, marine organisms produce potent bioactive compounds to fight off their competitors or to escape from micropredation [7,8]. According to the metabolic behaviour, the marine strains are dissimilar from their terrestrial counterparts, and thereby, they may produce unique bioactive compounds, which are not found in their terrestrial counterparts [9,10]. The capability to produce various types of antibiotics by *Bacillus spp*. has been reported by several genomic research. For example, the genomic sequence of the widely distributed *Bacillus* strains exposed that about 8% of the genome is devoted to producing antibiotics [11,12].Marine *Bacillus* isolates produce structurally diverse classes of secondary metabolites, such as lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, lipoamides, and isocoumarins. These structurally multipurpose compounds exhibit anextensive range of biological actions, such as antimicrobial, anticancer, antialgal, and anti-peronosporomycetal [13,14].In the present study, we aimed to isolate the antagonistic marine bacteria from soil samples collected from the seashore. Also aimed to study the antibacterial and anticancer activity of secondary metabolic extract of isolated antagonistic strain.

MATERIALS AND METHODS

Study area and sample collection

The east coast region of Pazhaiyar (11°21'06.7"N 79°50'13.1"E) in south Indian state of Tamil Nadu was selected as a sampling location for isolation of antagonistic bacteria. Approximately 10 to 30 g of sediment sample was collected in sterile airtight polyethene bags in the depth of 5-10 cm using sterile steel cooper. The soil samples were sieved through a 0.5 mm sieve to remove stones [15].Sieved soil samples were immediately transferred into the laboratory and stored under refrigerator condition for further use.

Isolation of marine bacteria

The dilution spread plate technique [16] was used to isolate the bacteria from collected soil samples. Soil sample weighing 1g was diluted in 10 ml of 50% seawater (1:1 v/v seawater (30 ppt): distilled water). Serial dilutions were obtained up to 10^{-5} and 0.1 ml of each dilution was spread on Nutrient agar medium was prepared with natural seawater. After incubation, plates were regularly examined to verify the growth of marine bacteria and the total number of bacterial colonies was counted by microbiological colony counter. The morphologically distinct colonies were selected, isolated and purified by standard microbiological plating and streaking methods. Accordingly, 5 morphologically distinct bacterial colonies were selected and purified by basic plating techniques. The purified bacterial isolates were subjected to microscopic analysis and deposited at the Microbiology Laboratory. Bacterial strains were stored in test tubes containing NA slants. The subcultures were made every month.

Collection of test bacterial strains

A total of five human pathogenic bacterial cultures *Escherichia coli* (MTCC- 1687), *Klebsiella pneumoniae* (MTCC-109), *Staphylococcus aureus* (MTCC- 6908), *Streptococcus pneumoniae* (MTCC - 5542), *Staphylococcus epidermidis* (MTCC- 2639) were obtained from the Microbial Type Culture Collection (MTCC), Chandigarh, India for the study.

Screening for antagonistic activity

The Cross streaking method was used to rapidly screen isolated microorganisms for antagonism [17,18]. Determinations of antimicrobial activities of 5 pure marine bacterial isolates were performed by the cross-streak method (CSM). Mueller-Hinton agar (MHA) plates were prepared and inoculated with test bacterial cultures was a streak in the full Petri dish and cross streak in the isolated marine bacteria after that incubated at 37°C for 24 hours. This was done to provide enough time for the active organism to produce the metabolites, which will diffuse into the agar medium [18].

Identification of potential antagonistic bacteria

The selected antagonistic bacterial genomic DNA was isolated using a total DNA extraction kit (OMEGA BioTek, Norcross, GA, USA) by following the guidelines of the kit provider. The isolated DNA was amplified at the 16S rRNA region using universal primers 27F (50- AGAGTTTGATCCTGGCTCAG-30) and 1492R (50-GGTTACCTTGTTACGACTT-30). The obtained nucleotide sequences of the strain were deposited to Gene Bank (Ass.No:MW250200). Using the NCBI database, the nucleotide sequences of strain was compared with known sequences and the phylogenic tree was created using a neighbour-joining algorithm (MEGA 6.0)

Extraction of secondary metabolites

The bacterial isolates which show the antagonistic activity against over the 5 bacterial pathogens were selected for further study. The inoculum of selected bacterial isolates was prepared by transferring bacterial culture into a 2000 mL conical flask containing 1000 mL of Nutrient Broth medium containing optimized nutrient levels. Inoculated flasks were incubated on a shaker at 200 rpm for 48 hours at 30^0C temperature range. After incubation, the Nutrient Broth medium was made cell free by centrifugation at 10,000 rpm for 20 minutes. Cold absolute ethanol was added to the supernatant in a ratio of 1:3 (v/v) and kept at 4°C for 24 hours for precipitation of secondary metabolites. The precipitates were recovered by centrifugation and purified by washing with Mille Q water and secondary metabolites pellets were dried at 60°C.

Assessment of Antibacterial activity

In Disc-diffusion assay (Kirby-Bauer Method), the test bacterial cultures were swabbed over the Muller Hinton agar. Then the discs loaded with different concentration of crude secondary metabolite extract (CSME) obtained from the antagonistic bacteria (100, 200, 300, 400 µg) and positive control (Standard antibiotic Erythromycin 20µg), and placed over the agar medium. All plates were incubated at 37°C for 24 hours and examined for a zone of inhibition. The diameter of the inhibition zones was measured and recorded in millimetres.

Determination of Minimal Inhibitory Concentration (MIC)

The MIC of CSME of PA4 was determined in bacterial metabolites showing antimicrobial activity by with minor modifications. Briefly, 100 µL Muller-Hinton broth (Himedia) and various concentrated bacterial metabolites were prepared and transferred to 90 well plates to obtain dilutions of the active extract from 43.7 to 700 mg/ml. Then, 10 µL of the test organisms were added to the new culture (final concentration of 1 x 10^6 CFU/ml). The plates were incubated for 24 h at 37 °C. The microbes were defined as the lowest concentration of the extract to control the visible growth of the tested organisms.

Antioxidant activity

DPPH free radical- scavenging activity

The antioxidant activity of CSME of PA4 was determined by DPPH free radical assay. The DPPH radical scavenging activity was measured according to the method described by [19]. 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 m M in methanol. DPPH reacts with an antioxidant compound the colour change will take place. The colour 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 [20].

$$Radicalscavengingactivity\,(AA\%) = \frac{Abscontrol - Abssample}{Abscontrol} \times 100$$

Cancer cell culture

The human oral squamous carcinoma KB cell cells were obtained from the National Centre For Cell Science (NCCS), Pune, India. Cells were cultured and maintained in DMEM and incubated at 37 °C in a 5% CO_2 and 95% air incubation (humidified condition). Before treatment to the cells, the CSME of PA4 completely dissolved in dimethyl sulfoxide (DMSO).

Cytotoxicity of CSME of PA4 on KB

The cytotoxicity of CSME of PA4 on KB cells was evaluated by employing MTT assay. The KB cells (1×10^5) were seeded in 96 wells plate and grown for 24 h at the humified incubator condition. After incubation, the cells were exposed to different concentrations of CSME (50, 60, 70, 80, 90, 100, 110, 120 and 130 µg/ml) for 24 h after replacing the old medium with the new medium. Afterwards, the 100 µL of MTT reagent (5 mg/ml in PBS) was subsequently added into each well, then the plate was kept in dark condition for 4 h. By adding the 100 µL of DMSO, the resulting for mazan was dissolved. Further, the absorption of dissolved formazan was determined at 595 nm wavelength using ELIZA plate reader (Tecan Multimode Reader, Austria). The concentrations of the test sample which showed 50% of cell death was calculated.

RESULTS

Isolation of marine bacterial strains

The collected sediment sample was serially diluted and inoculated on nutrient plates and incubated. After incubation, 25.24±1.62CFU/g x 10^5 colonies were counted on nutrient plates, from that 5 morphologically distinct bacterial colonies were observed and selected for the further study. The selected bacterial strains were named as PA1, PA2, PA3, PA4, PA5.

Antagonistic activity of isolated bacterial strains

The isolated bacterial strains were tested for its antagonistic nature against the test bacterial pathogens. Among the tested bacterial strains, the isolate PA4 showed significant antagonistic activity against the 4 tested bacterial pathogens. Other bacterial strains PA1 and PA5 exhibited significant growth inhibition activity against 2 bacterial pathogens. The isolates PA2 and PA3 showed growth inhibitory activity against one bacterial pathogen. The results indicate that the isolate PA4 exhibited significate antagonistic activity over the other bacterial isolates

Identification of antagonistic bacteria

The obtained nucleotide sequences of the isolated antagonistic bacterial strain were compared for the similarity with known sequenced using the BLAST search tool. The obtained sequences showed over 98% of similarity with the organism *Bacillus cereus*. Further, the strain was identified as genus *Bacillus* and species *cereus* (Fig. 2).

Antibacterial activity and MIC

The collected bacterial crude secondary metabolites were examined for its antibacterial activity on MHA plates. The crude secondary metabolites of isolate PA4 displayed greater antibacterial activity against all the selected pathogens and the zone of inhibition was varied significantly according to the concentration used. The maximum growth inhibitory activity of crude secondary metabolites of strain PA4 was observed against *Staphylococcus aureus* followed by *Streptococcus pneumoniae, Staphylococcus epidermidis* and *Escherichia coli*. However, the crude secondary metabolites of strain PA4 exhibited lesser inhibitory activity against *Klebsiella pneumoniae* while compared with other tested bacterial pathogens. The standard antibiotic Ciprofloxacin recorded zone of inhibition ranged from 21 to 26 mm. The MIC values ranged between 87.5 to 350 µg/ml and the lowest MIC value of87.5±2.5µg/ml was recorded against *Staphylococcus aureus, Escherichia coli* and *Streptococcus pneumoniae*. The inhibition of *Klebsiella pneumoniae* was started at the concentration of 350.0±6.4µg/ml

Antioxidant activity of CSME

The CSME of PA4 showed a concentration established anti-DPPH free radical scavenging activity. The crude secondary metabolites of strain PA4 possess hydrogen donating ability and the maximum inhibition of 29.76% was observed at 40 µg/ml concentration, which is considerably lesser than the inhibitory activity of 40 µg/ml of standard

Cytotoxicity of CSME on KB cells

The cytotoxicity of CSME on KB cells was studied by establishing the MTT assay. The results show that the concentration-dependent cytotoxicity on KB cells was demonstrated by CSME of PA4. The 50% inhibition of cell growth (IC50) was calculated as 87.32 µg/ ml

DISCUSSION

The search for new antibiotics from marine harboured microorganisms resulted in the isolation of more or less 10,000 metabolites [21] many of which endowed of pharmacological properties. A broad type of biological activities has been detected, such as an antibiotic, antifungal, cytotoxic, neurotoxic, activities. In this present study, the marine antagonistic bacterial strain was isolated from soil samples collected from the Pazhaiyar coastal region. The isolated strains showed significant antagonistic activity against tested bacterial pathogens. The potential antagonistic bacteria were identified as *Bacillus cereus*. Marine *Bacillus* species represent a potential source of structurally various classes of secondary metabolites including lipopeptides, polypeptides, macrolactones, fatty acids, polyketides, lipoamides, isocoumarins, and carotenoids. These structurally diverse natural products of marine isolates are derived from complex biosynthetic metabolic pathways. Some of these bioactive compounds have high potentials for the development of effective pharmaceutical and agrochemical products. Due to the genetic capability to adapt extreme environments, *Bacillus* strains isolated from unique niches (e.g., hydrothermal vent, deep sea, pH > 9.0 and salt lakes) may produce useful bioactive compounds [22].The crude metabolic extract, at 200 µg/disc, of *Bacillus cereus* strain exhibited promising antibacterial activity against the Gram-positive and gram- negative test strains. Gram-negative *Klebsiella pneumoniae* were resistant to the tested crude extract. This resistance may be attributed to the low permeability of the Gram-negative bacteria outer membrane and the lipopolysaccharide barrier for the hydrophobic compounds [23,24].Several studies reported that *Bacillus* strains were able to produce a large number of antimicrobial peptides with different chemical structures, such as bacteriocins 0and surfactin [25].MTT cytotoxicity tests were conducted to identify the anticancer property or

cytotoxicity of the extract against KB cells and A375 cells. It reflects strong cytotoxicity at an IC50 value of 87.32µg/mL for KB cells. Metabolites with lower IC50 value prove to be efficient in cytotoxicity even at low dose [26]. Active principles of cellular toxicity reside on the bioactive metabolites of the extracts. The present findings showed that the secondary metabolites separated form bacterial strain *Bacillus cereus* exhibited significant anticancer activity against tested human cancer cell lines.

CONCLUSIONS

Isolated antagonistic bacterial strain *Bacillus cereus* PA4 showed effective antagonistic activity against selected bacterial pathogens. The crude secondary metabolic extract of *Bacillus cereus*-PA4 showed effective antibacterial activity against gram-negative and gram-positive bacterial strains on MH agar plates. Moreover, a crude secondary metabolic extract of *Bacillus cereus*PA4 exhibited significant antioxidant activity anticancer activity against oral squamous carcinoma KB cell. The obtained results suggest that the crude secondary metabolic extract of antagonistic bacterial strain *Bacillus cereus*-PA4 possess the effective antibacterial, antioxidant and anticancer activity in *in-vitro* condition. Therefore, it could be used for infectious disease treatment and cancer treatment.

ACKNOWLEDGEMENT

The authors would like to thank Yaazh Xenomic laboratories, Coimbatore for excellent technical and bioinformatics assistance.

REFERENCES

1. Fenical W, Chemical studies of marine bacteria: developing a new resource. Chem Rev,1993, 93, 1673- 1683.
2. Kobayashi J, Ishibashi M. Bioactive metabolites of symbiotic marine micro-organisms. Chem Rev1993, 93: 1753-1769.
3. ProkschP, Edrada RA, Ebel R. Drugs from the seascurrent statusand microbiological and microbiologicalimplications.implications. App. Microbiol.Biotechnol 2002; 59: 125-134
4. BultelP, Berge V, Debitus C, Nicolas JL, GuyotM.Metabolites from the sponge-associatedbacterium *Pseudomonas*species. Mar. Biotechnol 1999; 1384-390.
5. Rinehart KL. Antitumor compounds from tunicates, Med. Res. Rev 2000; 20: 1-27.
6. Rampelotto, P.H. Resistance of microorganisms to extreme environmental conditions and its contribution to astrobiology. Sustainability 2010, 2, 1602–1623.
7. Sayem SMA, Manzo, E Ciavatta L, Tramice A, Cordone A, Zanfardino A, de Felice, M, Varcamonti M. Anti-biofilm activity of an exopolysaccharide from a sponge-associated strain of Bacillus licheniformis. Microb. Cell Fact. 2011, 10, 74.
8. Paul VJ, Arthur KE, Williams, RR, Ross C, Sharp K. Chemical defenses from compounds to communities. Biol. Bull. 2007, 213, 226–251.
9. Jensen PR,Fenical W. Strategies for the discovery of secondary metabolites from marine bacteria: Ecological perspectives. Annu. Rev. Microbiol. 1994, 48, 559–584.
10. Feling RH, Buchanan GO, Mincer TJ, Kauffman CA, Jensen PR, Fenical W,Salinosporamide A. A highly cytotoxic proteasome inhibitor from a novel microbial source, a marine bacterium of the new genus salinospora. Angew. Chem. Int. Ed. Engl. 2003, 20, 355–357.
11. Chen XH, Koumoutsi A, Scholz R, Eisenreich, A, Schneider K, Heinemeyer I, Morgenstern B, Voss, B, Hess WR, Reva O. Comparative analysis of the complete genome sequence of the plant growth—Promoting bacterium Bacillus amyloliquefaciens FZB42. Nat. Biotechnol. 2007, 25, 1007–1014.

12. Kunst F, Ogasawara N, Moszer I, Albertini, AM, Alloni G, Azevedo V, Bertero MG,Bessières P, Bolotin A, Borchert S. The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis.* Nature 1997, 390, 249–256.
13. Hamdache A, Lamarti A, Aleu J, Collado IG. Non-peptide metabolites from the genus *Bacillus*. J. Nat. Prod. 2011, 74, 893–899.
14. Baruzzi F, Quintieri L, Morea M, Caputo L. Antimicrobial Compounds Produced by Bacillus spp. and Applications in Food. In Science against Microbial Pathogens: Communicating Current Research and Technological Advances; Vilas, A.M., Ed.; Formatex: Badajoz, Spain, 2011; pp. 1102–1111.
15. Leslie JF, Summerell BA. The Fusarium Laboratory Manual. *Blackwell Publishing, Hoboken*, 2006, 1-2.
16. Warcup JH. The Soil-Plate Method for Isolation of Fungi from Soil. *Nature*, 1950, 166, 117-118.
17. Lertcanawanichakul M, Sawangnop S. A comparison of two methods used for measuring the antagonistic activity of Bacillus species WalailakJ. *Sci. Tech*., 2008, 5: 161-171.
18. Waksman selman A, Lechevalier, Hubert A.The Actinomycetes. Vol. III. Antibiotics of Actinomycetes. 1962, 8, 430.
19. Brand-Williams W, Cuvelier ME, Berset C. Use of free radical method to evaluate antioxidant activity. LebensmWiss Technology, 1995; 28:25-30.
20. Mensor LI, Menezes FS, Leitao GG, Reis AS, Dos Santos T, Coube CS, Leita SG. Screening of Brazillian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytotherapy Research 2001; 15:127-130.
21. Fusetani N. Introduction. In: Fusetani N (Ed.), Drugs from the Sea, Basel: Karger, p. 2000, 1-5.
22. Li JWH, Vederas JC. Drug discovery and natural products: End of an era or an endless frontier? Science 2009, 325, 161–165.
23. Delcour AH. Outer membrane permeability and antibiotic resistance. BiochimBiophys Acta., 2009, 1794: 808–816.
24. Wiener MC, Horanyi PS. How hydrophobic molecules traverse the outer membranes of Gram-negative bacteria. Proc Natl Acad Sci USA., 2011, 108: 10929-10930.
25. Benitez LB, Velho RV, da Motta A, Segalin J and BrandelliA..Antimicrobialfactor from Bacillus amyloliquefaciensinhibitsPaenibacillus larvae, the causative agent of American foulbrood. Arch Microbiol., 2012, 194: 177-185.
26. Maeda M, Mizuki E, Hara M."Isolation of *Bacillus thuringiensis* from intertidal brackish sediments inmangroves," Microbiological Research, 2001, 156(2),195–198.

Table-1. Antagonistic activity of bacterial strains isolated from collected soil samples

Bacterial isolates	Test bacterial pathogens				
	Escherichia coli	*Klebsiella pneumoniae*	*Staphylococcus aureus*	*Streptococcus pneumoniae*	*Staphylococcus epidermidis*
PA1	-	+	-	+	-
PA2	-		+	-	-
PA3	-	-	+	-	-
PA4	+	-	+	+	+
PA5	-	+	-	+	-

Note: +; positive, -; negative

Table-2 Antibacterial and MIC activity of crude secondary metabolites of *Bacillus cereus* PA4 strain against bacterial pathogens.

Test bacterial pathogens	Zone of inhibition (mm)					
	100 µg	200 µg	300 µg	400 µg	Erythromycin (20µg)	MIC
Escherichia coli	9.34±0.65	12.38±0.76	14.39±0.72	15.41±0.26	23.34±0.43	87.5±3.3
Klebsiella pneumonia	-	-	-	11.21±0.32	24.23±0.62	350.0±6.4
Staphylococcus aureus	10.43±0.42	14.23±0.24	16.31±0.24	18.54±0.42	23.75±0.24	87.5±2.5
Streptococcus pneumonia	11.32±0.54	13.15±0.65	14.82±0.64	16.74±0.17	24.32±0.64	87.5±1.4
Staphylococcus epidermidis	-	9.65±0.62	12.32±0.16	16.43±0.54	21.57±0.18	175.0±1.7

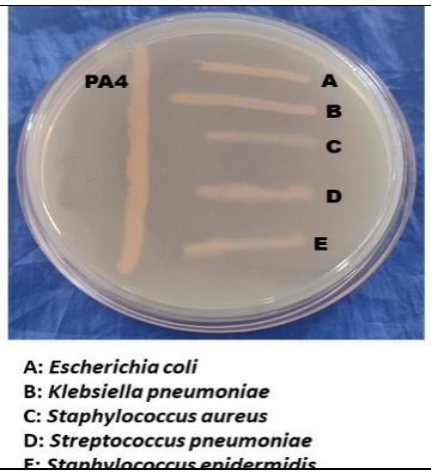


Fig. 1. Antagonistic activity of PA4 bacterial strain against teste bacterial pathogens.

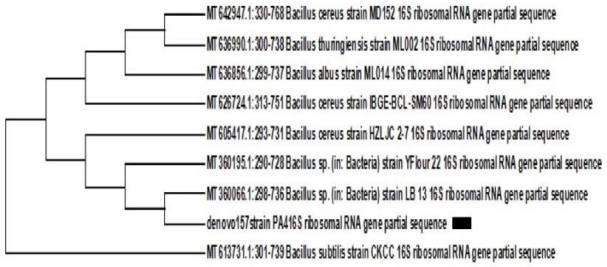


Fig. 2. Phylogenetic classifications of isolated antagonistic bacterial stain. The tree was constructed by the neighbour-joining method with known bacterial sequences using MEGA 6.0.

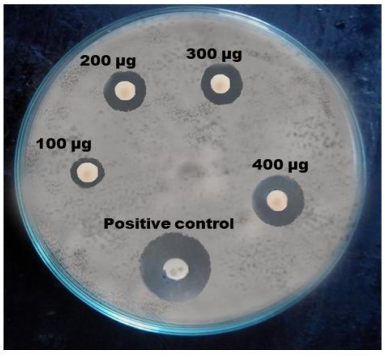


Fig. 3. Antibacterial activity of crude secondary metabolites of *Bacillus cereus* PA4 against *Staphylococcus aureus* bacterial pathogen on MH agar plate.

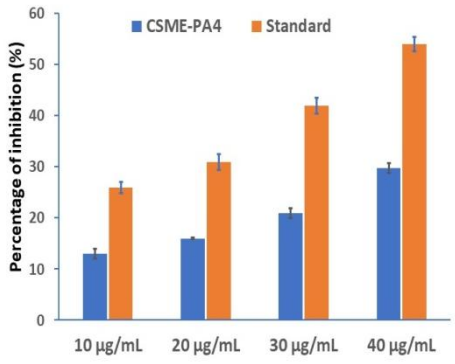


Fig. 4. DPPH free radical scavenging activity of crude secondary metabolites of *Bacillus cereus* PA4.

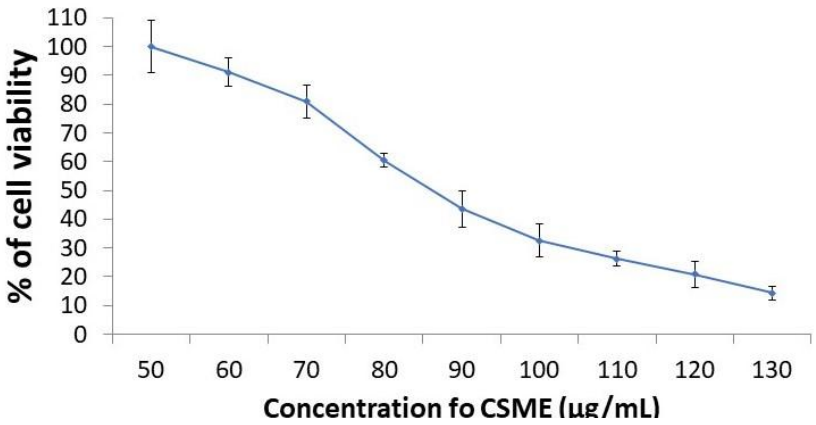


Fig. 5. Cytotoxic activity of crude secondary metabolites of *Bacillus cereus* PA4 against oral squamous carcinoma KB cell.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Analgesic, Antipyretic and Anti-inflammatory Efficacy of NSAIDs- A Review

Vasanth Murugesan[1]* and Raghu Srinivasan[2]

[1]Research Scholar, Vellore Institute of Technology, Vellore, Tamil Nadu, India.
[2]Assistant Professor, Vinayaka Mission's College of Pharmacy, Salem, Tamil Nadu, India.

Received: 17 Oct 2020 Revised: 19 Nov 2020 Accepted: 21 Dec 2020

***Address for Correspondence**
Vasanth Murugesan
Research Scholar,
Vellore Institute of Technology,
Vellore, Tamil Nadu, India.
Email: vasanthko1996@gmail.com

ABSTRACT

Non-steroidal anti-inflammatory drugs (NSAIDs) are the most commonly prescribed medications for Mild to moderate pain, fever and inflammatory disorders. NSAIDs are of 3 categories viz. Nonselective, selective and preferential COX inhibitors. As selective COX inhibitors selectively inhibit the enzyme COX-2 which is responsible for the production of prostaglandins during an inflammation, they are considered to be safe compared to nonselective COX inhibitors. Because nonselective COX inhibitors block both the enzymes COX -1 and COX-2, they can able to produce a wide range of adverse effects. Most of these drugs in this category are available over the counter as they are non-narcotic. But frequent and unwanted use of these drugs often causes certain adverse effects. Inappropriate use and self-selection of these drugs may also lead to mild to moderate adverse effects such as GI side effects, CNS effects, cardiovascular disturbances, nephrotoxicity, hepatotoxicity. In order to reduce this kind of adverse effects, we need to consider the efficacy of individual drugs and also their efficiency in the treatment and management of particular diseases. Comprehensive analysis and careful consideration are required to select an appropriate drug for the treatment of a particular condition or disorder. In this review, we analyzed the efficacy and effectiveness of individual drugs for the relief of mild to moderate pain, to reduce fever, and swelling in inflammatory disorders such as rheumatoid arthritis, osteoarthritis, gout, and musculoskeletal disorders.

Keywords: NSAIDs review, Therapeutic use of NSAIDs, Efficacy of NSAIDs, Non-steroidal anti-inflammatory drugs, Inflammation, Arthritis, Pain, Fever, NSAIDs.

INTRODUCTION

NSAIDs are the most commonly prescribed medication for the relief of pain, fever, and inflammation (1, 2). Typical properties of NSAIDs include analgesia, antipyretic activity, anti-inflammatory activity, antiplatelet activity, and occasionally uricosuric activity (3). Over the centuries, NSAIDs are commonly used to get relief from pain all over the world. Pain results from activation of nociceptive afferents which may be caused by an external stimulus or pathogen. To a lesser extent, it also occurs due to any abnormal or unwanted activity generated in the nervous system (4). Pain is of few types. Visceral pain can be a result of damage or injury to the internal organs. It may cause pain in certain regions of the body such as the chest, abdomen, pelvic. It can be diagnosed as pressure, discomfort, cramping, squeezing, aching in the affected region. In this pain, it is very hard to locate the affected area. Somatic pain may result from pain receptor stimulation rather than internal organ injury (5). It includes muscles, skin, bones, and joints which is much easier to pinpoint the area of pain (6). Pain that occurs due to abnormal activity in the nervous system is called neuropathic pain. It may result from damage to the nervous system which causes misfiring of neurons involved in the affected area (7). It leads to pain that seems almost impossible to pinpoint (5). Pain can be acute or chronic depends on the type and severity of the injury. NSAIDs are particularly useful for acute somatic pain, and not helpful to treat chronic, visceral, and neuropathic pain. There are few medications available to treat chronic, visceral pain. Opioid analgesics, especially morphine is useful to treat visceral pain. Inflammation is a tissue reaction to injury which is a host defense response to external stimuli or pathogen. It is characterized by redness, swelling, pain, heat and also there is a loss of functioning around the injured area. It all results from local immune response, vascular, and inflammatory cell responses (8). Though it is a host-defense mechanism (which happens to defend pathogens), when it is over expressed, it feels devastating to the host. People often consider it as an illness and want to get relief from inflammation. NSAIDs are the most commonly used medication to reduce swelling or inflammation.

Fever is an abnormal increase in body temperature above the normal level. It may be caused by hypothalamic set point, infectious agents, microbial products, cytokines, and also inflammatory cells. Pyrogen is a protein that increases the synthesis of prostaglandin (PG) in the hypothalamus, thereby increasing the temperature set-point. Cytokines such as Interleukin (IL)-1, IL-6, and Tumor Necrosis Factor (TNF)-α cause the synthesis of prostaglandin (PG)-E2 which binds to prostaglandin receptors in the hypothalamus and raise the thermostatic set point to febrile level. Fever is beneficial in a way that it inhibits the growth of some bacteria and viruses and also it enhances the immune response. Despite the fact that fever is counteracting the invaded pathogen or infectious agent, it is also considered an illness. People often tend to take medications to reduce a fever without considering a physician. Several NSAIDs are available over the counter for treating pain, inflammation, and fever. Injudicial use of these drugs may cause some adverse effects which may be mild to severe even sometimes requiring hospitalization. Thus, people more often tend to develop another illness by treating an illness that is literally a host defense. Therefore, a comprehensive approach and careful consideration are required regarding the selection and use of an individual drug for treating pain, fever, and inflammatory disorders with less or minimal adverse effects and improves the quality of life.

Classification

Classification is based on the enzymes they target or inhibit. Based on this, NSAIDs are classified into 3 categories namely nonselective COX, selective COX-2, and preferential COX-2 inhibitors. Nonselective COX inhibitors block or inhibit both the enzymes COX-1 and COX-2, thereby increasing the risk of getting adverse effects. Selective COX-2 inhibitors selectively inhibit the enzyme COX-2, while Preferential COS-2 inhibitors prefer to block the enzyme COX-2 over COX-1. Due to this selectivity or preference to COX-2 over COX-1, both the class of drugs reduce the risk of getting adverse effects produced by inhibition of COX-1 such as ulcer formation, and platelet inhibition. The classification of NSAIDs is given below.

Non-selective COX inhibitors

i) Salicylic acid derivatives
   Eg.Eg. Aspirin, Sodium salicylate
ii) Para-amino phenol derivatives
   Eg. Acetaminophen
iii) Aryl acetic acid derivatives
   Eg. Diclofenac, Aceclofenac
iv) Indole acetic acid derivatives
   Eg. Indomethacin, Sulindac, Etodolac
v) Pyrazolone derivatives
   Eg. Phenylbutazone, Oxyphenbutazone
vi) Propionic acid derivatives
   Eg. Ibuprofen, Keotprofen, Flurbiprofen, Naproxen, Carprofen
vii) Anthranilic acid derivatives
   Eg. Mefenamic acid, Flufenamic acid, Meclofenamic acid
viii) Enolic acid derivatives
   Eg. Piroxicam, Meloxicam, Tenoxicam

Selective COX-2 inhibitors

Eg. Celecoxib, Rofecoxib, Valdecoxib, Etoricoxib

Preferential COX-2 inhibitors

Eg.Nimesulide, Meloxicam

MECHANISM OF ACTION OF NSAIDS

During a cell injury or infection, arachidonic acid is liberated from phospholipids present in the cell membrane by an enzyme phospholipase A2. Liberated arachidonic acid is converted to prostaglandin (PG). This reaction is catalyzed by the enzyme cyclooxygenase (COX). COX is of two types COX-1 and COX-2. Both the enzymes produce prostaglandins hence they are also known as prostaglandin synthase. However, COX-1 produces PGs that are responsible for the protection of the stomach, intestinal lining, and also it activates platelets while COX-2 produces PG responsible for the conduction of pain and mediation of inflammation. PG sensitizes the neurons to receive signals for pain and inflammation, thereby mediating it(9). PG also plays a role in maintaining the temperature set point in the hypothalamus [90]. NSAIDs act by inhibiting COX, the enzyme which is responsible for PG synthesis from arachidonic acid. Thus, NSAIDs inhibit the mediation of pain and inflammation.

THERAPEUTIC EFFICACY OF NSAIDs

Nonselective COX inhibitors

Salicylic Acid Derivatives: Aspirin (Acetylsalicylic acid) is the prototype of salicylates and it is a salt of salicylic acid. In 400 B.C, The great Greek physician Hippocrates writes that willow bark and leaves relieve pain and fevers. Even some people still use willow bark as a natural remedy for minor pain, inflammation, and fever [12]. From then to till now, aspirin is one of the most prescribed medications to treat pain, inflammation, and fever [10]. From an evidence-based review, aspirin was found to be the best medication to get relief from pain (10). It is one of the important medications to treat mild fever, pain, and also inflammation to a lesser extent. But the use of this drug is limited as it may cause a gastric ulcer, dyspepsia, headache, dizziness, bleeding (11). One study suggested that aspirin and paracetamol showed almost the same efficacy (dose-related) and safety profile (12). It is also reported

that it is also useful to patients who have suffered from cardiovascular diseases such as heart attack, myocardial infarction, or stroke for the prevention of blood clot formation which might lead to further complications due to its antithrombotic action (13). It is the only NSAID that inhibits the enzymes COX-1 and COX-2 irreversibly while all the other NSAIDs inhibit the same reversibly. Hence, the analgesic, antipyretic, anti-inflammatory efficacy of aspirin is comparatively higher than the other NSAIDs. But some drugs may offer more analgesic/antipyretic/anti-inflammatory property but not all.

Para-amino Phenol Derivatives: Acetaminophen (Paracetamol) is a common and mostly used NSAID all over the world and it is also available since the 19th century. It is also available over the counter (OTC) so often it leads to drug interactions and some adverse effects. It is a potent analgesic/antipyretic drug but it has very weak anti-inflammatory properties (14). The analgesic, antipyretic action of acetaminophen is similar to that of aspirin but the anti-inflammatory activity is somewhat less compared to aspirin (15). Hence acetaminophen is preferred when there is fever and or pain. It is not preferable to treat arthritis since it has very weak anti-inflammatory activity. The major adverse effect that may affect health is liver damage induced by acetaminophen. But it is not reported in normal doses of acetaminophen. It occurs only when a higher dose of acetaminophen is ingested (14).

Aryl Acetic Acid Derivatives: Diclofenac is also a commonly used NSAID to treat a variety of acute and chronic pain and inflammatory conditions like rheumatoid arthritis. It also possesses the antipyretic property. These actions are not only due to COX inhibition and also by novel mechanisms of action (16). Diclofenac is proved to be the most useful NSAID for the treatment of rheumatic disorders. Therefore, Rheumatoid arthritis with moderate to severe pain should be treated with diclofenac instead of other NSAIDs. Diclofenac is a nonselective inhibitor of COX and thereby resulting in some of the gastrointestinal adverse effects, so they should be avoided in patients predisposed to gastrointestinal disorders. Aceclofenac is predominantly a COX-2 inhibitor and proved to be a better drug of choice in patients with gastrointestinal disorders (17). In such conditions, aceclofenac is preferred over diclofenac for its improved gastrointestinal tolerability. They both possess little or no effect on platelet function. So, they are not useful for cardiovascular disorders, unlike aspirin.

Indole Acetic Acid Derivatives: Etodolac was proved to be as significant in pain reduction as diclofenac and other NSAIDs and it is well tolerated with mild adverse effects (18). Indomethacin is a well known anti-inflammatory agent in certain conditions (19). It also possesses considerable antipyretic action. It has been clinically proven to be useful to treat pain in patients with ankylosing spondylitis. It also provided relief of pain, and reduction in swelling in patients with osteoarthritis and rheumatoid arthritis (20). Sulindac is also found to be useful in pain management, tenderness, sprains and strains, and swelling or inflammatory conditions (21). It is often used to treat rheumatoid arthritis and some other musculoskeletal disorders (22).

Pyrazolone derivatives: Phenylbutazone is a pyrazolone derivative that is most commonly employed in veterinary use. It is used in human medicine for the treatment of arthritis and inflammatory musculoskeletal disorders (23)[32]. It has the potential to treat chronic pain and lameness in equines and horses (24, 25)[29,30]. It is also found to be useful in ankylosing spondylitis(26)[31]. But it is discontinued for human use because it has harmful side effects which are even produced death in several individuals. Oxyphenbutazone, an analog of phenylbutazone and one of its active metabolites [34], has the same therapeutic potential compared to phenylbutazone but it was evident that oxyphenbutazone has much more potential to produce side effects, and also the death rate is even higher than phenylbutazone. So, both these drugs have been discontinued for human use [33].

Propionic Acid Derivatives: Ibuprofen is the first member of propionic acid derivatives which is introduced in 1969. It is also a nonselective COX inhibitor and considered to be safer than other NSAIDs (27). It has prominent analgesic and antipyretic properties but weaker anti-inflammatory activity (28). It is found to be very useful in restoring muscle function, rheumatoid arthritis, and other musculoskeletal disorders (29). It is one of the most widely

used NSAID to treat dental pain. It is also found to be useful in the treatment of orthostatic hypotension. Ketoprofen is also a propionic acid derivative which is very effective in treating pain, tenderness, swelling, and stiffness caused by arthritis (30). It is proven that it has greater efficacy in relieving pain in patients with rheumatic diseases than ibuprofen (31). Flurbiprofen is used in ophthalmic to reduce pain and swelling [40]. Carprofen is used in dogs to treat pain, fever, and inflammatory disorders [41].Naproxen is also used to relieve pain in joints, teeth, and muscles (32). It is also effective to treat inflammatory disorders such as rheumatoid arthritis, osteoarthritis, and gout [42]. It is also reported to be effective to relieve pain from back pain, sprains, and strains. It also possesses considerable antipyretic properties and it is well-tolerated (33). Reports are suggesting that naproxen is effective, safer than aspirin in the treatment of rheumatic fever (34).

Anthranilic Acid Derivatives: Mefenamic acid is used in the treatment of dysmenorrhea, irregular uterine bleeding, and premenstrual syndrome (35-37). It is also useful in the treatment of fever, inflammation, and pain, especially in menstrual pain. It is used to treat moderate to severe pain and to reduce blood loss from menstrual periods [50]. Flufenamic acid and meclofenamic acid are not widely prescribed because of their higher risk of GI adverse effects and low therapeutic index [49].

Enolic Acid Derivatives: Piroxicam is an enolic acid derivative aka oxicams which is used to treat pain and inflammation. It is also used to reduce joint stiffness in patients with arthritis (38). It is especially useful in treating postoperative pain, dental pain, and also low back pain (39, 40). Reports suggesting that it is an efficient alternative to aspirin in the treatment of rheumatoid arthritis and osteoarthritis (41). Meloxicam is also a member of this category which is also useful in the management of pain, fever, and inflammation (4). Reports are suggesting that it is significant in reducing postoperative pain like piroxicam (42, 43). It can also be used as an analgesic in orthodontic pain with fewer side effects (44). It is also recently approved to be used in the treatment of rheumatoid arthritis and osteoarthritis (45).

Selective COX-2 inhibitors

Celecoxib is a selective COX-2 inhibitor used in the treatment of rheumatoid arthritis. It blocks the enzyme COX-2 selectively, thereby reducing the risk of a few side effects associated with other NSAIDs (46). Several reports indicating that celecoxib is a high potential but expensive drug to treat rheumatoid arthritis (47, 48). It is also useful in postoperative pain, back pain (49, 50). It is also useful in the treatment of gouty arthritis (51). Rofecoxib is also a selective COX-2 inhibitor used in the treatment of postoperative pain (52, 53). It is reported that it has antipyretic properties (54). It is also useful in inflammatory disorders (55). Valdecoxib is used in the treatment of rheumatoid arthritis and osteoarthritis with less gastrointestinal toxicity (56). It also proved to be an efficient drug in the treatment of dysmenorrheal (57). Its analgesic power is much higher compared to rofecoxib (58). Therefore, it is useful to treat moderate to severe pain, postoperative pain, and also menstrual pain. Etoricoxib is useful in the management of low back pain, and also moderate to severe pain (59, 60). It is also used to treat rheumatoid arthritis (61). A report suggests that it is an efficacious alternative to treat arthritis and gout with significant gastrointestinal tolerability and the advantage of once-daily administration (62).

Preferential COX-2 inhibitors

Nimesulide is a preferential COX-2 inhibitor and it is useful in several inflammatory and pain conditions. It is also useful in dysmenorrhea, and postoperative pain (63). It was also reported that it has significant antipyretic property (64). It is also used in the management of gout, and symptomatic treatment of osteoarthritis (65, 66). Meloxicam, an enolic acid derivative that is used in the management of pain, fever, and inflammation. Reports also suggesting that it is also helpful in relieving postoperative pain. Meloxicam is also used to treat tenderness, swelling, stiffness caused by rheumatoid arthritis and osteoarthritis (4).It can also be used as an analgesic in orthodontic pain.

CONCLUSION

NSAIDs are the most commonly used medication for the management and treatment of pain, fever, and many inflammatory conditions and/or disorders all over the world. There are few classes of NSAIDs are available viz nonselective COX, selective COX-2, and preferential COX-2 inhibitors. Nonselective COX inhibitors often produce a wide range of adverse effects due to their inability to selectively block the enzyme COX-2 that is responsible for pain, fever, and inflammation. They are also available over the counter, so there is an increased risk of fallacious selection of drugs which may result in adverse effects. Since, COX-1 produces PGs that are responsible for the protection of the stomach, intestinal lining, and also activation of platelets, it produces adverse effects such as irritation or pain in the stomach, heartburn, ulcer, gas, diarrhea, constipation, and bleeding or bruising. Therefore, we need to consider the efficacy of individual drugs in order to select a suitable medication for the treatment of a particular condition or disorder. Understanding the therapeutic efficacy of a particular drug in a specific condition or disorder is essential to get the intended beneficiary effect with less adverse effect. In this review, we analyzed and compared the therapeutic efficacy of the individual drugs and gave some comprehensive information regarding their use in the management and treatment of pain, fever, and other inflammatory disorders.

REFERENCES

1. Wongrakpanich S, Wongrakpanich A, Melhado K, Rangaswami J. A Comprehensive Review of Non-Steroidal Anti-Inflammatory Drug Use in The Elderly. Aging Dis. 2018;9(1):143-50.
2. Hyllested M, Jones S, Pedersen JL, Kehlet H. Comparative effect of paracetamol, NSAIDs or their combination in postoperative pain management: a qualitative review. BJA: British Journal of Anaesthesia. 2002;88(2):199-214.
3. Wober W. Comparative efficacy and safety of nimesulide and diclofenac in patients with acute shoulder, and a meta-analysis of controlled studies with nimesulide. Rheumatology. 1999;38(suppl_1):33-8.
4. Fitzpatrick CE, Chapinal N, Petersson-Wolfe CS, DeVries TJ, Kelton DF, Duffield TF, et al. The effect of meloxicam on pain sensitivity, rumination time, and clinical signs in dairy cows with endotoxin-induced clinical mastitis. Journal of Dairy Science. 2013;96(5):2847-56.
5. Backonja M-M. Defining Neuropathic Pain. Anesthesia & Analgesia. 2003;97(3).
6. Miles MP, Clarkson PM. Exercise-induced muscle pain, soreness, and cramps. J Sports Med Phys Fitness. 1994;34(3):203-16.
7. Treede RD, Jensen TS, Campbell JN, Cruccu G, Dostrovsky JO, Griffin JW, et al. Neuropathic pain. Neurology. 2008;70(18):1630.
8. Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, et al. Inflammatory responses and inflammation-associated diseases in organs. Oncotarget. 2017;9(6):7204-18.
9. Stucky CL, Gold MS, Zhang X. Mechanisms of pain. Proceedings of the National Academy of Sciences. 2001;98(21):11845.
10. Ong CKS, Lirk P, Tan CH, Seymour RA. An evidence-based update on nonsteroidal anti-inflammatory drugs. Clin Med Res. 2007;5(1):19-34.
11. Lanas A, McCarthy D, Voelker M, Brueckner A, Senn S, Baron JA. Short-term acetylsalicylic acid (aspirin) use for pain, fever, or colds - gastrointestinal adverse effects: a meta-analysis of randomized clinical trials. Drugs R D. 2011;11(3):277-88.
12. Bachert C, Chuchalin AG, Eisebitt R, Netayzhenko VZ, Voelker M. Aspirin compared with acetaminophen in the treatment of fever and other symptoms of upper respiratory tract infection in adults: a multicenter, randomized, double-blind, double-dummy, placebo-controlled, parallel-group, single-dose, 6-hour dose-ranging study. Clin Ther. 2005;27(7):993-1003.
13. Ittaman SV, VanWormer JJ, Rezkalla SH. The role of aspirin in the prevention of cardiovascular disease. Clin Med Res. 2014;12(3-4):147-54.

14. Botting RM. Mechanism of Action of Acetaminophen: Is There a Cyclooxygenase 3? Clinical Infectious Diseases. 2000;31(Supplement_5):S202-S10.
15. Esh CJ, Mauger AR, Palfreeman RA, Al-Janubi H, Taylor L. Acetaminophen (Paracetamol): Use beyond Pain Management and Dose Variability. Front Physiol. 2017;8:1092-.
16. Gan TJ. Diclofenac: an update on its mechanism of action and safety profile. Current Medical Research and Opinion. 2010;26(7):1715-31.
17. Chunduri NS, Kollu T, Goteki VR, Mallela KK, Madasu K. Efficacy of aceclofenac and diclofenac sodium for relief of postoperative pain after third molar surgery: A randomised open label comparative study. J Pharmacol Pharmacother. 2013;4(2):144-5.
18. Vaghela JH, Shah JH, Patel JH, Purohit BM. Comparison of safety and analgesic efficacy of diclofenac sodium with etodolac after surgical extraction of third molars: a randomized, double-blind, double-dummy, parallel-group study. J Dent Anesth Pain Med. 2020;20(1):19-27.
19. Indomethacin as an Analgesic. New England Journal of Medicine. 1968;278(23):1291-2.
20. Hart FD, Boardman PL. INDOMETHACIN: A NEW NON-STEROID ANTI-INFLAMMATORY AGENT. Br Med J. 1963;2(5363):965-70.
21. Hayes T, Fyvie A, Janke P, Vandenburg M, Currie W. Sulindac versus ibuprofen in sprains and strains. British journal of sports medicine. 1984;18:30-3.
22. Moore RA, Derry S, McQuay HJ. Single dose oral sulindac for acute postoperative pain in adults. Cochrane Database Syst Rev. 2009;2009(4):CD007540-CD.
23. Worboys M, Toon E. Phenylbutazone (Bute, PBZ, EPZ): one drug across two species. Hist Philos Life Sci. 2018;40(2):27-.
24. OWENS JG, KAMERLING SG, STANTON SR, KEOWEN ML. Effects of ketoprofen and phenylbutazone on chronic hoof pain and lameness in the horse. Equine Veterinary Journal. 1995;27(4):296-300.
25. RAEKALLIO M, TAYLOR PM, BENNETT RC. Preliminary Investigations of Pain and Analgesia Assessment in Horses Administered Phenylbutazone or Placebo After Arthroscopic Surgery. Veterinary Surgery. 1997;26(2):150-5.
26. WORDSWORTH BP, EBRINGER RW, COGGINS E, SMITH S. A DOUBLE-BLIND CROSS-OVER TRIAL OF FENOPROFEN AND PHENYLBUTAZONE IN ANKYLOSING SPONDYLITIS. Rheumatology. 1980;19(4):260-3.
27. Moore N. Forty years of ibuprofen use. Int J Clin Pract Suppl. 2003(135):28-31.
28. Bushra R, Aslam N. An overview of clinical pharmacology of Ibuprofen. Oman Med J. 2010;25(3):155-1661.
29. Hasson SM, Daniels JC, Divine JG, Niebuhr BR, Richmond S, Stein PG, et al. Effect of ibuprofen use on muscle soreness, damage, and performance: a preliminary investigation. Med Sci Sports Exerc. 1993;25(1):9-17.
30. HOMMERIL J-L, BERNARD J-M, GOUIN F, PINAUD M. Ketoprofen for pain after hip and knee arthroplasty. BJA: British Journal of Anaesthesia. 1994;72(4):383-7.
31. Sarzi-Puttini P, Atzeni F, Lanata L, Bagnasco M. Efficacy of ketoprofen vs. ibuprofen and diclofenac: A systematic review of the literature and meta-analysis. Clinical and experimental rheumatology. 2013;31.
32. Dionne RA, Berthold CW. Therapeutic Uses of Non-Steroidal Anti-Inflammatory Drugs in Dentistry. Critical Reviews in Oral Biology & Medicine. 2001;12(4):315-30.
33. Uziel Y, Hashkes PJ, Kassem E, Padeh S, Goldman R, Wolach B. The use of naproxen in the treatment of children with rheumatic fever. The Journal of Pediatrics. 2000;137(2):269-71.
34. Hashkes PJ, Tauber T, Somekh E, Brik R, Barash J, Mukamel M, et al. Naproxen as an alternative to aspirin for the treatment of arthritis of rheumatic fever: a randomized trial. The Journal of Pediatrics. 2003;143(3):399-401.
35. Budoff PW. Use of Mefenamic Acid in the Treatment of Primary Dysmenorrhea. JAMA. 1979;241(25):2713-6.
36. Bonnar J, Sheppard BL. Treatment of menorrhagia during menstruation: randomised controlled trial of ethamsylate, mefenamic acid, and tranexamic acid. BMJ. 1996;313(7057):579-82.
37. MIRA M, McNEIL D, FRASER IS, VIZZARD J, ABRAHAM S. Mefenamic Acid in the Treatment of Premenstrual Syndrome. Obstetrics & Gynecology. 1986;68(3):395-8.

38. Amlie E, Weber H, Holme I. Treatment of acute low-back pain with piroxicam: results of a double-blind placebo-controlled trial. Spine (Phila Pa 1976). 1987;12(5):473-6.
39. Serpell MG, Thomson MF. COMPARISON OF PIROXICAM WITH PLACEBO IN THE MANAGEMENT OF PAIN AFTER TOTAL HIP REPLACEMENT. British Journal of Anaesthesia. 1989;63(3):354-6.
40. Desjardins PJ. Analgesic efficacy of piroxicam in postoperative dental pain. The American Journal of Medicine. 1988;84(5, Supplement 1):35-41.
41. Brogden RN, Heel RC, Speight TM, Avery GS. Piroxicam. Drugs. 1984;28(4):292-323.
42. Thompson JP, Sharpe P, Kiani S, Owen-Smith O. Effect of meloxicam on postoperative pain after abdominal hysterectomy. British Journal of Anaesthesia. 2000;84(2):151-4.
43. Benito-de-la-Víbora J, Lascelles BDX, García-Fernández P, Freire M, Gómez de Segura IA. Efficacy of tolfenamic acid and meloxicam in the control of postoperative pain following ovariohysterectomy in the cat. Veterinary Anaesthesia and Analgesia. 2008;35(6):501-10.
44. Zarif Najafi H, Oshagh M, Salehi P, Babanouri N, Torkan S. Comparison of the effects of preemptive acetaminophen, ibuprofen, and meloxicam on pain after separator placement: a randomized clinical trial. Progress in Orthodontics. 2015;16(1):34.
45. Ahmed M, Khanna D, Furst DE. Meloxicam in rheumatoid arthritis. Expert Opinion on Drug Metabolism & Toxicology. 2005;1(4):739-51.
46. Emery P, Zeidler H, Kvien TK, Guslandi M, Naudin R, Stead H, et al. Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: randomised double-blind comparison. The Lancet. 1999;354(9196):2106-11.
47. Garner SE, Fidan D, Frankish RR, Judd M, Shea B, Towheed T, et al. Celecoxib for rheumatoid arthritis. Cochrane Database of Systematic Reviews. 2002(4).
48. Simon LS, Weaver AL, Graham DY, Kivitz AJ, Lipsky PE, Hubbard RC, et al. Anti-inflammatory and Upper Gastrointestinal Effects of Celecoxib in Rheumatoid ArthritisA Randomized Controlled Trial. JAMA. 1999;282(20):1921-8.
49. Hiller JG, Sampurno S, Millen R, Kuruvilla N, Ho KM, Ramsay R, et al. Impact of celecoxib on inflammation during cancer surgery: a randomized clinical trial. Can J Anaesth. 2017;64(5):497-505.
50. Fabricio ASC, Veiga FH, Cristofoletti R, Navarra P, Souza GEP. The effects of selective and nonselective cyclooxygenase inhibitors on endothelin-1-induced fever in rats. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology. 2005;288(3):R671-R7.
51. SCHUMACHER HR, BERGER MF, LI-YU J, PEREZ-RUIZ F, BURGOS-VARGAS R, LI C. Efficacy and Tolerability of Celecoxib in the Treatment of Acute Gouty Arthritis: A Randomized Controlled Trial. The Journal of Rheumatology. 2012;39(9):1859-66.
52. Chiu WK, Cheung LK. Efficacy of preoperative oral rofecoxib in pain control for third molar surgery. Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontology. 2005;99(6):e47-e53.
53. Karamanhoglu B, Arar C, Alagöl A, Çolak A, Gemlik I, Süt N. Preoperative oral celecoxib versus preoperative oral rofecoxib for pain relief after thyroid surgery. European Journal of Anaesthesiology | EJA. 2003;20(6).
54. Schwartz JI, Chan C-C, Mukhopadhyay S, McBride KJ, Jones TM, Adcock S, et al. Cyclooxygenase-2 inhibition by rofecoxib reverses naturally occurring fever in humans. Clinical Pharmacology & Therapeutics. 1999;65(6):653-60.
55. Hudson M, Richard H, Pilote L. Differences in outcomes of patients with congestive heart failure prescribed celecoxib, rofecoxib, or non-steroidal anti-inflammatory drugs: population based study. BMJ. 2005;330(7504):1370.
56. Gierse JK, Zhang Y, Hood WF, Walker MC, Trigg JS, Maziasz TJ, et al. Valdecoxib: Assessment of Cyclooxygenase-2 Potency and Selectivity. Journal of Pharmacology and Experimental Therapeutics. 2005;312(3):1206-12.
57. Alsalameh S, Burian M, Mahr G, Woodcock BG, Geisslinger G. The pharmacological properties and clinical use of valdecoxib, a new cyclo-oxygenase-2-selective inhibitor. Alimentary Pharmacology & Therapeutics. 2003;17(4):489-501.

58. Fricke J, Varkalis J, Zwillich S, Adler R, Forester E, Recker DP, et al. Valdecoxib Is More Efficacious Than Rofecoxib in Relieving Pain Associated With Oral Surgery. American Journal of Therapeutics. 2002;9(2).
59. Pallay RM, Seger W, Adler JL, Ettlinger RE, Quaidoo EA, Lipetz R, et al. Etoricoxib reduced pain and disability and improved quality of life in patients with chronic low back pain: a 3 month, randomized, controlled trial. Scandinavian Journal of Rheumatology. 2004;33(4):257-66.
60. LIN H-Y, CHENG T-T, WANG J-H, LEE C-S, CHEN M-H, LEI V, et al. Etoricoxib improves pain, function and quality of life: results of a real-world effectiveness trial. International Journal of Rheumatic Diseases. 2010;13(2):144-50.
61. Matsumoto AK, Melian A, Mandel DR, McIlwain HH, Borenstein D, Zhao PL, et al. A randomized, controlled, clinical trial of etoricoxib in the treatment of rheumatoid arthritis. The Journal of Rheumatology. 2002;29(8):1623-30.
62. Brooks P, Kubler P. Etoricoxib for arthritis and pain management. Ther Clin Risk Manag. 2006;2(1):45-57.
63. Ward A, Brogden RN. Nimesulide. Drugs. 1988;36(6):732-53.
64. Reiner M, Massera E, Magni E. Nimesulide in the Treatment of Fever: A Double-Blind, Crossover Clinical Trial. Journal of International Medical Research. 1984;12(2):102-7.
65. Dreiser RL, Riebenfeld D. Nimesulide in the Treatment of Osteoarthritis. Drugs. 1993;46(1):191-5.
66. Barskova VG, Nasonova VA, Tsapina TN, Karateev AE, Iakunina IA, Eliseev MS, et al. [The effectiveness and safety of nimesulide (nimesile) in patients with gouty arthritis]. Klin Med (Mosk). 2004;82(12):49-54.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Effect of Growth Regulators on *In vitro* Regeneration of Plantlets from Leaf Explants of *Dendrobium aphyllum* (Roxb.) C.E.C Fischer - A Threatened Orchid from Darjeeling Himalaya, India

Mohammed Rahamtulla[1], Venkatesh Rampilla[2] and Shaik Mahammad khasim[1]*

[1]Department of Botany and Microbiology, Acharya Nagarjuna University, Nagarjuna Nagar, Guntur, Andhra Pradesh, India.
[2]Department of Botany, Dr. L.H.R Government Degree College, Mylavaram, Andhra Pradesh, India.

Received: 28 Nov 2020 Revised: 13 Dec 2020 Accepted: 23 Dec 2020

***Address for Correspondence**
Shaik Mahammad khasim,
Department of Botany and Microbiology,
Acharya Nagarjuna University,
Nagarjuna Nagar-522510, Guntur,
Andhra Pradesh, India.
Email : prof.smkhasim@gmail.com

ABSTRACT

Dendrobium aphyllum is a threatened orchid and very well known for its ornamental and ethnomedicinal importance. In view of the conservation aspect, the leaf segments of *D. aphyllum*, collected from the natural habitat of Takdah forest, Darjeeling, West Bengal were sterilized, inoculated on Murashige and Skoog (MS) medium augmented with 3% sucrose, 10% v/v coconut water and different growth regulators such as 2, 4-D, NAA, BAP, KN alone and in combinations of 2,4-D and BAP. Leaf explants showed better response (80%) to protocorm like bodies (PLBs) in 1 mg/L BAP and callus in 2 mg/L BAP, where as, in the presence of 0.5mg/L 2,4-D, only 30% of the explants responded within 6 weeks of culture. Combination of 0.5 mg/L 2,4-D and 2 mg/L BAP showed 100% response in the explants. Further, the leaf base segments showed greater morphogenetic potential in comparison to entire leaf and leaf tip. Furthermore, the successive subculture in basal medium supplemented with 0.5 mg/L 2,4-D and 1 mg/L BAP showed good differentiation of PLBs into plantlets with well-developed roots and shoots.

Key words: *Dendrobium aphyllum*, Growth regulators, Leaf explants, Protocorm like bodies (PLBs), Callus induction and Plantlet regeneration.

INTRODUCTION

First comprehensive survey of orchids carried out by Botanical survey of India (BSI) revealed that India is home to 1256 species of orchids belonging to 155 genera [1]. Nearly 479 orchid species are reported from Darjeeling

Himalaya, West Bengal [1]. *Dendrobium* is one of the largest diverse genera of orchids and more than 1100 species are present worldwide [2, 3]. Referring to the epiphytic habit of the genus, the name *Dendrobium* means living on a tree. More than 103 species of *Dendrobium* are reported mainly from North-East India [4,5,6]. *Dendrobium aphyllum* is reported from North East region [7,8], Western Ghats [9]and Eastern Ghats [10] of India. *D. aphyllum* is well known as leafless orchid because at its blooming stage the leaves are shed off and the whole branch gets covered by pale pinkish and fragrant flowers [11,12].This leafless orchid is a floricultural potential orchid for its magnificent flowers of great delicacy, beauty [13,14,15] and also possess ethnomedicinal significance [13,15,16]. Rampant collection, destruction of natural habitat, over exploitation for medicinal purposes and unauthorized trade has made this species to register its name in IUCN red list of threatened species [17]. The present status of *D. aphyllum* is least concern (LC) and the population trend is decreasing at an alarming rate. Literature studies reveal that *D. aphyllum* is also reported as endangered in China in Chinese red list [18,20]. Orchids are propagated vegetatively as well as by seeds. In sympodial orchids propagation occurs by separation of psuedobulbs [21,22]. *Dendrobium*s are sympodial and propagated through cuttings, separation of off shoots and keikis produced from the old stems [13]. In orchids *in vivo* seed propagation is very slow process [23, 24]. A persual of literature on *in vitro* propagation of *D. aphyllum* reveals that explants such as seeds [12,13,26], nodal segments [28] and shoot tips [29] have been employed for the multiplication of plantlets. However, leaf segment explants are superior above other kind of explants because they can be procured without sacrificing the mother plant and available throughout the year [30]. Therefore, micropropagation by using insignificant parts such as leaves of the plant would be profitable [25, 31]. Several authors [3,32,33,34] successfully regenerated plantlets from leaf explants of other *Dendrobium* species. Therefore leaf explants can be used for the production of larger number of uniform plants, In view of the background of *D. aphyllum*, the present study was undertaken so as to develop high frequency plantlet formation from *in vitro* culture of *D. aphyllum* leaf explants.

MATERIALS AND METHODS

Fortunately, while carrying out an ethnobotanical survey of orchids in Takdah, Darjeeling, west Bengal, authors noticed *D. aphyllum* (Fig. 1a, b) on the bark of sal (*Shorea robusta* Gaestn. F.). Keeping in view of *D. aphyllum's* ethnomedicinal significance and IUCN status, authors collected only two plants from the natural populations. After collecting the plants, they were transferred to the green house in the Botanical garden of Acharya Nagarjuna University. For the present investigation young leaves of *D. aphyllum* were used as explants. The collected leaves were surface sterilized by washing sequentially with 1 % (v/v) Teepol for 5 minutes, distilled water containing a few drops of Dettol for 5 min, 0.1 % (v/v) Mercuric chloride ($Hgcl_2$) for 2 minutes and five times with autoclaved distilled water (3 minutes for each wash). Later the leaf margins exposed to Mercuric chloride ($Hgcl_2$) were removed carefully and remaining part of the leaf was cut in to small pieces of 1-2 cm [34]. Leaf segments (1-2 cm) were inoculated on full strength of Murashige and Skoog (MS) medium [35] supplemented with 3 % sucrose and10 % v/v coconut liquid endosperm served as basal medium. Culture media were augmented with auxins [1-*Napththalene acetic acid* (*NAA*) and *2,4-Dichlorophenyloxyacetic acid* (2,4-D)] and/or cytokinins [6-Benzylaminopurine (BAP) and 6-Furfurylaminopurine (KN)] at 0.5 mg/L to 2.5 mg/L and coded accordingly as R_1 to R_{21} (Table 1). Later the pH of the medium was adjusted to 5.5 and further solidified with 0.8% agar. In order to determine the morphogenetic potential of segmented leaf explants, leaf bases and leaf tips (0.3-0.5cm) were inoculated in basal medium supplemented with 0.5 mg/L each of 2,4-D and BAP. Ten flasks with 4 explants in each were maintained as replicates. Protocorm like bodies (PLBs) and plantlets were sub cultured in MS medium containing 1 mg/L each of 2,4-D and BAP. Culture flasks were subjected to incubation under 2000 lux of 14 hour illumination at 25±2℃ temperature [36]. Systematic observations were done from time to time and comparison of response was made at the end of 6th week of culture.

RESULTS

Leaf explants (Fig. 2a, b) of *D. aphyllum* started showing signs of growth at their bases within 5 weeks of culture and these generated PLBs, callus and leaf primordia in 24 weeks depending upon the type of growth regulator used (Table 1) from leaf base, lamina, leaf tip and even on all over the surface of leaf. The media augmented with different concentrations of 2,4-D (R_1 to R_3) and NAA (R_4 to R_6) have shown poor response (below 30%) for the formation of PLBs, callus and leaf primordia. Media fortified with 2,4-D (R_1 to R_3) is somewhat better when compared to NAA (R_4 to R_6). Interestingly the media supplemented with BAP (R_7 to R_9) and KN (R_{10} to R_{12}) showed good response (40 to 80 %) (Table 1, Fig. 3). However, the most satisfactory results was observed only when 2,4-D and BAP were used in combination; the medium (R_{15}) having 0.5 mg/L 2,4-D and 2.0 mg/L BAP produced 100 % response. This R_{15} medium not only supported PLBs (Fig. 4b) but also callus and leaf primordia formation. Almost similar results were also obtained in R_{14} medium containing 0.5 mg/L 2,4-D and 1 mg/L BAP. R_{18} medium supplemented with 1 mg/L 2,4-D and 2 mg/L BAP showed 80 % response with respect to PLBs and callus but failed in case of leaf primordia. R_{19} medium supplemented with 2 mg/L 2,4-D and 0.5 mg/L BAP showed formation of maximum callus. It is interesting to observe that medium R_9 supplemented with 2 mg/L BAP produced good callus (Fig. 4a). R_8 medium containing (1 mg/L BAP) showed 80 % response with respect to leaf primordia as comparable to that of R_{13} medium (0.5 mg/L 2,4-D + 0.5 mg/L BAP). Apart from the above results, the experiment carried out to find out the morphogenetic response of different portions of leaf segments such as leaf base, leaf tip and entire leaf on the medium R_{13} (0.5 mg/L 2,4-D + 0.5 mg/L BAP) showed that leaf base produced maximum PLBs in comparison to leaf tip and entire leaf. 40 % whole leaf explants produced PLBs and callus where as leaf tip explants produced only leaf primordia (Table 2). Further, the leaf explants showed more response in adaxial portion of leaf when compared to abaxial portion. It is also noteworthy to mention that prolonged cultures of PLBs (more than 4-5 months) in the same medium resulted in their differentiation in to plantlets (Fig. 4c). Further, It is also observed that when the PLBs were sub cultured on R_{14} medium (0.5 mg/L 2,4-D +1 mg/L BAP) they become differentiated into well developed plantlets with in 14 weeks of subcultures (Fig. 4d).

DISCUSSION

In the present investigation, addition of external PGRs in appropriate concentrations promoted leaf proliferation from the explants cultured in the MS medium. . Different studies in orchids reveal that external addition of PGRs was largely influence the initiation and proliferation of explants in orchids [22,37,39]. Dohling *et al.,* (2012) developed PLB regeneration from explants is one of the accepted methods of *in vitro* propagation of medicinal orchids [40]. In the present study, the response of explants to the media containing cytokinins (BAP) in combination with auxins (2,4-D) varied according to concentration. The media containing auxins (R_1 to R_3) alone responded least (below 30 %) whereas the medium augmented with cytokinins (R_4 to R_6) responded well (40-80 %) with respect to formation of PLBs, callus and leaf primordia. Sinha and Hegde (1999) reported similar results in case of *in vitro* leaf culture of *Renades* arunoday hybrid [36]. Tanaka and Sakanishi (1980) also reported similar results in case of *Phalaenopsis* [41]. In our present findings we observed combined treatment of auxin 2,4-D and cytokinin (BAP) responded well i.e., media R_{13} to R_{21} in inducing proliferation in the leaf culture of *D. aphyllum.* The type and concentration of cytokinins used is closely related to proliferation and regeneration of explants [42]. Better results were obtained by using the combination of auxin and cytokinin in leaf cultures of *Vanda*, *Satyrium*, *Luisia* species [37], *Vanda coerulea* [44]and also in *Dendrobium formosum* [32]. It is interesting to note that in *D. aphyllum* cytokinin alone was enough to produce PLBs. Noteworthy works of Gu *et al.,* (1987), Churchill *et al.,* (1972; 1973) and Tanaka and Sakanishi (1978) suggest that if explants were cultured in suitable medium with appropriate combination of growth regulators, then it is not necessary to transfer callus mediated PLBs to media containing different growth regulators for the shoot induction [44,43] In the present study the prolonged culture of *D. aphyllum* PLBs (more than 5 months) in same medium, resulted in their differentiation into plantlets. Furthermore, the leaf bases regenerated PLBs, callus and leaf

primordia. Previous study reports that frequency of proliferation from the leaf base is higher than other leaf parts [48,36] The reason for high frequency of proliferation from leaf base is may be due to high meristematic nature of monocot leaf base [36,49] The above view is agreeable in the leaf culture of D. *aphyllum*. The response of *in vitro* cultured explants on medium supplemented with 0.5 mg/L 2,4-D + 2 mg/L BAP (R_{15}) was maximum (100 %) as compared to their effect used alone. Interestingly, it is also noticed that leaf explants of *D. aphyllum* responded better in adaxial surface of leaf. Similar findings of adaxial surface leaf explant were reported in *Oncidium* [39,50].

CONCLUSION

Data obtained from present study indicates that MS medium augmented with 0.5 mg/L 2,4-D and 2 mg/L BAP appears to be suitable for PLB induction from leaf culture. Further these are to be sub cultured on MS medium containing 0.5 mg/L 2,4-D and 1 mg/L BAP for differentiation in to plantlets.

ACKNOWLEDGEMENTS

Author (MR) acknowledges the University Grants Commission, New Delhi, for awarding Maulana Azad National Fellowship (MANF).

REFERENCES

1. https://www.thehindu.com/sci-tech/energy-and-environment/india-is-home-to-1256-species-of-orchid-says-first-comprehensive-survey/article28429797.ece
2. Leitch IJ, Kahandawala I, Suda J, Hanson L, Ingrouille MJ, Chase MW & Fay MF, Genome size diversity in orchids: consequences and evolution. *Annals of Botany*, 104 (2009) 469.
3. Goswami K, Yasmin S, Nasiruddin KM, Khatun F & Akte J, *In vitro* regeneration of *Dendrobium* sp. of orchid using leaf tip as explant. *Journal of Environmental Science and Natural Resources*, 8 (2015) 75.
4. Singh KP, Phukan S & Bujarbarua P, Orchidaceae. In: *Floristic Diversity and Conservation Strategies in India*, (Ed. NP Singh & DK Singh; Botanical Survey of India, Kolkata, India), 2001, 1735.
5. Chattopadhyay P, Banerjee N & Chaudhary B. Genetic characterization of selected medicinal *Dendrobium* (Orchidaceae) species using molecular markers. *Res J Biol*, 2 (2012) 117.
6. Ramesh G, *Morpho-anatomical and molecular studies in Dendrobieae (Orchidaceae) from India*. Ph.D. thesis, Acharya Nagarjuna University, Guntur, India, 2014.
7. Lokho A, Diversity of *Dendrobium* Sw. its distributional patterns and present status in the North-East India. *International Journal of Scientific and Research Publications*, 23 (2013) 1.
8. Rahamtulla M, Roy AK & Khasim SM. Orchid Diversity in Darjeeling Himalaya, India: Present Status and Conservation. In: *Orchid Biology: Recent Trends & Challenges*, (Ed. SM Khasim, SN Hegde, MT Gonazalez-Arnao, K Thammasiri; Springer Nature, Singapore), 2020, 155.
9. Sinu PA, Kuriakose G & Chandrashekara K, Epiphytic orchid diversity in farmer-managed Soppinabetta forests of Western Ghats: implications for conservation. *Current Science*, (2011) 1337.
10. Venkaiah M, Rao JP, Naidu MT, Prameela R, Rao PJ & Padal SB. Orchid Diversity in the Eastern Ghats of Northern Andhra Pradesh, India. In: *Orchid Biology: Recent Trends & Challenges*, (Ed. SM Khasim, SN Hegde, MT Gonazalez-Arnao, K Thammasiri; Springer Nature, Singapore), 2020, 189.
11. Bose TK, Bhattacharjee SK, Das P & Basak UC, *Orchids of India* (Naya Prakash, Calcutta), 1999,1.
12. Dutta S, Chowdhury A, Bhattacharjee B, Nath PK & Dutta BK, *In vitro* multiplication and protocorm development of *Dendrobium aphyllum* (Roxb.) CEC Fisher. *Journal of Science and Technology*, 7 (2011) 57.
13. Hossain MM, Sharma M & Pathak P, *In vitro* propagation of *Dendrobium aphyllum* (Orchidaceae)- seed germination to flowering. *Journal of Plant Biochemistry and Biotechnology*, 22 (2013)157.

14. Subedi A, Kunwar B, Choi Y, Dai Y, Van Andel T, Chaudhary RP, de Boer HJ & Gravendeel B, Collection and trade of wild-harvested orchids in Nepal. *Journal of Ethnobiology and Ethnomedicine*, 9 (2013) 64.
15. Hossain MM, Traditional therapeutic uses of some indigenous orchids of Bangladesh. *Medicinal and Aromatic Plant Science and Biotechnology*, 42 (2009) 101.
16. Rahamtulla M, Pradhan UC, Roy AK, Rampilla V & Khasim SM. Ethnomedicinal Aspects of Some Orchids from Darjeeling Himalaya, India. In: *Orchid Biology: Recent Trends & Challenges*, (Ed. SM Khasim, SN Hegde, MT Gonazalez-Arnao, K Thammasiri; Springer Nature, Singapore), 2020, 451.
17. Romand-Monnier F, *Dendrobium aphyllum*. The IUCN Red List of Threatened Species (2013) e.T15053780A44433456. https://www.iucnredlist.org/species/15053780/44433456
18. Fu L & Chin CM, *Dendrobium aphyllum* in *China plant red data book* (Science Press, Beijing, China), 1992.
19. Gao JY, Liu Q & Yu DL, Orchids of Xishuangbanna: diversity and conservation. *China Forestry*, (2014).
20. Zi XM, Sheng CL, Goodale UM, Shao SC & Gao JY, *In situ* seed baiting to isolate germination-enhancing fungi for an epiphytic orchid, *Dendrobium aphyllum* (Orchidaceae). *Mycorrhiza*, 24 (2014) 487.
21. Henderson MR & Addison GH, *Dendrobium aphyllum* in *Malayan orchid hybrids* (US Government Printing Office, USA), 1956.
22. Reddy J, *Biotechnology of orchids* (IK International Publishing House, New Delhi, India), 2008.
23. Pierik RLM, *In vitro culture of higher plants* (Martinus Nijhoff Publ., Dordrecht, Boston, Lancaster), 1987.
24. Pant B & Thapa D, *In vitro* mass propagation of an epiphytic orchid, *Dendrobium primulinum* Lindl. through shoot tip culture. *African Journal of Biotechnology*, 11 (2012) 9970.
25. Jainol JE & Gansau JA, Embryogenic callus induction from leaf tip explants and protocorm-like body formation and shoot proliferation of *Dimorphorchis lowii*: Borneon endemic orchid. *Journal of Agricultural Science*, 39 (2016) 1.
26. Du G, Lai TC & Yang HY, Study on the tissue culture of *Dendrobium aphyllum* (Roxb) CEC Fisch. *North Hort*, 8 (2012)140.
27. Talukdar A, Multiple shoot induction in *Dendrobium aphyllum* Roxb. *J Orchid Soc India*, 15 (2001) 35.
28. Bhadra SK, Barua AK, Bhattacharjee B & Hossain MM, *In vitro* germination and micropropagation of *Dendrobium aphyllum* (Roxb.) C. E. C., Fischer. *Bangladesh J Genet Biotechnol*, 3 (2002) 47.
29. Nayak NR, Rath SP & Patnaik S, *In vitro* propagation of three epiphytic orchids, *Cymbidium aloifolium* (L.) Sw., *Dendrobium aphyllum* (Roxb.) Fisch. and *Dendrobium moschatum* (Buch-Ham) Sw. through thidiazuron-induced high frequency shoot proliferation. *Scientia Horticulturae*, 71 (1997) 243.
30. Sarmah D, Kolukunde S, Sutradhar M, Singh BK, Mandal T & Mandal N, A review on: *in vitro* cloning of orchids. *International Journal of Current Microbiology and Applied Sciences*, 6 (2017)1909.
31. Chugh S, Guha S & Rao IU, Micropropagation of orchids: a review on the potential of different explants. *Scientia Horticulturae*, 122 (2009) 507.
32. Nasiruddin KM, Begum R &Yasmin S, Protocorm like Bodies and Plantlet Regeneration from *Dendrobium formosum* leaf callus. *Asian Journal of Plant Sciences*, 2 (2003) 955.
33. Chung HH, Chen JT & Chang WC, Plant regeneration through direct somatic embryogenesis from leaf explants of *Dendrobium*. *Biologia plantarum*, 51 (2007) 346.
34. Reddy J, Micropropagation of *Dendrobium* Queen Sonia from leaf explants. *International Research Journal of Natural and Applied Sciences*, 3 (2016) 220
35. 35 Murashige T & Skoog F, A revised medium for rapid growth and bio assays with tobacco tissue cultures. *Physiologia plantarum*, 15 (1962) 473.
36. Sinha SK & Hegde SN, Regeneration of Plantlets from *in vitro* leaf culture of *Renades* Arunoday hybrid (*Aerides rosea* Loddeges ex Paxt. x *Renanthera imschootiana* Rolfe). *J Orchid Soc India*, 13(1999)19.
37. Vij SP & Promila P, Micropropagation of orchids through leaf segments. *J Orchid Soc India*, 4 (1990) 69.
38. Yam TW & Weatherhead MA, Leaf-tip culture of several native orchids of Hong Kong. *Lindleyana* 6 (1991)147.
39. Pyati AN & Murthy HN, *In vitro* propagation of *Acampe praemorsa* (Roxb.) Blatt. & McCann from leaf explants. *J Orchid Soc India*, 13 (1999) 33.
40. Dohling S, Kumaria S & Tandon P, Multiple shoot induction from axillary bud cultures of the medicinal orchid, *Dendrobium longicornu*. *AoB Plants*, (2012) 1.

41. Tanaka M & Sakanishi Y, *Clonal propagation of Phalaenopsis through tissue culture* (Proc. 9th World Orchid Conference, Bangkok, Thailand), 1980, 215.
42. Amoo SO, Aremu AO, Moyo M, Szüčová L, Doležal K & Van Staden J, Physiological effects of a novel aromatic cytokinin analogue in micropropagated *Aloe arborescens* and *Harpagophytum procumbens*. *Plant Cell, Tissue and Organ Culture*,116 (2014)17.
43. Seeni S, Micropropagation of blue Vanda using leaf bases (Proc. Current Research Trends in Indian orchids with special reference to tissue culture technology, Shillong, India) 1988, 22.
44. Churchill ME, Ball EA & Arditti J, Tissue culture of orchids I. Methods for leaf tips. *New Phytologist*, 72 (1973)161.
45. Churchill ME, Ball EA & Arditti J, Tissue culture of orchids II. Methods for root tips. *American Orchid Society Bulletin*, 41 (1972) 726.
46. Gu Z, Arditti J & Nyman LP, *Vanilla planifolia*: callus induction and plantlet production *in vitro*. *Lindleyana*, 2 (1987) 48.
47. Tanaka M & Sakanishi Y, Factors affecting the growth of *in vitro* cultured lateral buds from *Phalaenopsis* flower stalks. *Scientia Horticulturae*, 8 (1978)169.
48. Seeni S & Latha PG, Foliar regeneration of the endangered red vanda, *Renanthera imschootiana* Rolfe (Orchidaceae). *Plant Cell, Tissue and Organ Culture*, 29 (1992) 167.
49. Zimmer K & Pieper W, Clonal propagation of *Phalaenopsis* by excised buds. *Orchid Review*, 86 (1978) 223.
50. Chen JT & Chang WC. Effects of tissue culture conditions and explant characteristics on direct somatic embryogenesis in *Oncidium* 'Gower Ramsey'. *Plant Cell, Tissue and Organ Culture*, 69 (2002) 41.

Table 1. Regeneration response of *D. aphyllum* leaf explants on MS medium and its various combinations with growth regulators

Media code (R)	Growth regulators (mg/L) used				% Response	Type of response		
	2,4-D	NAA	BAP	KN		PLB	Callus	Leaf primordia
Control	-	-	-	-	-	-	-	-
R_1	0.5	-	-	-	30	+	-	-
R_2	1.0	-	-	-	25	+	+	-
R_3	2.0	-	-	-	10	+	-	-
R_4	-	0.5	-	-	20	+	-	-
R_5	-	1.0	-	-	10	-	-	-
R_6	-	2.0	-	-	15	+	-	-
R_7	-	-	0.5	-	60	++	+	-
R_8	-	-	1.0	-	80	++	+	++
R_9	-	-	2.0	-	80	+	++	+
R_{10}	-	-	-	0.5	40	+	-	-
R_{11}	-	-	-	1.0	70	++	+	+
R_{12}	-	-	-	2.0	50	+	+	-
R_{13}	0.5	-	0.5	-	80	++	+	++
R_{14}	0.5	-	1.0	-	95	+++	++	++
R_{15}	0.5	-	2.0	-	100	+++	++	+++
R_{16}	1.0	-	0.5	-	70	++	+	-
R_{17}	1.0	-	1.0	-	60	+	+	-
R_{18}	1.0	-	2.0	-	80	++	+	-
R_{19}	2.0	-	0.5	-	60	+	+++	-
R_{20}	2.0	-	1.0	-	40	-	+	+
R_{21}	2.0	-	2.0	-	30	-	-	+

+++ Excellent, ++ Good, + Poor, - None

Table 2. Comparative response of entire/or segmented leaf explants on R_{13} medium (0.5 mg/L 2,4-D + 0.5 mg/L BAP).

S. No	Explant	% Response	Type of response		
			PLBs	Callus	Leaf primordia
1.	Whole leaf	40	++	+	-
2.	Leaf base	80	++	++	+
3.	Leaf tip	20	-	-	+

Fig. 1. *Dendrobium aphyllum*: a. Habit, b. flower

Fig. 2. Leaf explants of *Dendrobium aphyllum* a. whole leaf, b. leaf base inoculated in the MS medium

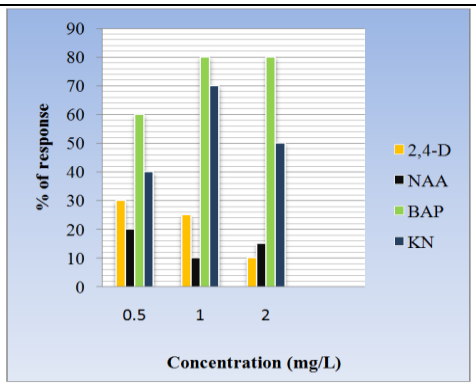


Fig. 3. *In vitro* response of *D. aphyllum* leaf explants to different concentrations of 2,4-D, NAA, BAP and KN.

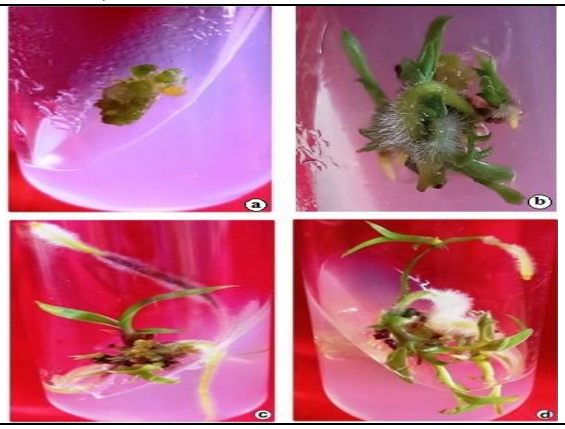


Fig.4. Leaf culture of *D. aphyllum* a. Callus from leaf base on R_{19} medium (MS + 2 mg/L 2,4-D and 0.5 mg/L BAP) after 8 weeks of culture; b. PLBs on R_{15} medium (MS + 0.5 mg/L 2,4-D and 2 mg/L BAP) after 8 weeks of culture; c. PLBs with differentiating plantlets on R_{13} medium (MS + 0.5 mg/L 2,4-D and 0.5 mg/L BAP) after 16 weeks of subculture; d. seedlings with well developed roots and shoots (0.5 mg/L 2,4-D + 1mg/L BAP) after 14 weeks of culture.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Candida auris Infection and Its Natural Remedies

Elizabeth N Xavier[1]* and Shincy Mariya Francis[2]

[1]Assistant Professor, Department of Pharmaceutics, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India.
[2]Department of Pharmaceutics, Nirmala College of Pharmacy, Muvattupuzha, Kerala, India.

Received: 21 Nov 2020 Revised: 02 Dec 2020 Accepted: 24 Dec 2020

***Address for Correspondence**
Elizabeth N Xavier
Assistant Professor,
Department of Pharmaceutics,
Nirmala College of Pharmacy,
Muvattupuzha, Kerala, India
Email : elsuonline4chat@gmail.com

ABSTRACT

Antimicrobial resistance is a major problem in medical field. The antifungal resistance although considered secondary is another area which need focused approach. There is a surge in researches which test the antimicrobial potential of various chemicals. Agents from natural sources find more potential as antimicrobials. Plants have a wide range of pharmacological activities including antifungal and antibacterial activities. This review article summarizes the emergence of *Candida auris* infections in India, diagnosis in *Candida auris* infections, its infection sites, virulence factors, and the mechanisms of antifungal resistance for this multi-resistant *Candida* species. This article also reviews the natural remedies for *Candida auris* infections.

Keywords: Plants, *Candida auris,* antifungal, natural, infections

INTRODUCTION

Annual death from fungal infections count to 1.6 million and serious fungal infections affect 300 million people. Of the 1.5 million fungal species present in worldwide, more than eight thousand are known to cause disease in plants and 300 to be pathogenic to humans[1].Candida is one of the most common fungal pathogens and it is known as the major cause of health-care related infections among both immunocompetent and immunosuppressed hosts. Candida represents the major cause of opportunistic mycoses worldwide. The frequency of healthcare-related candidemia is considered as the most common bloodstream infections in the intensive care units[2]*Candida albicans* is the major *Candida* species and *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. aurisare the* non-*albicans Candida* species in Hospital acquired invasive candidiasis globally[3].The crude mortality rate from *C.auris* infections has been documented ranging from 30% to 72% [4]. *Candida auris* was first isolated in 2009 from the external ear canal of a

patient in Japan and also from 15 patients with otitis media in South korea [5]. Since then, *C. auris* has been reported from 32 countries spanning six continents. The emergence of *C. auris* in each geographical region is independent and not spread from a single source, suggested from the studies related to different clades from different geographical areas [4].

The essential oils extracted from various medicinal and aromatic plants(MAPs) consist of various naturally associated compounds like terpenes, aromatic compounds, and terpenoids [6,7] are promising source of alternative antifungals [8,9].Such plant derived components are found to be effective against antibiotic-resistant strains [10]. The proteins and peptides derived from plants shows a therapeutic activity against the fungi. For example, *Impatiens balsamina* plant seeds contain a group of antifungal peptides (Ib-AMP1 through Ib-AMP4) that comprise about 0.5% of the total protein in the mature seed [11].The mode of antifungal activity of plant extracts could be due to their destructive effects on the fungal cell wall structures and the leakage of the cytoplasm from the cells. The essential compounds from the extract could interfere in the fungal morphogenesis and then leads to the stopping of their growth [12]Many traditional medicinal plants have antimicrobial activities. The Ashwagandha (*Withania somnifera*) is an important herb in the Ayurvedic and indigenous medicine system and it belongs to the family Solanaceae. The plant parts from Ashwagandha having a huge number of medicinal properties including antifungal and antibacterial activities [13]. The extract from plant herbals and antibiotics combination approach provide significant therapy for the development of potential treatment for microbial infections. The most frequently used herbs that have antimicrobial properties are garlic, cloves, cinnamon, mustard, neem, curcumin, tea and also include many others[14].Some South Indian plants have also shown to be potent antifungal like medicinal action. Various plants and their phytoconstituents have been used to eradicate different candida species [15].

Emergence In India

C. auris bloodstream infections began to be reported in India from 2013, with the earliest *C.auris* culture dating back to 2009. A tertiary care general hospital, a paediatric centre, a hospital intensive care unit, and a university hospital located in Northern and Southern India are the initially identified four affected facilities in India [16]. In India, a study of 27 intensive care units across the country found 5.7% of Candidemia cases from April 2011 to September 2012 were due to *C. auris* [17].

Diagnostics

C. auris can survive for at least two weeks on plastic surfaces. So, the Infection prevention guided by rapid detection in health care settings is vital, because *C. auris* is difficult to treat as it is commonly resistant to multiple antifungal drug classes [18].*Candida auris* is a budding yeast that forms white pink, or purple colonies on CHROMagar and can be difficult to distinguish from other Candida species. In contrast with most other Candida species, it grows at higher temperatures (40-42^{o}) and can tolerate salt concentrations in the culture medium of up to 10%[19].Identifying Candida to the species level is important for various reasons. Species level identification can detect or identify *C. auris* and trigger necessary infection control measures that are needed to prevent its spread in health care settings [20]. Misdiagnosis of *C.auris* as other yeast poses a problem for its diagnosis [21]. MALDI-TOF MS, rDNA sequencing and PCR methods can be used for identification of candida species [5]. The different diagnostic techniques for *C. auris*are summarized in Table 1.

Phenotypic methods

Sabouraud agar with smooth white to cream-colored colonies appear as beige to pink colonies on CHROMagar Candida medium or it might be necessary to confirm such identification result, by rDNA sequencing *C.auris* specific PCR/qPCR or MALDI-TOF MS, especially for high fluconazole minimal inhibitory concentration (MIC) or multidrug resistant isolates. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry can reliably differentiate *C. auris* from other *Candida* species, provided *C. auris* spectrum is included in the reference database and by selecting appropriate extraction method MALDI-used as their fingerprints. Since the identification

process is based on comparison of spectra acquired for a tested sample and a database of spectra of known species, accurate result is reliant on the presence of the sample organism spectrum in the database[22].

Molecular methods

The specific PCR assays for *C. auris* and for *C. auris*related species using cultured colonies arise as a promising tool for its rapid and accurate identification [23]. The accurate identification of *C. auris* and differentiation from other yeast were provided by the sequencing of rDNA genetic loci, namely internal transcribed spacer and D1/D2 region of large subunit, that are amplified with standard primers. A range of molecular techniques, including amplified fragment length polymorphism (AFLP analysis), pulsed-field gel electrophoresis (PFGE), M13 DNA fingerprinting and sequencing of genetic loci, have been used for the typing of *C. auris* isolates [24].

Infection Sites and Spread

The most frequently reported site is the bloodstream, with isolation from urinary or respiratory tract close behind. *C. auris* is the first fungal pathogen categorised as a public health threat due to its ability to readily colonize skin, spread rapidly among patients, and cause severe disease. Another factor contributing to the spread and transmission of *C.auris* is the propensity of the species to persist on the surfaces of hospital rooms and on medical devices [25]. *C.auris* may spread through contact with contaminated environmental surfaces, conditions and fomites. People affected or infected with *C. auris* shed the organism [16].The global warming related changes in the environment might have played a major role in *C. auris's* emergence [26].

Infection Control

Patients with *C.auris* infection, persons colonized with or suspected to have such infections or patients transferred from hospitals with a history of *C.auris* infections should be kept in separate wards or rooms under strict contact precautions as described by different authorities[27].The prevention of transmission of any pathogen in healthcare settings can be done by the basic methods which are Good standard infection control, including environmental cleaning, adequate cleaning and reprocessing of medical devices, and adequate capacity of microbiological laboratories, as well as sufficient capacity of health facilities for patient isolation[28].Hand hygiene is one of the most basic method for infection control. Hand hygiene can be performed with soap and water, alcohol-based hand rubs, or alcohol and chlorhexidine hand rubs[16].For patients having *C. auris* infection, thorough daily and terminal cleaning and disinfection of room surfaces using a sporicidal disinfectant has been recommended. The Candida species on surfaces can be reduced by both mechanical removal due to wiping and sporicidal and hydrogen peroxide-based disinfectants [29]. Infection control regimens are often based on the use of disinfectant or cleaner combined with diverse materials [30]. Patients or healthcare workers coming in close contact with infected persons should be placed under strict contact precautions until they consistently provide negative cultures over 3 weeks [27].

Drug Resistance

Antifungal drug resistance

One of the reasons for the emergence of *C. auris* has been so alarming is the potential for these organisms to maintain or develop the multidrug resistance [5].

Multidrug resistance

C. auris is a multidrug resistant pathogen because it can resistant to multiple antifungal drugs with some isolates resistant to all three major classes that include azoles, polyenes, and echinocandins [16]. Unlike other Candida species *C. auris* is associated with skin rather than gastrointestinal tract colonization and consists an intrinsic resistance to conventional front-line antifungal agents, antiseptics, and disinfectants [31].

Drug resistance and their mechanisms

Antifungal agents research is limited due to low occurrence of fungal infections, eukaryotic nature of fungus makes finding antifungal targets difficult [32]. Structure activity relationship of natural antifungals pave a way for developing antifungal drug leads. Amphotericin and capsofungin are examples of naturally derived antifungals in today's frontline therapy [33]. Some *C. auris* strains exhibit elevated minimum inhibitory concentration (MIC) for three main classes of antifungal drugs, they are azoles (e.g., itraconazole, voriconazole, Posaconazole and isavuconazole), polyenes and echinocandins. Recently, reports have documented high MICs to amphotericin B, voriconazole and capsofungin. Antifungal susceptibility testing of clinical blood isolates and isolates recovered from various sites that is environmental and body swabs from hospitals in Colombia revealed that all isolates had low MICs to voriconazole, itraconazole, isavuconazole and echinocandins. The variable rate of azole resistance in different geographic regions suggest localized evolvement of resistance [28]. The resistance mechanism of different classes of antifungal drugs against *C. auris* are summarized in Table 2.

Azole

Point mutations within the ERG11 gene that encodes, the target of the azoles that is lanosterol 14a-demethylase, increase transcription of this gene, leading to increased amounts of the enzyme, or the efflux pumps, such as Cdr1 and Cdr2, also affect this class of antifungals [34]. Former leads to inhibition of ergosterol biosynthesis [35]. and latter are proteins that transport components across cell membrane which may be a drug in this case and reduce their concentration an defect[36].The clinically approved azoles include fluconazole, itraconazole, voriconazole, posaconazole and isavuconazole and have mainly fungistatic activity against yeasts, such as *Candida* and *Cryptococcus* species. As anexception, voriconazole displays fungicidal activity against *A. fumigates* [35]. The three main mechanisms through which *Candida* species may become resistant to azoles are, the first is the introduction of multidrug pumps in the fungal cell wall. This allows the cell to pump out the drug, decreasing the inhibition of enzymes and alteration of the fungal cell wall. The second is that can lead to azole resistance is through the alteration or up-regulation of the gene encoding for the enzyme being targeted, ERG11.The third mechanism is for the fungal cell to develop bypass pathways as a result of mutations[37].

Echinocandins

Echinocandins are a group of semisynthetic, cyclic lipopeptides, the drugs included in this class are caspofungin, micafungin and anidulafungin. Echinocandins act by the inhibition of β (1, 3)-D- glucan synthase which is a key enzyme necessary for integrity of the fungal cell wall. Due to high cost it isnot in the firstline therapy but areserve drug for most invasive candida infection [38]. Targeting Fks1, the gene coding for glucan synthase disrupts the synthesis of the majorcell wall biopolymer (1,3)-β-D-glucan, resulting in loss of cell wall integrity and imparting a severecell wall stress on the fungi [35].

Amphotericin

Polyenes bind and extract the major fungal membranesterol ergosterol from cell membranes, preventing ergosterol from serving its many essential cellular functions. Mutations in ERG 2, 3, 5, 6 or 11 have been shown to have this effect [35].

Flucytosine(5-flurocytosine)

Flucytosine (a nucleoside analog) inhibits nucleic acid synthesis. Flucytosine has to be activated to have an antifungal effect, after a cell entry occurs. In resistant *C. auris* strain specific missense mutation observed a F211I amino acid substitution in the FUR1 gene[35].

Treatment and Managment of Patients

Because of its high rates of antifungal resistance *C. auris* poses a high treatment challenge. Fluconazole is the most widely available antifungal treatment for candidiasis. But *C. auris* isolates were resistant to fluconazole. The

alternatives like amphotericin B and echinocandins, are expensive and not easily available in the countries with more limited resources. Amphotericin B is also causes severe side effects [16]. Echinocandins are the first-line therapy for *C. auris* infection, given resistance to azoles and amphotericin B. To detect therapeutic failure and eventual development of resistances, the patients should undergo close follow-up and microbiological culture- based reassessment. In cases of unresponsiveness to echinocandins, liposomal amphotericin B (as single or combination therapy with an echinocandins) should be prescribed [25]. Micafungin demonstrated the highest efficacy in comparison to fluconazole and amphotericine B in some studies of *C.auris* candidemia conducted in mice. However, as echinocandin use is becoming more widespread [39]. The use of combination of micafungin and voriconazole drugs exhibited synergistic activity against multidrug-resistant *C. auris*, suggesting that this is an another approach to overcome antifungal drug resistance [40].

Virulence

Germination, adherence, biofilm formation, phospholipase, and proteinase production are all known to contribute to Candida pathogenesis [41]. Numerous virulence attributes of *C. auris* resemble with *C. albicans* which includes enzyme secretion, tissue invasion, nutrient acquisition, iron acquisition, histidine kinase-2 component system, multidrug efflux, genes and pathways involved in cell wall modelling and nutrient acquisition. The study conducted on an isolate of *C. auris* from a case of vulvoginitis showed proteinase, phospholipase and haemolysin activity. In another study, conducted on 16 different *C. auris* isolates collected from different geographical regions showed that phospholipases and proteinases production are strain dependent [4].

Promising Natural Candidates for *C. Auris* Infection

Some studies conducted on four monoterpene phenols, carvacrol, thymol, eugenol and methyl eugenol with and without conventional antifungal drugs. The results showed that had antifungal activity against *C. auris* with carvacrol being the most effective. Besides having low MIC, at sub-inhibitory concentrations carvacrol also significantly possible mechanism of antifungal activity are inhibited the adherence to epithelial cells and reduced the proteinase production. This shows that carvacrol can inhibit the growth of *C. auris*at MIC values. Additional long-lasting effects will be provided by rendering the pathogens to avirulent forms. Combination of carvacrol with the antifungal drugs in equal ratios (1:1) indicates additive, synergistic or indifferent interactions, while no antagonistic interaction was observed. With CAR-FLU, 16% of the *C. auris* isolates exhibit synergistic effect of which all were resistant to fluconazole. With CAR-AMP, 28% of the *C. auris* isolates exhibit synergistic effect of which only 12% were resistant to AMP. With CAR-NYS, 28% of the *C. auris* isolates gives synergistic effect of which all were resistant to NYS. With CAR-CAS, only one *C. auris* isolate exhibit synergistic effect and it was resistant to CAS. Combination of antifungal agent and phenolic compound had no effect on the *C. albicans* control strain and showed indifferent interactions in all the combinations. Related studies shown that carvacrol was the most active compound with the lowest MICs, *C. auris* isolates were exposed to different concentration of carvacrol to determine the anti-adherence activity. Overall carvacrol reduced the adherence ability of *C. auris* significantly and the reduction was concentration dependent [42].

Defensins

Defensins, expressed uniquely in granulocytes and epithelia of Old World monkeys, are pleotropic effectors of innate immunity that possess wide-spectrum antimicrobial activities and immunomodulatory properties Rhesus defensin 1 (RTD-1), the prototype defensin. In the studies they evaluated the four most active defensins (RTD-1, RTD-2, BTD-2, and BTD-8), as well as BTD-4, the least active peptide, against a panel of drug-resistant non-*albicans* Candida species, including *C. glabrata, C. parapsilosis, C. tropicalis, C. krusei,* and *C. auris.* Of note, RTD-1, RTD-2, BTD-2, and BTD-8 were highly activeagainst two strains of fluconazole and caspofungin-resistant *C. auris.* All defensins tested were active against fluconazole and echinocandin-resistant Candida species. The results showed that Defensins were active against two caspofungin-resistant *C. auris* clinical isolates. Ehinocandins are the treatment of choice for *C. auris* infections. Of note, the two *C. auris*isolates killed by defensins are echinocandin resistant [43].

Rocaglates

Rocaglates occur in Aglaia genus as secondary metabolites [44]. They have cytotoxic and antiproliferative activities. They include a series of structurally unique group of cyclopenta benzofurans [45].The two most potent rocaglates, namely, the synthetic rocaglate analogue CMLD010515 [(-)-RHT]and the natural product CMLD010853 [(-)-methyl rocaglate] are well studied translation inhibitors, and each displayed antifungal activity against *C. auris* at concentrations below 12.5 M. Therefore, rocaglates display potent antifungal activity against *C. auris* inhibited translation initiation and activated a cell death program in *C. auris*. Rocaglates act by its affinity for eIF4A proteinis part of the eIF4F heterotrimeric complex which act as RNA helicase in ribosome scanning. Programme cell death is the result of translation inhibition in yeast. Cell death occurs by apoptosis and autophagy [46].

REFERENCES

1. Microbiology N. Stop neglecting fungi. Nat Microbiol 2: 17120.
2. Sameh S. M. Soliman, Mohammad H. Semreen, Ali A. El-Keblawy, Arbab Abdullah, Priya Uppuluri, Ashraf S. Ibrahim. Assessment of herbal drugs for promising anti-Candida activity. BMC Complementary and Alternative Medicine 2017; 17:257
3. Bongomin F, Gago S, Oladele RO, Denning DW. Global and multi-national prevalence of fungal diseases-estimate precision. Journal of fungi 2017;3(4):57
4. Lone SA, Ahmad A. Candida auris-the growing menace to global health. Mycoses. 2019;62(8):620-37.
5. Spivak ES, Hanson KE. Candida auris: an emerging fungal pathogen. Journal of clinical microbiology. 2018;56(2):e01588-17.
6. Reginaldo dos Santos Pedroso, Brenda Lorena Balbino, Géssica Andrade, Maria Cecilia Pereira Sacardo Dias, Tavane Aparecida Alvarenga, Rita Cassia Nascimento Pedroso et al. In Vitro and In Vivo Anti-Candida spp. Activity of Plant-Derived Products. Plants 2019; 8:494
7. Giulia Potente, Francesca Bonvicini , Giovanna Angela Gentilomi, Fabiana Antognoni. Anti-Candida Activity of Essential Oils from Lamiaceae Plants from the Mediterranean Area and the Middle East. Antibiotics 2020; 9:395
8. Mallappa Kumara Swamy, Mohd Sayeed Akhtar,Uma Rani Sinniah. Antimicrobial Properties of Plant Essential Oils against Human Pathogens and Their Mode of Action: An Updated Review. Evidence-Based Complementary and Alternative Medicine Volume 2016;1-21
9. HamzehMafakheri, Sayid Mahdi Mirghazanfari. Antifungal activity of the essential oils of some medicinal plants against human and plant fungal pathogens. Cell Mol Biol (Noisy le Grand) 2018;64(15)
10. Puneet Sudan, Manish Goswami. A Critical Review Summarizing the Role of Traditional Plants as Potent Fungicidal. PAIDEUMA JOURNAL.2019;12(12)
11. A.J. De Lucca, T.E. Cleveland, D.E. Wedge. Plant-derived antifungal proteins and peptides. Can. J. Microbiol. 2005; 51: 1001–1014
12. M. Hashem, S.A. Alamri, A.A. Shathan, S.R.Z. Alshehri, Y.S. Mostafa, A. El-kott Antifungal efficacy of wild plants against human opportunistic pathogens. Journal de MycologieMedicale2019;29:168–173
13. Nisha Khanchandani, Prachi Shah, Twinkle Kalwani, Anujkumar Ardeshna, Darshan Dharajiya. Antibacterial and Antifungal Activity of Ashwagandha (*Withania somnifera* L.): A review. Journal of Drug Delivery & Therapeutics. 2019; 9(5-s):154-161
14. Ankit Saini, Bhupendra Singh, Ashis Suttee, Surajpal Verma, Lavi Kumar Garg, Sourav Thakur. A review on phytoconstituents: The potent synergistic approach in antimicrobial formulations. Journal of Pharmacy Research. 2018;12(2):151-159
15. Swati Rawat, Akansha Jain, Shubham Jain. Recent Herbal Trends against Fungal Infections: A Review. Pharmacognosy Reviews [Phcog Rev.] 2008;2(4):257-265
16. Forsberg K, Woodworth K, Walters M, Berkow EL, Jackson B, Chiller T, Vallabhaneni S. Candida auris: The recent emergence of a multidrug-resistant fungal pathogen. Medical mycology. 2019;57(1):1-2.

17. Anuradha Chowdhary, Cheshta Sharma, Shalini Duggal, Kshitij Agarwal, Anupam Prakash, Pradeep Kumar Singh, et al. New Clonal Strain of Candida auris, Delhi, India.Emerging infectious diseases. 2013;19(10):1670.
18. Rory M. Welsh, Meghan L. Bentz, Alicia Shams, Hollis Houston, Amanda Lyons, Laura J. Rose, et al. Survival, Persistence, and Isolation of the Emerging Multidrug-Resistant Pathogenic Yeast Candida auris on a Plastic Health Care Surface. Journal of Clinical Microbiology 2017;55(10):2996-3005
19. Nikki Kenters, Martin Kiernan, Anuradha Chowdhary, David W. Denning, Javier Peman, Katja Saris. Control of Candida auris in healthcare institutions: Outcome of an International Society for Antimicrobial Chemotherapy expert meeting. International Journal of Antimicrobial Agents.2019;54:400–406
20. Shawn R. Lockhart, Brendan R. Jackson, Snigdha Vallabhaneni, Luis Ostrosky-Zeichner, Peter G. Pappas, Tom Chillera. Thinking beyond the Common Candida Species: Need for Species-Level Identification of Candida Due to the Emergence of Multidrug-Resistant Candida auris. Journal of Clinical Microbiology.2017;55(12): 3324-3327
21. Sears D, Schwartz BS. Candida auris: an emerging multidrug-resistant pathogen. International Journal of Infectious Diseases. 2017;63:95-8.
22. Kordalewska M, Perlin DS. Identification of drug resistant Candida auris. Frontiers in microbiology. 2019;10:1918.
23. Cortegiani A, Misseri G, Fasciana T, Giammanco A, Giarratano A, Chowdhary A. Epidemiology, clinical characteristics, resistance, and treatment of infections by Candida auris. Journal of intensive care. 2018;6(1):69.
24. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, Manuel R, Brown CS. Candida auris: a review of the literature. Clinical microbiology reviews. 2018;31(1):e00029-17.
25. Jeniel E. Nett. Candida auris: An emerging pathogen "incognito"?.PLoS pathogens. 2019;15(4).1-6
26. Arturo Casadevall, Dimitrios P. Kontoyiannis, Vincent Robert. On the Emergence of Candida auris: Climate Change, Azoles, Swamps, and Birds. American society for microbiology. 2019;10(4):e01397-19
27. John Osei Sekyere. Candida auris: A systematic review and meta-analysis of current updates on an emerging multidrug-resistant pathogen. Microbiologyopen. 2018;7(4):e00578.1-29
28. Cortegiani A, Misseri G, Fasciana T, Giammanco A, Giarratano A, Chowdhary A. Epidemiology, clinical characteristics, resistance, and treatment of infections by Candida auris. Journal of intensive care. 2018;6(1):69.
29. Jennifer L. Cadnum, Aaron A. Shaikh, Christina T. Piedrahita, Annette L. Jencson, Emily L. Larkin, Mahmoud A. Ghannoum, et al. Relative Resistance of the Emerging Fungal Pathogen Candida auris and Other Candida Species to Killing by Ultraviolet Light. Infect Control Hosp Epidemiol2018;39:94–96
30. Katarzyna Ledwoch, Jean-Yves Maillard. Candida auris Dry Surface Biofilm (DSB) for Disinfectant Efficacy Testing. Materials 2019; 12(18):1-10
31. Mariagrazia Di Luca, Anna Koliszak, Svetlana Karbysheva, Anuradha Chowdhary, Jacques F. Meis, Andrej Trampuz. Thermogenic Characterization and Antifungal Susceptibility of Candida auris by Microcalorimetry. J. Fungi 2019;5(103):1-14
32. Scorzoni L, Paula e Silva AC, Marcos CM, Assato PA, Melo WC, Oliveira HC, et al. Antifungal therapy: new advances in the understanding and treatment of mycosis. Frontiers in microbiology. 2017;23(8):36.
33. Vengurlekar S, Sharma R, Trivedi P. Efficacy of some natural compounds as antifungal agents. Pharmacognosy reviews. 2012;6(12):91.
34. Wiederhold NP. Antifungal resistance: current trends and future strategies to combat. Infection and drug resistance. 2017;10:249
35. Robbins N, Caplan T, Cowen LE. Molecular evolution of antifungal drug resistance. Annual review of microbiology. 2017;71:753-75.
36. Chaabane F, Graf A, Jequier L, Coste AT. Review on antifungal resistance mechanisms in the emerging pathogen Candida auris. Frontiers in Microbiology. 2019;10:2788.
37. Pristov KE, Ghannoum MA. Resistance of Candida to azoles and echinocandins worldwide. Clinical Microbiology and Infection. 2019;25(7):792-8.
38. Grover ND. Echinocandins: A ray of hope in antifungal drug therapy. Indian journal of pharmacology. 2010;42(1):9.
39. Jeffery-Smith A, Taori SK, Schelenz S, Jeffery K, Johnson EM, Borman A, Manuel R, Brown CS. Candida auris: a review of the literature. Clinical microbiology reviews. 2018;31(1):e00029-17.

40. Hamed Fakhim, Anuradha Chowdhary, Anupam Prakash, AfsaneVaezi, Eric Dannaoui, Jacques F. Meis, et al. In Vitro Interactions of Echinocandins with Triazoles against MultidrugResistant Candida auris. Antimicrobial Agents and Chemotherapy. 2017;61 (11) e01056-17:1-5
41. Chatterjee S, Alampalli SV, Nageshan RK, Chettiar ST, Joshi S, Tatu US. 2015. Draft genome of a commonly misdiagnosed multidrug resistant pathogen Candida auris. BMC Genomics 16:686
42. Shaban S, Patel M, Ahmad A. Improved efficacy of antifungal drugs in combination with monoterpene phenols against Candida auris. Scientific Reports. 2020 Jan 24;10(1):1-8.
43. Basso V, Garcia A, Tran DQ, Schaal JB, Tran P, Ngole D, Aqeel Y, Tongaonkar P, Ouellette AJ, Selsted ME. Fungicidal potency and mechanisms of θ-defensins against multidrug-resistant Candida species. Antimicrobial agents and chemotherapy. 2018;62(6):e00111-18
44. An FL, Wang XB, Wang H, Li ZR, Yang MH, Luo J, Kong LY. Cytotoxic rocaglate derivatives from leaves of Aglaia perviridis. Scientific reports. 2016;6:20045.
45. Hidayat AT, Farabi K, Harneti D, Nurlelasari N, Maharani R, Mayanti T, Supratman U, Shiono Y. A Cytotoxic Rocaglate Compound from The Stembark of Aglaia argentea (Meliaceae). Molekul. 2017;12(2):146-52.
46. Iyer KR, Whitesell L, Porco JA, Henkel T, Brown LE, Robbins N, Cowen LE. Translation Inhibition by Rocaglates Activates a Species-Specific Cell Death Program in the Emerging Fungal Pathogen Candida auris. mBio. 2020;11(2).

Table 1: Different Methods For Diagnosing *C.Auris* Fungal Infections

Sl.no	Methods	Examples
1.	Phenotypic methods	• rDNA sequencing • *C. auris*-specific PCR/qPCR • Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry
2.	Molecular methods	• Amplified fragment length polymorphism (AFLP analysis) • Pulsed-field gel electrophoresis (PFGE) • M13 DNA fingerprinting • Sequencing of genetic loci

Table 2: Different Classes of Drugs and Their Resistance Mechanisms

Sl.no.	Class of Drug	Resistance mechanism	Examples for drugs
1.	Azole	• Point mutations within the ERG11 gene that encodes lanosterol 14a-demethylase • The target of the azoles increase transcription of this gene, leading to increased amounts of the enzyme, or the efflux pumps (Cdr1 and Cdr2) also affect this class of antifungals. • And leads to inhibition of ergosterol biosynthesis. • Then proteins that transport components across cell membrane which may be a drug in this case and reduce their concentration and effect.	• Fluconazole • Itraconazole • Voriconazole • Posaconazole • Isavuconazole

2.	Echinocandins	• Act by inhibition of β (1, 3)-D- glucan synthase. • Targeting Fks1, the gene coding for glucan synthase disrupts the synthesis of the major cell wall biopolymer(1,3)-β-D-glucan. • Then resulting in loss of cell wall integrity and imparting a severe cell wall stress on the fungus.	• Caspofungin • Micafungin • Anidulafungin
3.	Amphotericin	• Polyenes bind and extract the major fungal membrane sterol ergosterol from cell membranes. • This leads to preventing ergosterol from serving its many essential cellular functions	
4.	Flucytosine (5-flurocytosine)	• This inhibits nucleic acid synthesis. • After cell entry, flucytosine has to be activated to have an antifungal effect. • This activation requires, the protein encoded by the gene FUR1.	

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Leucinodes orbonalis Life Cycle and its Developmental Stages

Saraswathi, Sowrabha Ramachandra, and Doddamane Manjulakumari*

Department of Microbiology and Biotechnology, Bangalore University, Bengaluru, Karnataka, India.

Received: 19 Nov 2020 Revised: 21 Dec 2020 Accepted: 23 Jan 2021

***Address for Correspondence**
Doddamane Manjulakumari
Department of Microbiology and Biotechnology,
Bangalore University,
Bengaluru, Karnataka, India.
Email: manjulakumari_doddamane@yahoo.co.in

ABSTRACT

L. orbonalis Guenee (Lepidoptera: Crambidae) is a monophagous pest of brinjal. It is known to destroy brinjals grown in South & South East Asia.The wild samples of *Leucinodes orbonalis* populations were collected from fields in and around Hesaraghatta, Bengaluru, Karnataka, India, to maintain a stock culture. The insects were reared in the laboratory conditions and insect colony has been established successfully in the laboratory with five generations of a population. During this period, the life cycle stages of *L. orbonalis* were recorded. In our study, the adults pre-oviposition and oviposition period lasted for 1.2±0.41 and 2.26±0.45 days, respectively and fecundity was 247.4±21.25 in numbers. The total incubation period of *L. orbonalis* lasted for 4.06±0.70 days and larval period lasted for 14.86±1.06 days. The entire pupal period has lasted for 8.46±0.74 days. Adults were found to be active at night, mating was observed on the same day of emergence, and it lasted for 1hour. Effect of adult Nutrition on egg-laying indicated that females fed on 10% honey and 10% sucrose solution laid significantly more eggs than the water-fed and food-deprived moths. The longevity of males and females was found to be 3.62±0.56 and 5.28±0.67 days, respectively. The total developmental period of *L. orbonalis* was found to be 32.6±1.99.

INTRODUCTION

Brinjal/ Eggplant/ *Solanum melongena* L. is the most important vegetable throughout the world. China occupies the first largest brinjal producer that contributes nearly 68.7% while India occupies the second position in production with 23.3 % of the world's. It is found to be an Indian origin and cultivated worldwide (Choudhary, 1970; Pareet, 2006). This crop is majorly exposed by several pest insects starting from the nursery stage until harvesting (Regupathy et al., 1997). Among which the most destructive pest on brinjal is *Leucinodes orbonalis Guenee* or brinjal fruit and shoot borer (*Leucinodes orbonalis* Guenee) (Lepidoptera: Crambidae) and claimed to be monophagy in nature (Boopal et al., 2013). *Leucinodes orbonalis* creates a severe problem due to its higher dynamic potential, quick

generational turnover, and brinjal cultivation in both wet and dry seasons throughout the year. Initially, the *L. orbonalis* larvae start bore into tender shoots, leading to the zig-zag feeding tunnels in fruits. Further, they are blocked with frass that marks fruits unfit for marketing and consumption. The total percentage of brinjal yield loss has been reported up to 92 % (Chakraborti and Sarkar, 2011). A deep understanding of the insect's system and its functioning forms an essential part in successfully tackling any pest insect. Here in the present study, we have attempted to outline the *L. orbonalis* life cycle and reproductive behavior.

MATERIALS AND METHODS

Sample collection and mass rearing of *L. orbonalis*

Parental stock

Infested brinjal fruits were collected from fields in and around Hesaraghatta, Bengaluru, Karnataka, India, to maintain a supply of wild *L. orbonalis*. Infested fruits were kept in a tray (dimension: 7.1x 30.7 x 42 cm)(Figure 1)and maintained under room temperature with relative humidity until the fully-grown larvae came out of the infested fruit. A suitable substratum for pupation was provided by placing the brinjal's dry leaves on an autoclaved soil bed. The pupae were maintained in a separate box (size: 7.7x 25.6 cm) and left undisturbed until the adults emerged. After adult emergence from the pupae, the male and female moths were transferred to a transparent plastic bottle (size: 15 x 9.6 cm) and allowed to mate. The bottle's opening was covered with black cloth, which served as a substratum for the female to perch on and lay eggs firmly, and the black cloth provides a dark background for the white eggs and thus facilitates the counting of eggs.

Mass rearing of *L. orbonalis*

Once the eggs hatched, the neonate larvae were released to a rearing container (size: 9.7 X 9.1 cm) using a fine camel hairbrush on potato slices. The potatoes were sterilized with 2% Sodium hypochlorite solution and air-dried before use as a feed. The potato served as a diet for larvae, a suitable alternative host for brinjal shoot and fruit borer (Boopal et al., 2013; Mannan et al., 2015). The *L. orbonalis* neonate larvae were reared under environmentally controlled laboratory conditions at 25±2 ºC with 65±5% humidity. The potato slices were changed occasionally, approximately 3 to 4 days when they started decaying, to avoid bacterial and fungal contamination. Late instar larvae burrowed into the soil and pupated, while some of them pupated on muslin cloth covering the opening of the box, as well as tissue paper kept inside the box to absorb excess moisture. The pupae were collected and maintained in a pupae collection box after pupation until the adult's emergence. As adults emerged from pupae, they were sexed, separated by observing the body size and tuft of hairs at the abdomen tip.

Study of the Life cycle and reproductive behavior of *L. orbonalis*

Incubation period: The black cloth containing eggs kept in a plastic container and allowed the eggs to hatch. The eggs were under observation for hatching every day to record the incubation period.

Larval period: The neonate larvae were transferred to individual small plastic cups (size: 4.2 X 4.5) containing fresh potato slices. Until the completion of larval period, the progress into every instar was recorded (n=30).

Pupal period: The fully grown fifth instar larvae were transferred to small vials having a moist layer of autoclaved sand and daily observed to study the pupal duration of *L. orbonalis* from pupation to adult emergence (n=30)

Mating Behavior: Once the adults start emergence, the male and female moths were transferred to a box and allowed to mate. The mating behavior was recorded by observing the movements of both males and females. The time and duration of mating were recorded for several pairs (n=25).

Oviposition behaviour: Oviposition behaviour was studied by taking the egg count every day after egg laying until its death. The total number of eggs laid and the temporal pattern of egg-laying was recorded in both mated and virgin females (n=20).

Effect of adult Nutrition on egg-laying: The total number of eggs laid was recorded in mated females provided with different diets such as 10% honey, 10% sucrose, and distilled water. The results were compared with a group deprived of food (n=20).

The longevity of the adult moths: The longevity of virgin and mated moths was calculated by recording the time interval between moth's emergence from the pupa and its death (n=25).

Statistics Analysis: Data on oviposition, longevity, and adults mating were analyzed using nonparametric statistical tests like the Wilcoxon Signed Rank test and Kruskal-Wallis test (One-way ANOVA on Rank)and Rejection level was set to 5% ($\alpha < 0.05$) using SPSS, version 17.4.18.

RESULTS AND DISCUSSION

Rearing of *L. orbonalis* was done under laboratory conditions at 25±2°C. *Leucinodes orbonalis* insect colony has been established successfully in the laboratory with five generations of a population. During this period, the life cycle stages of *L. orbonalis* were recorded. The eggs of *L. orbonalis* were laid around 5 to 6 in batches or singly on the black cloth used to cover the container. The shape of freshly laid eggs were oval with creamy white in colour. Before hatching, the egg of this pest is turned into deep orange colour and also showing a noticeable black spot at the tip of the egg, which was the larvae developing head. The incubation period of eggs of the *L. orbonalis* lasted for 4.06±0.70 days (Table 1). The average incubation period for eggs of *L. orbonalis* has been reported as 3.90±0.88 (Padwal et al., 2018), which is in close concurrence with the present finding. Similarly, Maravi *et al.* (2013) reported the *L. orbonalis* incubation last for about 3.49 days. Eggs were oval with creamy white in colour. Our present observation is similar to that of Wankhede *et al.* (2009) and Maravi *et al.* (2013). The *L. orbonalis* neonate larvae are usually creamy white in colour with a dark brown head, three pairs of thoracic legs, and five pro-legs (Figure 1a). Normally, larvae molted four times and passed through five instars. The size of the larva increased with different instar and changed its colour. During the last instar, the larvae appear to be cylindrical with a pinkish color, and the pupating behavior was more distinct at this stage. The larvae were reared on potatoes as a diet under the laboratory conditions (Figure 1b) till the completion of five instars (Figure 1c). The mean duration of the first instar to till fifth instar larvae is shown in Table 1. The total larval duration is 14.86±1.06 days (Table 1). Mannan *et al.* (2015) showed that the larval period was 14.50±0.18days. Several studies showed that larvae passed five instars in 18.66 days (Singh and Singh, 2001a), 12.80 days (Gupta and Kauntey, 2007), and 16.32 days (Kavitha et al., 2008).

The final instar larvae come out of the infested fruits and pupates on the substratum provided, either an autoclaved soil bed or muslin cloth. The initial colour of *L. orbonalis* pupae was whitish-yellow and then converted to yellow-orange colour as stage advanced (Figure 1d). The pupae were elongated, oval in shape, gradually tapering posterior with the almost straight abdomen, and wing margins extended to the abdominal segment's posterior margin. The entire pupal period has lasted for 8.46±0.74 days (Table 1). The present investigation is similar to Padwal & Srivastava (2018), who reported 8.80±1.14 days. The adult moths are white with head and thorax covered with greyish and brown scales. The fore wings are creamish white with large patches of light brown colour over it (Figure 1e). In the hind wing, a faint black wavy line is observed close to the apical margin. The wings are slightly fringed at

the margins. The female moth's size is generally more massive than the male and has a tuft of hair at the abdomen's tip. The male moths' abdomen was slender and blunt at the posterior end. During the day time, adults remained inactive, and they are found to be active at night for their mating and oviposition purposes (Figure 1f). Adults were found to be active at night, mating was observed on the same day of emergence, and it lasted for 1hour. The present study clearly showed that the peak period of mating in *L. orbonalis* was at 1-3 AM (50%). According to Mannan *et al.* (2015), mating duration was found to be 43.27 minutes late at night, where 90.80% of mating occurred on the same day of emergence.One-Way ANOVA (Kruskal-Wallis) test showed a statistically significant difference in the number of moths mated between the different time intervals, $\chi^2(2) = 22.319$, $p = 0.00$.(Figure 2). Some researchers like Yasuda and Kawashaki (1994) observed the copulation of males and females at 4.40 AM, which lasted for 43 minutes. Similarly, Kavitha *et al.* (2008) showed that mating took place on the same day after emergence. Prabhat and Johnsen (2000) reported that the mating lasted for about 16 minutes. The above authors' findings supported the present investigation on the mating behaviour of adult moths of *L. orbonalis* that the insect is nocturnal. Some of the lepidopterans mate on the same day of emergence, whereas in other lepidopterans, it happens on the second day. The mating duration is 45 minutes in *H. armigera* (Chaitra et al., 2020) and 40 minutes in *Spodoptera litura* (Mamtha et al., 2018, 2019).

Egg-laying behaviour was also observed, and it always occurs at night. Oviposition started on the next day after the mating. The oviposition behaviour of *L. orbonalis* is recorded as the total number of eggs laid by female moths and which is represented as a percentage. Significant difference between the two groups was observed after Wilcoxon Rank-Sum test ($Z = -3.922$) ($p < 0.05$) (Figure 3). Mated moths have laid more eggs on each day than virgins, and the number of eggs gradually decreased each day. Mannan *et al.* (2015) reported that 85.90% of females start egg-laying on the first day, and 14.10% were on the second day. Gupta and Kauntey (2007) said that the average oviposition period of *L.orbonalis* was 2.46 days. The adults' pre-oviposition and oviposition periods lasted for 1.2±0.41 and 2.26±0.45 days, respectively, and fecundity was 247.4±21.25. Singh and Singh (2001a) reported an average pre-oviposition and ovipositional period of 1.35 and 2.09 days, respectively. Mannan et al. (2015) said that the average number of eggs laid by females was 288.05±2.32. In comparison, Kavitha *et al.* (2008) showed that the female's average number of eggs during its lifetime was 170.

Mated females fed on 10% honey and 10% sucrose solution laid significantly more eggs than the water-fed and food-deprived moths. The One-Way ANOVA (Kruskal-Wallis) test showed a statistically significant difference in the number of eggs laid between the different diets- fed with mated females $\chi2(2) = 91.896$, $p = 0.00$ (Figure 4). Adult feeding plays a role in insects' fecundity (Boggs,1986). Most of the Lepidopteran adults feed extensively on floral nectar. Adult feeding has been considered essential for egg-laying in *H. armigera* (Hardwick, 1965) and in *Heliothiszea* (Callahan, 1962). The present study results on an adult diet fed on 10% honey, and a 10% sucrose solution laid significantly more eggs than the water-fed and food-deprived mated moth. A similar experiment was also reported in *H. armigera* Song et al. (2007) suggest that diets with sugars significantly increase fecundity. Hence, oviposition is greatly influenced by the quantity of carbohydrate provided. The lifespan of adult *L. orbonalis* male moths was usually shorter than the female moths. The longevity of virgin males and mated males was found to be 3.62±0.56 and 2.4±0.5 days, respectively (Figure 5). The lifespan of female adult moths was longer than the male moths. The longevity of virgin female moths was 5.28±0.67 days, and mated females were found to be 3.44±0.58 days(Figure 5). The virgin moths lived longer than the mated moths. A Wilcoxon Signed-Ranks test indicated that the virgin female (mean rank =12.87) was ranked more favourably than the virgin male (mean rank = 4.0), $Z = -4.285$, $p = 0.00$. A report on the longevity of male and female moth by Kavitha *et al.* (2008) was 3.5 and 5.70 days, respectively, similar to the present study results. Padwal & Srivastava (2018) showed that males' longevity was 3.40± 0.84 and 5.00 ±0.94 days for females. All the reports suggest that males' longevity is shorter than females, as is recorded even during the present study. As adults, their only function is reproduction; once the male transfers sperms to the female, its job is done, unlike the female, which has to lay eggs. Hence female lives are longer than the male in many insect species. Over all the life cycle stages of *L.orbonalis* is given in Figure 1g.

ACKNOWLEDGEMENT

We would like to thank the University Grants Commission (F1-17.1/2016-17/RGNF-2015-17-SC-KAR-19221), Government of India, for providing financial assistance and the Department of Microbiology and Biotechnology for Infrastructure facility.

REFERENCES

1. Boggs, C. L. (1986). Reproductive strategies of female butterflies: variation in and constraints on fecundity. *Ecological Entomology*, *11*(1), 7-15.
2. Boopal, K., Arya, V. V., Reddy, P. R., & Hanur, V. S. (2013). Potato a suitable alternative host for Brinjal fruit shoot and fruit borer *Leucinodes orbonalis* Guenee (lepidoptera: pyralidae). *International Journal of Agricultural Science and Research*, *3*(4), 179-184.
3. Callahan, P. S. (1962). Techniques for rearing the corn earworm, Heliothiszea. *Journal of Economic Entomology*, *55*(4), 453-457.
4. Chaitra, B.S, Saraswathi, Mamtha, R. Tannavi Kiran and D. Manjulakumari (2020). Tracking the changes in protein profile during mating in male accessory glands of *Helicoverpa armigera* (H) J. ent. Res., 44 (1): 1-6.
5. Choudhary B. Vegetables. National Book Trust, India, 1970, 25-50.
6. Gupta, Y. C., & Kauntey, R. P. S. (2007). Biology of shoot and fruit borer, *Leucinodes orbonalis* Guenee of brinjal, *Solanum melongena* Linn. at Mathura, Uttar Pradesh, India. *Asian J. Exp. Sci*, *21*(1), 155-160.
7. Hardwick, D. F. (1965). The corn earworm complex. *The Memoirs of the Entomological Society of Canada*, *97*(S40), 5-247.
8. Kavitha, V. S., Revathi, N., & Kingsley, S. (2008). Biology of brinjal pest, *Leucinodes orbonalis* Guen. of Erode region in Tamil Nadu. *Journal of Entomological Research*, *32*(3), 255-257.
9. Mamtha, R., Kiran, T. and Manjulakumari, D. 2018. An evidence of a secretory protein transferred to female during mating from the male accessory glands of *Spodoptera litura* (F.). *Int. J. Recent Sci. Res*. **9**, 23739-23743.
10. Mamtha, R., Kiran, T., & Manjulakumari, D. (2019). Accessory gland proteome of male tobacco cutworm, *Spodoptera litura* (F.)-An approach to identify proteins influencing reproductive physiology and behaviour. *Journal of Asia-Pacific Entomology*.
11. Mannan, M. A., Islam, K. S., Jahan, M., & Tarannum, N. (2015). Some biological parameters of brinjal shoot and fruit borer, *Leucinodes orbonalis* guenee (lepidoptera: pyralidae) on potato in laboratory condition. *Bangladesh Journal of Agricultural Research*, *40*(3), 381-390.
12. Maravi, M. S., Dubey, V. K., &Paikra, M. K. (2013). Biology of *Leucinodes orbonalis* Guen. on Brinjal (*Solanum melongena)* crop. *Indian Horticulture Journal*, *3*(3and4), 91-94.
13. Padwal, K. G., & Srivastava, C. P. (2018). Comparative biology of *Leucinodes orbonalis* Guenee from different regions of India in particular reference to its developmental stages. *Journal of Experimental Zoology, India*, *21*(1), 461-466.
14. Pareet DJ. Bio-rational approaches for the management of brinjal shoot and fruit borer. M.Sc. (Agri.) Thesis, University of Agricultural Sciences, Dharwad, Karnataka, India, 2006.
15. Prabhat, K., & Johnsen, S. (2000). Life cycle studies on fruit and shoot borer (*Leucinodes orbonalis*) and natural enemies of insect-pests of eggplant (*Solanum melongena). Journal of Applied Biology*, *10*(2), 178-184.
16. Regupathy A, Palanisamy S, Chandramohan N, Gunathilagaraj K. A guide on crop pests. Sooriya Desk Top Publishers, Coimbatore, 1997, 264-267.
17. Singh, Y. P., & Singh, P. P. (2001). Biology of shoot and fruit borer *Leucinodes orbonalis* Guen of egg plant Solanummelongena L at medium high altitude hills of Meghalaya A Morphological characters of the developmental stages. *Indian Journal of Entomology. September*, *633*, 221-226.
18. Song, Z. M., Li, Z., Li, D. M., Xie, B. Y., & Xia, J. Y. (2007). Adult feeding increases fecundity in female *Helicoverpa armigera* (Lepidoptera: Noctuidae).

19. Wankhede, S. M., Kale, V. D., & Gangurde, S. M. (2009). Biology of *Leucinodes orbonalis*: an important pest of brinjal. *International Journal of Plant Protection*, *2*(2), 258-260.
20. Yasuda, K., & Kawasaki, K. (1994). Mating - behavior of eggplant fruit-borer, *Leucinodes orbonalis* Guenee (Lepidoptera, Pyralidae) and capture of males in virgin-female traps. *Japanese Journal of Applied Entomology and Zoology*, *38*(4), 302-304.

Table No. 1: Life cycle of *Leucinodes orbonalis* with different stages

Sl.No	Parameters observed	Mean±S.D
1	Pre-Oviposition period (days)	1.2±0.41
2	Oviposition period (days)	2.26±0.45
3	Fecundity	247.4±21.25
4	Incubation period of eggs (days)	4.06±0.70
5	Larval instar period (days)	
	1st instar	2.2±0.41
	2nd instar	2.73±0.59
	3rd instar	2.86±0.35
	4th instar	3.66±0.48
	5th instar	3.4±0.50
	Total larval period (days)	14.86±1.06
6	Total pupal period (days)	8.46±0.74
7	Adult longevity (days)	
	Male	3.62±0.56
	Female	5.28±0.67
8	Total life cycle (days)	32.6±1.99

Figure 1:Infested brinjal fruits collected from Hesaraghatta, Bangalore, Karnataka

Figure 1a: Neonate larvae of *L. orbonalis*

Figure 1b: Mass rearing of *L. orbonalis*

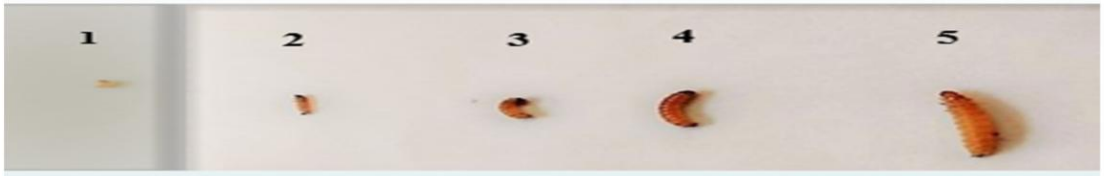


Figure 1c: Larvae of *Leucinodes orbonalis* from 1st to 5th instar

Figure 1d: The Pupae of *L. orbonalis*

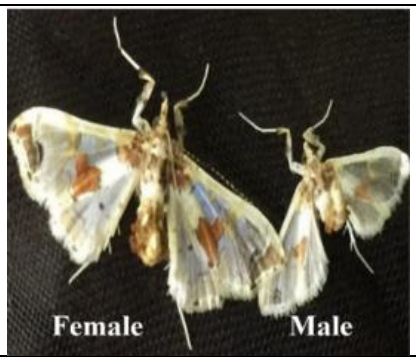


Figure 1e: *L. orbonalis* Adults

Figure 1f:A mating pair of *L. orbonalis*

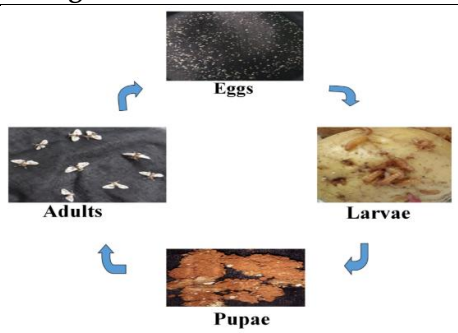


Figure 1g: Life cycle of *L. orbonalis*

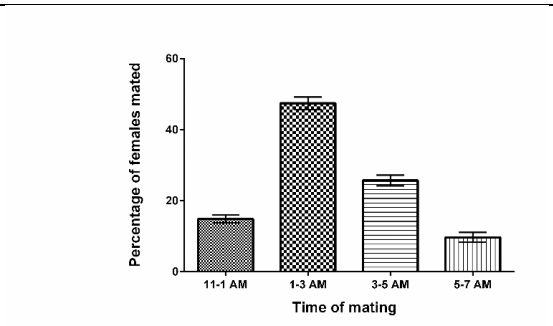


Figure 2: Time at which *L. orbonalis* females mated: Percentage of *Leucinodes orbonalis* female's adults mated, recorded from the time of the first female mated. One-Way ANOVA test showed that there is a statistically significant difference in the number of moths mated between the different time intervals, (*P* < 0.05). Values are means (*n* = 25) ± SD

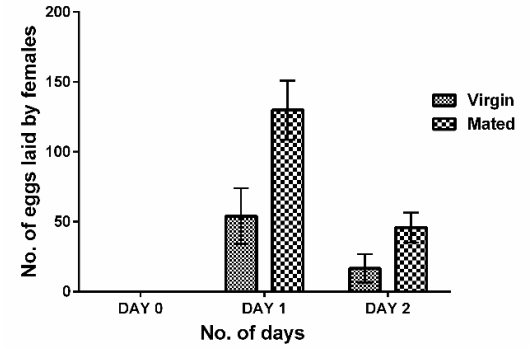


Figure 3: Oviposition behavior: Number of eggs laid by virgin and mated female during its life time, recorded on day basis. Mated moths have laid more number of eggs on each day as compared to virgins. Significant difference between the two groups was observed after Wilcoxon Rank-Sum test (Z = -3.922) (*P* < 0.05). Values are means (*n* = 20) ± SD.

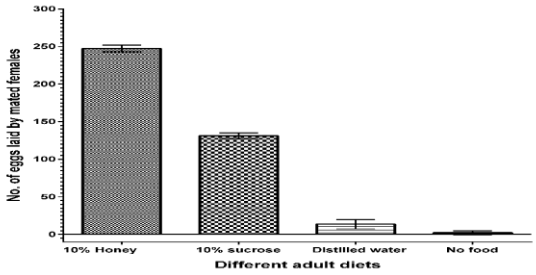


Figure 4: Effect of adult Nutrition on egg-laying: One-Way ANOVA test showed that there is a statistically significant difference in the number of egg laid between the different diet fed with mated females, (*P* < 0.05). Values are means (*n* = 25) ± SD.

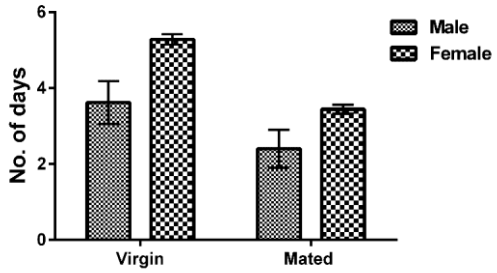


Figure 5: Longevity of *L. orbonalis* adults: Longevity of virgin and mated females during its lifetime recorded daily. There is a mean difference in longevity of virgin males and virgin females. (Wilcoxon Rank-Sum test, Z = -4.285, p < 0.05). There is a mean difference in longevity of mated males and females (Wilcoxon Rank-Sum test, Z = -3.389, p < 0.05), Values are means (*n* = 25) ± SD.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Phytochemical Analysis and Green Synthesis of Silver Nano Particles by Kariveppillai Fruits Extract - Biological Activity Study

K. R. Padmapriya[1], S. Divya Barathi[1], M. Pandeeswaran[1]*, N. Renuga Devi[2] and M. Muthumari[3]

[1]Department of Chemistry, GTN Arts College, Dindigul (Autonomous) - 624005, Tamil Nadu, India.
[2]Department of Zoology, GTN Arts College, Dindigul (Autonomous) - 624005, Tamil Nadu, India.
[3]Department of Botany, GTN Arts College, Dindigul (Autonomous) - 624005, Tamil Nadu, India.

Received: 21 Nov 2020 Revised: 23 Dec 2020 Accepted: 25 Jan 2021

***Address for Correspondence**
M. Pandeeswaran
Department of Chemistry,
GTN Arts College (Autonomous),
Dindigul, Tamil Nadu, India.
7) E mail: pandeeswaran@gmail.com

ABSTRACT

Plant-interacted green synthesis of nanoparticles has increasingly desired popularity due to its eco-friendly and low cost. In this study we synthesized silver nanoparticles and phytochemical analysis using *Murraya koenigii* fruit extract. These silver nanoparticles were characterized by various spectroscopic techniques like UV, FT-IR and SEM. These results indicated the formation of silver nanoparticles. For example UV study confirmed the nanoparticles by the peak formation at 416 nm. FT-IR study suspected that the *Murraya koenigii Fruit* (MKF) extract and $AgNO_3$ solution functioned as stabilized silver nanoparticles. Moreover, as synthesized silver nanoparticles examined for their anti bacterial properties. These result shows that silver nanoparticles against bacterial growth. Phytochemical screening analysis was carried at aqueous extract of MKF extract. The Preliminary phytochemical analysis of these extract showed the presence of many biologically active constituents. These constituents are glycosides, saponins, tannins, phenols, Flavonoids and terpinoids.

Keywords: Silver nanoparticles (AgNPs), Curry fruit extract, *Murraya koenigii*, Antimicrobial activity, Antibacterial activity, Green synthesis, Phytochemical analysis.

INTRODUCTION

Nanotechnology deals with small structures or small-sized materials. A nanometer (nm) is one billionth of a meter, or 10^{-9} m. One nanometer is approximately the length equivalent to 10 hydrogen or 5 silicon atoms aligned in a line. Small features permit more functionality in a given space [1,2]. Materials in nanometer exhibit physical properties

distinctively different from that in bulk. Nano materials are one of the main products of nanotechnology as nano-scale particles, tubes, rods, or fibers [3]. Nanoparticles are normally defined as being smaller that hundred nanometers in at least one dimension. Materials reduced to nano scale can suddenly show very different properties compared to what they exhibit on a macro scale, enabling unique applications. For instance, Copper which is an opaque substance become transparent, Platinum which is an inert material become catalyst. Aluminium which is a stable material turns combustible. Silicon insulators become conductors. Gold which is solid, inert and yellow in room temperature at micro scale becomes liquid and red in colour at nano scale in room temperature. It also gets unusual catalytic properties not seen at macro scale [4]. Nanoparticles are generally classified based on their dimensionality, morphology, composition, uniformity, and agglomeration. These are one dimensional in the nanometer scale, typically thin films or surface coatings and include the circuitry of computer chips and the anti-reflection and hard coatings on eyeglasses [5]. 1D Nanoparticle are used in Electronics, Chemistry, and Engineering. Two-dimensional nanomaterials have two dimensions in the nanometer scale. These include 2D nanostructure films, with nanostructures firmly attached to a substrate or nanopore filters used for small particle separation and filtration. Materials that are nanoscale in all three dimensions are considered 3D nanomaterials. These include thin films deposited under conditions that generate atomic-scale porosity, colloids, and free nanoparticles with various morphologies [6-7].

Nanoparticles are generally characterized by their size, morphology and surface charge, using such advanced microscopic techniques as scanning electron microscopy (SEM), transmission electron microscopy (TEM) and atomic force microscopy (AFM) [8-9]. Chemical synthesis method leads to presence of some toxic chemical absorbed on the surface that may have adverse effect in the medical applications. Green synthesis provides advancement over chemical and physical method as it is cost effective, environment friendly, easily scaled up for large scale synthesis and in this method there is no need to use high pressure, energy, temperature and toxic chemicals [10-11]. Medicinal plants have bioactive compounds which are used for curing of various human diseases and also play an important role in healing. Phytochemicals have two categories primary and secondary constituents. Primary constituents have chlorophyll, proteins, sugars and amino acids. Secondary constituents contain phenols, tannins, glycosides and terpinoids. Medicinal plants have many biological activities. The phytochemical analysis of the plants is very important commercially and has great interest in pharmaceutical companies for the production of the new drugs for curing various diseases. It is expected that the important phytochemical properties recognized by our study in the indigenous medicinal plants will be very useful in the curing of various disease in the region [12].

Currently, bactericide is mainly used to prevent and control bacterial diseases. However, the necessity for developing novel prevention and control strategies is increasing due to serious environmental pollution and bacterial resistance by the excessive use of chemicals in rice-growing countries across the world. Application of biosynthesized AgNPs is considerable interest in the field of agriculture because of their antioxidant and wide spectrum of antimicrobial activity along with their eco-friendly, biocompatible, and cost-effective nature. The nanoparticles are not only reported for plant improvement but exhibit different bactericidal mechanisms [13-15]. It is therefore of keen interest to scrutinize the inhibitory effect of synthesized AgNPs, which can be used in the field of nanotechnology as a cost-efficient, environmentally friendly and safe strategy. Therefore, we aim to synthesize AgNPs using fresh fruit extract of *Murraya koenigii* and evaluate their antibacterial properties. In the kitchen, use of leaves for a warm, appetising aroma and a subtle, spicy flavour with meat, seafood or vegetable curries, chutneys, pickles, coconut sauces, relishes, omelettes, marinades and vegetarian cuisine. The method of using the leaves (preferably fresh ones) in stir-fries and curries is to heat some oil, butter or ghee in a pan, add the curry leaves along with a little ginger and garlic and sauté until brown. The flavour of the curry leaf is enhanced when fried. Fresh curry leaves will keep for a week if kept in a dry plastic bag in the fridge [16]. *Kadipatta* or curry leaves are a rich source of iron and folic acid. Interestingly, anaemia is not only about the lack of iron in your body but also about the body's inability to absorb iron and use it. This is where folic acid comes into play. Folic acid is mainly responsible for iron absorption and since *kadipatta* is a rich source of both the compounds it is your one-stop natural remedy to beat anaemia. If you are a heavy drinker, eat a lot of fish or indulge in other activities that could be damaging your liver,

then you must eat curry leaves. The curry leaves were protect your liver from oxidative stress and harmful toxins that build-up in your body due to the presence of kaempferol, a potent antioxidant [17].

Plant description

Tree

Murraya koenigii is semi deciduous, unarmed aromatic small spreading shrub or tree with strong woody stem but slender with the stem which is dark green to brownish in colour the tree is 4–8.7m (13–31 feet) tall, with a trunk up to 81cm[18] diameter[19]. The diameter of main stem is about 16cm [19,20] .

Flower

The flowers of curry leaves is small, white fragrant and funnel- shaped, regular, pentamerous, stalked, complete, ebracteate, hypogynous, persistent, inferior, green, corolla, polypetalous, androecium, polyandrous, lanceolate, stigma, bright, sticky, style, short, ovary, inflorescence, a terminal cyme, the diameter of a flower is 1.12cm in the fully opened form, each cluster bear approximately 60 to 90 flowers at a time after flowering at once, 5-lobed calyx, with petals in having length 5 mm and the petals are 5 in number, with stamen in number 10 and in small in size approximate number 4 mm, dorsifixed, arranged into circles, with long superior gynoecium with size 5 to 6mm[21].Curry tree flowers have a sweet fragrance, bisexual with self-pollinated for produce black berries in small size with shiny appearance containing a large visible seed with the number 1[22].

Leaf

Curry leaves are aromatic in nature having characteristic aroma, leaves of curry leaves are shiny and smooth with paler undersides [23]. Leaves are pinnate, exstipulate, having reticulate venation and having ovate lanceolate with an oblique base,30 with 11-21 leaflets whose size description is each leaflet is 0.79–1.57inch long and 0.39–0.79 inch broad. Leaflets are short stalked, alternate, gland dotted and having 0.5-cm-long petiole The leaf margins are irregularly serrate[21,24]. The yield of a bush in approximately found about 480 g in three to four pickings [25].

Stem and bark

The stem of *Murraya koenigii* is brown to dark green in colour, with dots on the bark like small node on it, when the bark was peeled off longitudinally under the exposing the white wood underneath; the girth of the main stem is 16cm up to 6 meters in height and 15 to 40cm in diameter[26].

Fruit

Fruits of the *Murraya koenigii* occur in cluster form varies in 32 to 80 in number[27].The fruits are in the ovoid or subglobose and small in size in the spinach green colour seed in one or two number which are enclosing each other in thin pericarp[28]. The fruits are 1 to 1.2cm in the diameter with length 1.4 to 1.6cm, purple black after ripening and they are edible and yields 0.76% of a yellow volatile oil[29]. Curry leaf fruit is 11mm long and weigh about 445mg Fruits. The plant produces small white flowers which can self-pollinate. The weight of pulp is 880mg and the volume is 895 microliters [30]. The seeds of the *Murraya koenigii* are poisonous in nature and should not be consumed for any purpose [28,31].

MATERIALS AND METHODS

Botanical Description

In the present study we have taken up the studies on synthesizing silver nanoparticle, phytochemical analysis and antibacterial studies of *Murraya koenigii* (Fig. 1). Fruit extract (MKF)

Synonyms	: Limro (or) curry leaves
Binomial name	: *Murraya koenigii*
Family	: Rutaceae
Genus	: Murraya
Species	: Koenigii
English name	: Curry leaves
Tamil name	: கறிவேப்பிலை மரம்

Present Study

In the present study we synthesis silver nanoparticles (AgNPs) from aqueous extract of *Murraya koenigii* Fruit (MKF) using microwave assisted method and antibacterial activities with Phyto chemical analysis.

Chemicals

Silver nitrate ($AgNO_3$) was purchased from Sigma-Aldrich, India. All solutions were prepared in Millipore water and all apparatus were rinsed with aqua regia (3:1 solution of HCl, HNO_3) and then washed with Millipore water before use. All reagents and solvents used in this study were of guaranteed reagent grade.

Preparation of MKF aqueous extract by Microwave Method

About 10 gm of the fruits were soaked in 100 ML of Millipore water and irradiated with Microwave for 10 minutes. The extract was then filtered and stored at 4°C which is used for further experiments.

Phytosynthesis of MKF –AgNPs

For the phytosynthesis of AgNPs, about 5 mL of MKF aqueous extract was added to 5 mL of 0.1M $AgNO_3$ aqueous solution and kept in microwave oven at Micro level 60^0C with continuous microwave irradiation for 90 seconds. Rapid reduction of Ag^+ ions to Ag^0 was observed by the change in the color of the solution from yellowish brown to dark brown colour of MKF -AgNPs synthesized were taken up for further study.

Phytochemical Screening Analysis

Preparation of plant extract

Murraya koneigi Fruit (MKF) was taken and then washed under running tap water to remove dust. The Fruit was crushed into beaker and distilled water was added and the solution boiled for 30 minutes. Now the solution filtered with the help of filter paper and filtered extract were taken and used for further phytochemical analysis [32-35].

Test for reducing sugars

Equal volume of Fehling A and Fehling B reagents were mixed together and 2ml of it was added to crude extract and gently boiled. A brick red precipitate appeared at the bottom of the test tube indicated the presence of reducing sugars.

Test for glycoside

4ml of extract solution was dried till to 2ml. To it was added 1-ml of ammonium hydroxide and shaken. Appearance of cherish red color indicates the presence of glycosides.

Salkowski's test

Extract was mixed with 2ml of chloroform then add 2ml of concentrated H_2SO_4 was added carefully and shaken gently. A reddish brown colour indicated the presence of glycosides.

Test for phenols and Tannins

Extract was mixed with 2ml of 2% solution of $FeCl_3$. A blue –greenish blue-black indicates presence of polyphenols and tannins.

Test for flavonoids

Extract was mixed with 2ml of aqu.NaOH. A yellow orange colour indicates presence of flavonoids.

Test for saponins

Extract was mixed with 5ml of distilled water in a test tube and it was shaken vigorously for 30 seconds. The formation of stable foam even after 30 minutes was taken as an indication for the presence of saponins.

Test for steroids

Extract was mixed with 2ml of chloroform and concentrated H_2SO_4 was added sidewise. A red colour produced in the lower chloroform layer indicates presence of steroids.

Test for terpenoids

Extract was dissolved in 2ml of chloroform and evaporated to dryness. To this, 2ml of concentrated H_2SO_4 was added; a reddish brown coloration at the interface indicated the presence of terpenoids.

RESULTS AND DISCUSSION

Colour change of solution

The sequential colour change indicates the formation of Ag Nanoparticles by our plant materials. This is the primary test for the checking of formation of AgNPs. The colour reduction of $AgNO_3$ into nanoparticles was visibly evident from the colour change leaf extract was added into a silver nitrate solution [36]. Within few minutes the appearance of brown colour was observed which indicates the formation of AgNPs. The color was changed from yellow to brown (Fig. 2). This colour change indicates the formation of AgNPs [37,38].

The polyphenols, flavonoids and phytochemicals present in MKF extract not only reduces the silver salt to metallic silver but also acts as a capping agent to avoid the aggregation of silver nanoparticles[39]. This dispersion of the Ag nanoparticles in water is very clear and stable for more than one month without settling down which shows that the particles are small in size and no aggregation of particles is taking place[40].

UV- Visible spectroscopy

UV- Vis absorbance spectroscopy has been proved to be a very useful technique for the analysis of nanoparticles formation and stability of metal nanoparticles in aqueous solution. In order to determine completion of the reaction, $AgNO_3$ was reacted with MKF aqueous extract and spectra were recorded. Fig. 3 shows the UV- Vis spectra of the synthesized silver nanoparticles. The dark brown colour of AgNPs is attributed to Surface Plasmon Resonance (SPR) appearing at 416 nm [39,41].

In extract concentration variation study

UV–visible absorbance spectroscopy has been proved to the peak positions and shapes are sensitive to particle size. Figure 3a, shows the UV–Visible absorption spectra of the silver nanoparticles with different concentration of leaf extract addition amount of 0.2, 0.4, 0.6 and 0.8 ml [42]. MKF extract as well as silver nitrate solution (1 mM) were used as controlled condition. All experiments were carried out in triplicates and representative data is presented here.

Light brown and dark reddish brown colours were observed at salt concentrations of 0.6 and 0.8 mM respectively, and darker shades of reddish brown colour were observed at silver nitrate concentrations ranged from 0.2 to 0.8mM. The SPR peak of silver nanoparticles became distinct with increasing the concentration of silver nitrate, the maximum peak intensity was obtained at 0.8mM of AgNO3 (Fig. 3b). A variation in the biological material and metal salt concentration is known to influence nanoparticle synthesis [43,44]. The reaction mixtures containing 0.2, 0.4, 0.6 and 0.8 ml of MKF developed a light reddish brown colour, while those containing 0.2 up to 0.8 ml of MKF developed darker reddish brown colour. The SPR peaks were proportionally more intense and the maximum peak intensity was observed at MKF content of 0.8 ml (Figure 3b).

Time dependent study

Effect of the reaction time on MKF extract of AgNPs synthesis was also evaluated with UV-Visible spectra and it is noted that with an increase in time the peak becomes sharper. The increase in intensity could be due to increasing number of nanoparticles formed as a result of silver ions presented in the aqueous solution are shown in Figure 3c. Electronic absorption spectrum of silver nanoparticle is shown in Fig. 3c. Broad bell-shaped spectrum curve was obtained from UV–Visible spectra analysis. Various metabolites from MKF plant extract introduced to solution make the plasmon band broad because they may be read in this spectrophotometric range, too. Surface Plasmon Resonance (SPR) of silver occurs at 416 nm. This peak increased with time up to 30 min. According to Mie theory [45], spherical nanoparticles show only a single SPR band. The number of peaks increases by increasing diversity of particles shapes [46,47]. Then, it can be concluded that biosynthesized AgNPs are unanimously spherical in nature.

FT-IR study

Figure 4, the FT-IR spectrum of the extract, gives information regarding the chemical transformation of the functional groups involved in the reduction of the silver ions. Some pronounced absorbance bands centered at 652, 1045, 1419, 1561, 2924 and 3432 cm^{-1} were observed in the region 400 - 4000 cm^{-1}. Among them, the absorbance bands at 652, 1045, 1561, 2924 and 3432 cm^{-1} were associated with the stretch vibration of –C-O, -C-H, -C=C, CH_2, NH and O-H respectively [32]. These absorbance bands could be attributed to the reducing sugars, flavonoids, saccharides and proteins in the extract, while the absorbance band at 1057 cm^{-1} could be regarded as a fingerprint of the biomolecules. In the present study, the band at 3440 cm^{-1} may be the result of the –O-H groups of the reducing sugars, flavonoids, saccharides and proteins in the extract. The reducing sugars among the saccharides acted as reduction while the other saccharides as protecting agents. The functional groups associated with these saccharides as well as the protein matter may thus be involved in reducing the Ag^+ to Ag^0. Biological components are known to interact with metal salts via these functional groups and mediate their reduction to nanoparticles [47].

SEM Study

The shape of the synthesized silver nanoparticles was analyzed by SEM, representative SEM micrographs of control and treated MKF magnified at 10 μm and 2 μm are shown in Fig. 5a and 5b, respectively. Monodispersed spherical silver nanoparticles were formed on the surface of MKF derived biological materials as indicated in Fig.5b. The image obtained by the FESEM also showed spherical nanoparticles (Fig.5b), confirming the result obtained by SEM.

Phytochemical study

The change of colour was observed when the test reagent was added to the prepared sample for the photochemical test. The result was recorded as present (+) or absent (-) depending on the outcome of the test. The entire test was repeated. The result denoted in the following table 1. Analysis of the plant extract revealed the presence of phytochemicals such as phenols, saponin, glycosides, tannins, flavonoids, steroids and terpinoids. These phytochemicals which are exhibit medicinal and biological activities. The evidence of workers had reported the analgesic, antispasmodic, antibacterial properties of alkaloids. Glycosides have lowered the blood pressure. Phenolic compound revealed many biological activities such as apoptosis, anti-aging, anti-carcinogen, anti-inflammation, anti-atherosclerosis, cardiovascular protection, and improvement of endothelial function as well as inhibition of

angiogenesis. Saponins was cured the inflammatory effect. Steroids have been the antibacterial effect and also this compound important function involved in sex hormones. Tannins can be inhibited the growth of fungi, yeast, bacteria and viruses. Terpinoids were attributed for analgesic and anti-inflammatory effect [14]. The results obtained in this study the identified phytochemical compounds proving to be an increasingly valuable reservoir of bioactive compounds of plants.

Antibacterial activity of MKF, MKF-AgNPs and the standard

The results of antibacterial activity of the photo synthesized silver nanoparticles with a larger surface area against Gram positive (*Streptococcus*) microorganisms are presented in Fig. 6a. The details of inhibition zone around the disk of approximately 6 mm each with MKF aqueous extract, MKF-AgNPs and standard against the test strains at test concentration are shown in Table 2. Flavones, polyphenols and isoflavones, were the major constituents of MKF, and these compounds were most likely responsible for the antibacterial efficacy of the plant [39]. The inhibition results indicated that polyphenols from MKF might be responsible for the increased antimicrobial activity of MKF capped silver nanoparticles. Both the MKF extract and MKF-AgNP showed only moderate antibacterial activity when compared with the standard drug (*Streptococcus aureus*). From the antibacterial study results for MKF, MKF-AgNPs and the standard when tested at different concentrations against four different organisms (Fig. 6 a,b) it is clear that both MKF and MKF-AgNPs showed appreciable antibacterial activity only at a higher concentration of 10mg/ml and exhibited only mild antibacterial activity at all other lower concentrations. This finding indicated that the larger surface area of the nanoparticles can act more effectively on the bacterial cells through the membrane and its charge-related aspects [39].

The result showed that AgNPs were effective against both strains, in gram positive bacteria *Streptococcus,* the maximum zone of inhibition was 18 mm at 10 µL concentration. The *Staphylococcus aureus* showed 6 mm zone of inhibition, the gram negative bacteria *E.coli* was 7 mm of zone inhibition and absence of inhibition effect in parasitic fungi of candida [48]. Silver nanoparticles showed more bactericidal activity compared with the silver salt, the inhibition zone diameter were (12-18 mm) and (6 mm), respectively [49,50]. The high bactericidal activity of silver nanoparticles is due to their extremely large surface area, which provides better contact with microorganisms. Moreover, silver nanoparticles act as reservoirs for the Ag^+ bactericidal agent [44,51].

CONCLUSION

In our study, it has been observed thus that MKF – AgNPs synthesized under Microwave assisted condition using MKF were spherical in shape and they can be a good capping as well as a stabilizing agent for synthesis of MKF – AgNPs. The observation that the MKF – AgNPs synthesized could be stored for two months at room temperature indicated that these particles are much more stable than the silver nanoparticles synthesized by other synthetic and biological routes. This green chemistry MKF approach towards the synthesis of MKF – AgNPs has many advantages such as, ease with which the process can be scaled up, high-yield, speed, and low cost etc., The nanoparticles were characterized by UV–visible, SEM, and FT-IR measurements. These silver nanoparticles were of high purity, making them potentially useful for biological applications [52]. The MKF–AgNPs significant bactericidal activity against Gram-positive (*Streptococcus)* pathogens. Analysis of the plant extract revealed the presence of phytochemicals such as alkaloids, saponins, glycosides, tannins, reducing sugars, flavonoids, steroids and terpinoids. These phytochemicals exhibit medicinal and biological activities. The results of the present study indicated that the aqueous extract of MKF have a rich source of phytochemicals and exhibit biological properties of the plants studied in the treatment of different ailments. The traditional medicine practice is recommended strongly using these plants as well as it is suggested that further work should be carried out to isolate, purify and characterize the active constituents responsible for the activity of these plants[51]. Also additional work is encouraged to see whether these plants have said health benefits, especially as anti cancer drugs and elucidate the possible mechanism of action of these extracts.

ACKNOWLEDGEMENT

One of the authors (Dr. M. Pandeeswaran) is thankful to UGC (Autonomous Grant) for providing financial assistance to purchase of UV-visible spectrophotometer.

REFERENCES

1. Mohanpuria, P., Rana N.K. and Yadav, S.K., J. Nanopart. Res.10, 507–517 (2008).
2. Singh, Ravi Chand, Manmeet Pal Singh, and Hardev Singh Virk. Solid State Phenomena, 201, 131-158 (2013).
3. Kohler, J.M., Csaki, A., Reichert, R., Straube, W. and Fritzche, W., Sens. ActB. 76, 166-172, (2001).
4. Schatz, G.C., Lazarides, A.A., Kelly, K.L. and Jensen T.R. J. Mol. Structure (Theochem), 529, 59-63 (2000).
5. Kim, B S., Song, J Y., CRC Press, Boca Raton, pp 399–407 (2009).
6. Zygmunt Sadowski, Wroclaw University of Technology, Poland, CRC Press, Boca Raton, pp 399–407 (2009).
7. Mafune, F., Kohno, J., Takeda, Y., Kondow, T., *J. PhysChem B*, 107, 4218-23, (2003)
8. Krutyakov, Y., Olenin, A., Kudrinskii, A., Dzhurik, P., Lisichkin, G., Nanotechnol *Russia*, 3, 303-308 (2008).
9. Bai, J., Li, Y., Du, J., Wang, S., Zheng, J., Yang, Q., Chen Mater, X., PhysiChem, 106, 412-418 (2007).
10. Mafune, F., Kohno, J., Takeda, Y., Kondow, T., J. PhysChem. *B*, 107, 4218-23, (2003).
11. Mohanpuria, P., Rana, N.K., Yadav, S.K., J Nanopart Res, 10, 507-17 (2008).
12. Mann, S., Biomimetic Materials Chemistry. New York: VCH, 43, (1996).
13. Jain D., Kumar Diama H., Kachhwaha S., Kothari S.L. *Digest* J.Nanomat. Biostruct. 4(3), 557-563, (1999).
14. Padmapriya K.R., Divya Barathi S., Pandeeswaran M., Int. J. All Resear. Edu. Scientific Methods, 8(11), 1-5, (2020).
15. Aziz, N., Faraz, M., Pandey, R., Shakir, M., Fatma, T., Varma, A., Langmuir 31, 11605–11612. (2015).
16. Rengasamy Balakrishnan ., Dhanraj Vijayraja ., Song-Hee Jo . , Palanivel Ganesan .., In Su-Kim ,Dong-Kug Choi ., journal of antioxidants,(2020).
17. Mahawar, H., and Prasanna, R., Environ. Nanotech. Monitor Manage, 10, 477–485, (2018).
18. Dutta NL, Quasim C, Wadia MS.. *Indian T Chem*. 7:1061–1062. (1969)
19. Ganesan P, Phaiphan A, Murugan Y, et al. *Journal of Chemical and Pharmaceutical Research*. 5(11):590–594.(2013)
20. Nagappan T, Ramasamy P, Effendy M, et al. Lines. *Molecules*. 16(11):9651–9664.(2011)
21. Prakash V, Natarajan CP. *Food Sci and Technol*. 11(6):284–28.(1974)
22. Narasimha NS, Paradkar MV, Chitguppi VP, et al. *Indian Jour Chem*. 13(10):993–999.(1975)
23. Gahlawat DK, Jakhar S, Dahiya P. *Murraya koenigii* (L.) Spreng*Journal of Pharmacognosy and Phytochemistry*.3(3):109–119.(2014)
24. Leela KS, Natarajan AV, Devi K, et al *International Journal of Pharmaceutical & Biological Archives*.;4(3):493–497.(2013)
25. Roy MK, Thalang VN, Trakoontivakorn G, et al. *Biochem Pharmacol*. 2004;67(1):41–51.
26. Sastri BN.The Wealth of India, *Council of Scientific and Industrial Research (New Delhi)*.;6:446–447.(1949)
27. Prabhu KA, Tamilanban T. *International Journal of Research in Pharmacology and Pharmacotherapeutics*.1(2):165–168.(2012)
28. S, Prakash J. *Plant Foods Hum Nutr*.;64(1):39–45.(2009)
29. Sathaye S, Amin PD, Mehta VB, et al. *International Journal of Pharma and Bio Sciences*. 3(1):430–438. (2012)
30. Khosa RL, Prasad S. *J Res Indian Med*.;9(3):105. (1974)
31. Manshu Jain., Ritu Gilhotra., Ravindra Pal Singh., Jitendra Mitta, *MOJ Biology and Medicine* 2 (3) – (2017)
32. Oves, M., Aslam, M., Rauf, M. A., Qayyum, S., Qari, H. A., Khan, M. S., Mater. Sci. Eng. C Mater. Biol. Appl. 89, 429–443 (2018).
33. Dinsmore, A.D., Hsu, M.F., Nikolaides, M.G., Marquez, M., Bausch, A.R., Weitz, D.A., *Science*, 298, 1006-1009 (2002).
34. Elghanian, R., Storhoff, J.J., Mucic, R.C., Letsinger, R.L., Mirkin, C.A., *Science*, 277, 1078-1081 (1997).
35. Magnusson. M., Deppert, K., Malm, J., Bovin, J., Samuelson, L., J Nanopar Res, 1, 243-249 (1999).
36. Chandran, S.P., Chaudhary, M., Pasricha, R., Ahmad, A., Sastry, M., 22, 577–583 (2006).
37. Kumar, P., Selvi, S.S., Govindaraju, M., Appl. Nanosci. 3, 495–500 (2013).
38. Muniyappan, N., Nagarajan, N.S., Process Biochemistry, 49(6), 1054-1061 (2014).

39. Nikesh Gupta, Henam Premananda Singh, Rakesh Kumar Sharma. Colloids and Surfaces A: Physicochemical and Engineering Aspects, 367(1-3), 102-107 (2010).
40. Banerjee, P., Satapathy, M., Mukhopahayay, A., Das, P., Bioresour. Bioprocess. 1, 1–10 (2014).
41. Shikha Jain, Ankita Jain, Pranav Kachhawah, Vijay Devra. Transactions of Nonferrous Metals Society of China, 25(12), 3995-4000 (2015).
42. Hayatam.M.M. Ibrahim. Journal of Radiation Research and Applied Sciences, 8(3), 265-275 (2015).
43. Raut Rajesh, W., Lakkakula Jaya, R., Kolekar Niranjan, S., Mendhulkar Vijay, D., Kashid Sahebrao, B., Curr. Nanosci. 5, 117–122, (2009).
44. Ibrahim M.M.H., J. Rad. Resear. Appl. Sci., 8, 265-275, (2015).
45. Ghaforyan, H., Ebrahimzadeh, M., Bilankohi, S.M., World appl. programming, 5(4), 79-82 (2015).
46. Bankar, A., Joshi, B., Kumar, A. R., Zinjarde, S., Colloids and Surfaces A-Physicochemical and Engineering Aspects, 368, 58-63, (2010).
47. Bar, H., Bhui, D. K., Sahoo, G. P., Sarkar, P., Pyne, S., & Misra, A., Colloids and Surfaces A-Physicochemical and Engineering Aspects, 348, 212-216, (2009).
48. Wiegand, I., Hilpert, K., & Hancock, R. E. W., Nature Protocols, 3(2), 163-175, (2008).
49. Bilal, M.; Rasheed, T.; Iqbal, H. M.; Li, C.; Hu, H.; Zhang, X., Int. J. Biol. Macromol., 105, 393–400, (2017).
50. Infant Tresa J., Pandeeswaran M., Renugadevi N., Dharaneedharan S., Emperor Int. J. Finance and Manag. Resear., 4(2), 142-148, (2018).
51. Ashokkumar, S., Ravi, S., Velmurugan, S., Spectrochimica Acta Part A Molecular and Biomolecular Spectroscopy, 115, 388-392 (2013).
52. Marambio-Jones, C., Hoek, E. M. V., J. Nanoparticle Research, 12, 1531-1551, (2010).

Table 1. Result of preliminary qualitative Phyto chemical analysis

S.No	Phytoconstituents	Result
1	Carbohydrates	--
2	Phenols	++
3	Alkaloids	--
4	Glycosides	++
5	Flavonoids	++
6	Protiens	--
7	Quinones	--
8	Saponins	++
9	Sterols	--
10	Tannins	++
11	Terpinoids	++
12	Ketones	--

Note: ++ indicates present, -- indicates absent.

Table.2 Antibacterial activity MKF-AgNPs

S.No	Test	Zone of inhibition (mm)			
		Streptococcus (Gram +ve)	*Staphylococcus aureus* (Gram +ve)	*E. coli* (Gram -ve)	*Candida* (Fungi)
1	Plant extract	4	3	4	4
2	Plant extract + $AgNO_3$	18	6	7	5

Figure 1. ***Murraya koenigii***

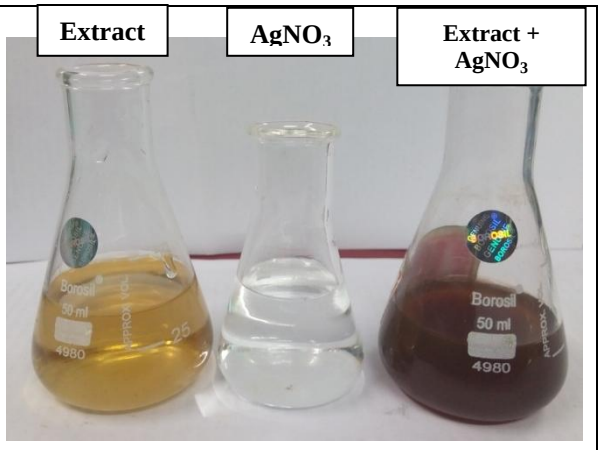


Figure 2. Colour change indicating synthesis of Nanoparticles

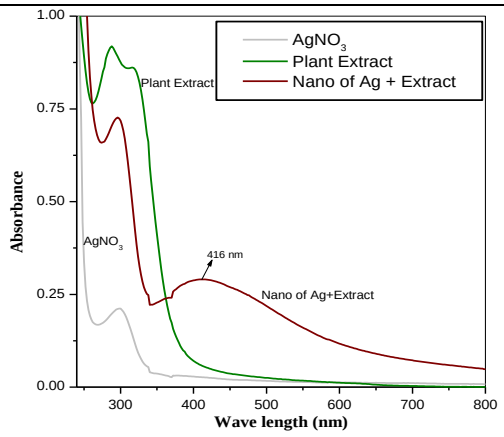


Figure 3. UV- Visible spectra for aqueous extract MKF and MKF-Silver nanoparticles at room temperature

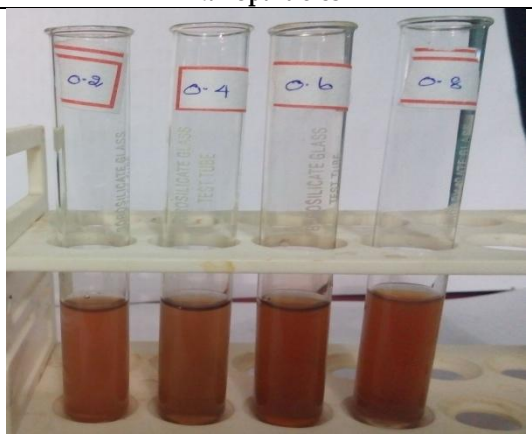


Figure .3a UV–Visible absorption spectra of the silver nanoparticles with different concentration of leaf extract

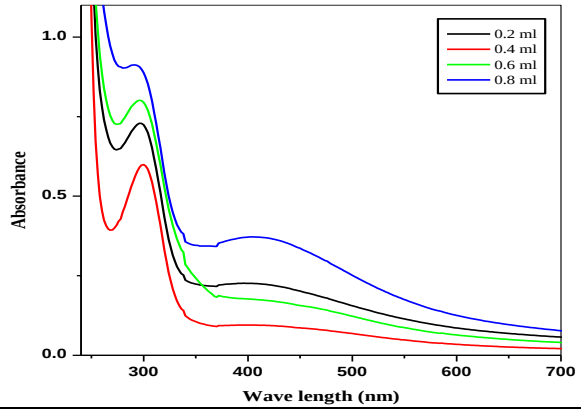


Figure. 3b UV–Visible absorption spectra of silver nanoparticles biosynthesized by MKF extract during reaction

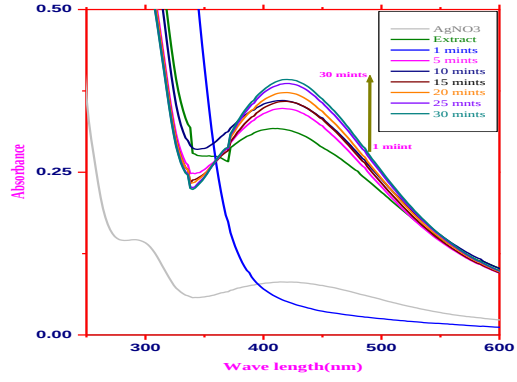


Figure 3c. Time variation graph for MKF-Ag nanoparticles for 1 minute to 30 minutes at room temperature

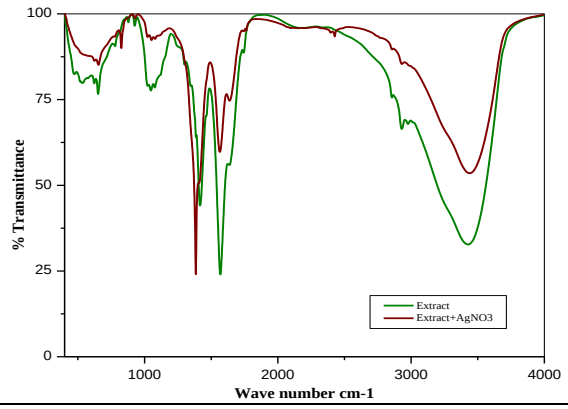


Figure 4. FT-IR Spectra for MKF extract and Silver nanoparticles with MKF aqueous extract

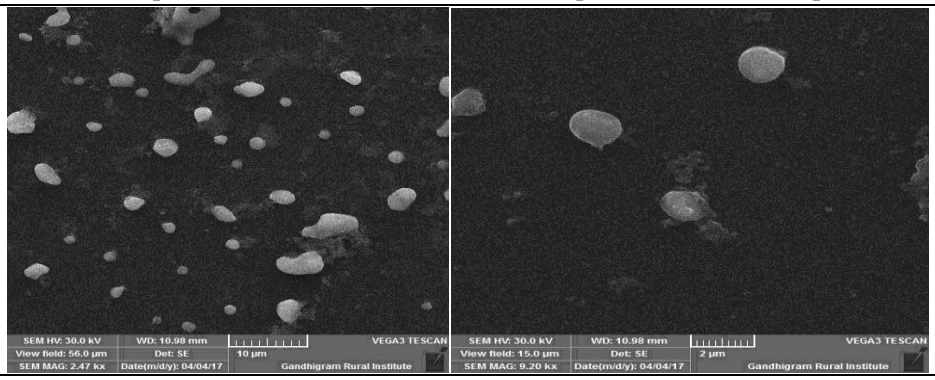


Figure 5a, 5b. SEM image of aqueous extract of MKF with silver nanoparticles

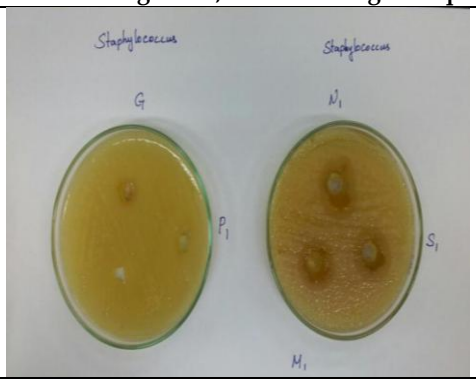


Fig. 6a. Antimicrobial activity of MKF and AgNPs and the standard Against *Streptococcus*

Fig. 6b. Antimicrobial activity of MKF and AgNPs and the standard Against *Staphylococcus aureus*

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

A Review on Targeting of Drugs to Brain

Nihar Ranjan Kar*

Centurion University of Technology and Management, Odisha, India.

Received: 21 Nov 2020 Revised: 06 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
Nihar Ranjan Kar
Centurion University of Technology and Management,
Odisha, India.
Email: nihar_795@rediffmail.com

ABSTRACT

The stand up to in drug focusing on isn't just the focusing of medication to a positive site yet in addition holding it for the favored term to acquire pharmacological accomplishment. Medication focusing to brain by going around the physiological boundaries is a precondition for drugs following up on Central Nervous system (CNS) and mending capability of numerous medications can be improved by adequately focusing on the drug(s) to brain. The brain is a pitiful organ, and nature has capably secured it. Medication discharge into the brain was difficult because of the continuation of blood brain barrier, which just permits a couple of atoms to stretch out beyond openly. The brain is secured lined up with conceivably by the presence of two hindrance frameworks: the blood brain barrier (BBB) and the blood cerebrospinal fluid barrier (BCSFB). The blood brain boundary is confronting the blood side and is the significant hindrance for moving of macromolecular medications from blood to brain. In this way, focusing on methodologies to the BBB are fundamental to specifically and explicitly transport macromolecular medications to the brain.

Keywords: Drug Targeting, Brain, Blood Brain Barrier, Blood Cerebrospinal fluid barrier, macromolecules.

INTRODUCTION

Sicknesses of the Central Nervous System (CNS) are plentiful and impact a significant piece of the world's occupants. Stroke, ischemia, human immunodeficiency virus 1 (HIV-1) contamination, epilepsy, and other mental issues, for example, nervousness, melancholy and schizophrenia are upsetting conditions that unmistakably impact the dismalness and demise in current society. The neurodegenerative sicknesses, for example, Alzheimer's disease (AD), Parkinson's disease (PD) and numerous sclerosis are described by manifestations identified with development, memory, and dementia because of the progressive loss of neurons. Brain tumors, including gliomas, astrocytomas and glioblastomas, comprise a pertinent and unsolved clinical issue and the therapy of brain malignancies are

significant difficulties.[1] Tragically, barely any sheltered and compelling techniques are known for conclusion and treatment of CNS problems and this is predominantly because of the anatomical qualities of the CNS. The narrow microvessels of the brain have advanced to keep the entry of atoms and cells among blood and brain, on condition that a characteristic opposition against coursing harmful or irresistible specialists. The similar impermeability of the Blood–Brain Barrier (BBB) results from firm intersections and disciple intersections including slender endothelial cells framed by cell bond particles. Brain endothelial cells likewise contain few exchange transport pathways (e.g., fenestra, transendothelial channels, pinocytotic vesicles), and pass on significant levels of dynamic efflux transport proteins, including P-glycoprotein (P-gp, MDR-1 or ABCB1) and bosom malignant growth obstruction protein (BCRP, ABCG2). The BBB keeps up basic mind homeostasis yet as a result, speaks to a vital check to the proficient fix of many brain diseases [2].

Advantages

1. Result and harmfulness decreases.
2. Portion of medication diminishes by focusing on organ.
3. Dodges debasement of medication (first pass metabolism).
4. Bioavailability increases.
5. Variance in focus diminishes.
6. Porousness of proteins and peptide increments [3]

Disadvantages

1. Upgrades freedom from target.
2. Hard to target tumor cells.
3. Progressed strategies necessities.
4. Skill persons required.
5. Now and again it might causes poisonousness.
6. Hard to keep up strength of measurement structure, for example Resealed erythrocytes must be put away at 4^0c [4]

Barriers to CNS Drug Delivery

The malfunction of systemically delivered drugs to efficiently extravagance lots of CNS ailments can be streamlined by bearing in brain a digit of barriers that reduce drug delivery to the CNS.

Blood-Brain Barrier

It is at present all around perceived that the BBB is a sole membranous divider that solidly isolates the brain from the circling blood. The CNS comprise blood vessels which are fundamentally different from the blood vessels in different tissues; these auxiliary contrasts bring about a penetrability divider flanked by the blood inside brain vessels and the extracellular liquid in brain tissue. Vessels of the vertebrate brain and spinal cord do not have the little pores that permit fast development of solutes from dissemination into different organs; these vessels are fixed with a layer of extraordinary endothelial cells that need fenestrations and are fixed with tight intersections. Tight epithelium, comparable in nature to this obstruction, is additionally found in different organs (skin, bladder, colon, and lung). This porousness boundary, containing, the brain capillary endothelium, is known as the BBB. Ependymal cells covering the cerebral ventricles and glial cells are of three sorts. Astrocytes structure the auxiliary casing work for the neurons and control their biochemical climate. Astrocytes foot cycles or appendages that spread out and adjoining one other, embody the vessels are firmly connected with the veins to frame the BBB. Oligodendrocytes are answerable for the arrangement and upkeep of the myelin sheath, which encompasses axons and is fundamental for the quick transmission of activity possibilities by saltatory propagation. Microglias are blood inferred mononuclear macrophages. The tight intersections between endothelial cells brings about a high trans-endothelial electrical obstruction of 1500-2000 $\Omega.cm^2$ contrasted with 3-33 Ω. cm^2 of different tissues which diminishes the watery based para-cell diffusion that is seen in different organs. Miniature vessels make up an expected 95% of the complete

surface zone of the BBB, and speak to the chief course by which synthetic compounds enter the brain. Vessels in mind were found to have to some degree more modest breadth and more slender divider options to keep transport from the albuminal extracellular fluid (ECF) to the brain ECF. The choroid plexus might be of significance while considering the vehicle of peptide drugs, since it is the significant site of cerebrospinal-fluid (CSF) creation, and both the CSF and brain ECF unreservedly trade. The BBB likewise has an extra enzymatic angle. Solutes crossing the cell membrane are thusly presented to debasing chemicals present in huge numbers inside the endothelial cells that contain huge densities of mitochondria, metabolically profoundly dynamic organelles. BBB catalysts likewise perceive and quickly debase most peptides, including normally happening neuropeptides. At long last, the BBB is additionally strengthened by a high convergence of P-glycoprotein (Pgp), dynamic – drug-efflux-carrier protein in the luminal layers of the cerebral capillary endothelium. This efflux carrier effectively eliminates an expansive scope of medication atoms from the endothelial cell cytoplasm before they cross into the brain parenchyma [5-6].

Blood-Cerebrospinal Fluid Barrier

The second barrier that a systemically administered drug encounters before entering the CNS is known as the blood-cerebrospinal fluid barrier (BCB). Since the CSF can exchange molecules with the interstitial fluid of the brain parenchyma, the passage of blood-borne molecules into the CSF is also carefully regulated by the BCB. Physiologically, the BCB is found in the epithelium of the choroids plexus, which are arranged in a manner that limits the passage of molecules and cells into the CSF. The choroid plexus and the arachnoid membrane act together at the barriers between the blood and CSF. On the external surface of the brain the ependymal cells fold over onto themselves to form a double layered structure, which lies between the dura and pia, this is called the arachnoid membrane. Within the double layer is the subarachnoid space, which participates in CSF drainage. Passage of substance from the blood through the arachnoid membrane is prevented by tight junctions. The arachnoid membrane is generally impermeable to hydrophilic substances, and its role is forming the Blood-CSF barrier is largely passive. The choroid plexus forms the CSF and actively regulates the concentration of molecules in the CSF. The choroid plexus consist of highly vascularized, "cauliflower like" masses of pia mater tissue that dip into pockets formed by ependymal cells. The preponderance of choroid plexus is distributed throughout the fourth ventricle near the base of the brain and in the lateral ventricles inside the right and left cerebral hemispheres. The cells of the choroidal epithelium are modified and have epithelial characteristics. These ependymal cells have microvilli on the CSF side, basolateral interdigitations, and abundant mitochondria. The ependymal cells, which line the ventricles, form a continuous sheet around the choroid plexus. While the capillaries of the choroid plexus are fenestrated, non-continuous and have gaps between the capillary endothelial cells allowing the free-movement of small molecules, the adjacent choroidal epithelial cells form tight junctions preventing most macromolecules from effectively passing into the CSF from the blood.

However, these epithelial- like cells have shown a low resistance as compared the cerebral endothelial cells, approximately 200 $\Omega.cm^2$, between blood and CSF. In addition, the BCB is fortified by an active organic acid transporter system in the choroids plexus capable of driving CSF-borne organic acids into the blood. As a result a variety of therapeutic organic acids such as the antibiotic penicillin, the anti-neoplastic agent methotrexate, and the antiviral agent zidovudine are actively removed from the CSF and therefore inhibited from diffusing into the brain parenchyma. Furthermore, substantial inconsistencies often exist between the composition of the CSF and interstitial fluid of the brain parenchyma, suggesting the presence of what is sometimes called the CSF-brain barrier. This barrier is attributed to the insurmountable diffusion distances required for equilibration between the CSF and the brain interstitial fluid. Therefore, entry into the CSF does not guarantee a drug's penetration into the brain than vessels in other organs. Also, the mitochondrial density in brain micro-vessels was found to be higher than in other capillaries not because of more numerous or larger mitochondria, but because of the small dimensions of the brain micro-vessels and consequently, smaller cytoplasmic area. In brain capillaries, intercellular cleft, pinocytosis, and fenestrae are virtually nonexistent; exchange must pass trans- cellularly. Therefore, only lipid-soluble solutes that can freely diffuse through the capillary endothelial membrane may passively cross the BBB. In capillaries of other parts of the body, such exchange is overshadowed by other nonspecific exchanges. Despite the estimated total length of

650km and total surface area of 12 m^2 of capillaries in human brain, this barrier is very efficient and makes the brain practically inaccessible for lipid- insoluble compounds such as polar molecules and small ions. As a consequence, the therapeutic value of many promising drugs is diminished, and cerebral diseases have proved to be most refractory to therapeutic interventions. Given the prevalence of brain diseases alone, this is a considerable problem. Practically all drugs currently used for disorders of the brain are lipid-soluble and can readily cross the BBB following oral administration. Although antimicrobial b-lactam antibiotics, when administered intracerebroventricularly, cause severe convulsion, fortunately these antibiotics, When administered intravenously or orally, do not cause such central nervous system (CNS) side effect because their limited transport across the blood–brain barrier (BBB). Further, in spite of being well distributed into various tissues, a lipophilic new quinolone antimicrobial agent, grepafloxacin, cannot enter the brain, resulting in the avoidance of CNS side effects such as headache and dizziness due to the displacement of g-aminobutyric acid (GABA) from the GABA receptor binding sites. On the other hand, benzodiazepines such as diazepam have been used as sedative-hypnotic agents, because these lipophilic drugs readily cross the BBB. However, the BBB transport of an immunosuppressive agent, cyclosporin A, which is more lipophilic than diazepam, is highly restricted. Similarly, almost all of the lipophilic anticancer agents such as doxorubicin, epipodophylotoxin and Vinca alkaloids (e.g., vincristine and vinblastine) hardly enter the brain, causing difficulty in the treatment of brain tumors. Although levodopa, which is useful for treatment of Parkinson's disease, is very hydrophilic, it can readily penetrate the BBB. In order to avoid overlap with this section, the drug transport across the BBB of small-molecular drugs by carrier-mediated transport and of peptide drugs by the adsorptive-mediated transcytosis are discussed respectively. Some regions of the CNS do not express the classical BBB capillary endothelial cells, but have micro-vessels similar to those of the periphery.

These areas are adjacent to the ventricles of the brain and are termed the circumventricular organs (CVOs). The CVOs include the choroid plexus, the median eminence, neurohypophysis, pineal gland, organum vasculosum of the lamina terminalis, subfornical organ, subcommisaral organ and the area postrema. Though in the CVO brain regions the capillaries are more permeable to solutes, the epithelial cells of the choroid plexus and the tanycytes of other regions form tight junctions to prevent transport from the abluminal extracellular fluid (ECF) to the brain ECF. The choroid plexus may be of importance when considering the transport of peptide drugs, because it is the major site of cerebrospinal-fluid (CSF) production, and both the CSF and brain ECF freely exchange. The BBB also has an additional enzymatic aspect. Solutes crossing the cell membrane are subsequently exposed to degrading enzymes present in large numbers inside the endothelial cells that contain large densities of mitochondria, metabolically highly active organelles. BBB enzymes also recognize and rapidly degrade most peptides, including naturally occurring neuropeptides. Finally, the BBB is further reinforced by a high concentration of P-glycoprotein (Pgp), active –drug-efflux-transporter protein in the luminal membranes of the cerebral capillary endothelium. This efflux transporter actively removes a broad range of drug molecules from the endothelial cell cytoplasm before they cross into the brain parenchyma [7-9].

Increasing Permeability of Drug through BBB

Low molecular weight, unionized at physiological pH and better lipophilicity require for better permeation from blood brain barrier. For example heroin crosses the BBB about 100 times faster than morphine due to its lipophilic nature. Hydrophilic drug can't cross BBB easily as compared with lipophilic drug. Incorporation lipophilic groups to hydrophilic drugs penetration of BBB enhances. The two techniques of drug permeation enhancement are:

Lipophilic Approach: Lipophilicity requires for the drug penetration through the BBB. The drug molecule should have Log P value of approximately 1.5 to 2.5 with an optimum octanol-water partition coefficient [10].

Prodrugs Approach: To improve the drug's pharmacokinetic properties prodrug form of them may be used. A prodrug consists of a drug covalently linked to an inert chemical moiety. The active drug is formed when the attached moiety in prodrug is cleaved by hydrolytic or enzymatic processes. In prodrugs the attaching chemical

moieties should be such that it enhances the lipoidal nature of the drug. For example,various analogues of morphine. BBB is not readily crossed by morphine whereas acetylated product of morphine (heroin) readily traverses the BBB, and on subsequent cleavage of the acetyl groups by hydrolysis yields morphine. Hence in brain there is accumulation of morphine because of its hydrophilicity. Prodrug formation of a drug improves the brain uptake of drugs. For example, chemical modifications such as esterification of hydroxyl group, amidation of hydroxyl-, or carboxylic- groups of a drug, may increase the lipophilicity of drug and, as a result of which entry into the brain enhances [11-12].

Carriers Drug Delivery System

There are various carriers system used in brain targeting. Some of they include the following

Liposomal Technology

A liposome is a spherical vesicle having at least one lipid bilayer. The liposome can be used as a vehicle for administration of nutrients and pharmaceutical drugs. Liposome's are most often composed of phospholipids, especially phosphatidylcholine, but may also include other lipids, such as egg phosphatidylethanolamine, so long as they are compatible with lipid bilayer structure. A liposome design may employ surface ligands for attaching to unhealthy tissue. The major types of liposome' s are the multilamellar vesicle (MLV, with several lamellar phase lipid bilayers), the small unilamellar liposome vesicle (SUV, with one lipid bilayer), the large unilamellar vesicle (LUV), and the cochleate vesicle. A less desirable form are multivesicular liposome's in which one vesicle contains one or more smaller vesicles. Liposomes should not be confused with micelles and reverse micelles composed of monolayers. LUVs and MLVs form by aqueous sucrose solution added slowly in the flask in slightly tilt position and drain over lipid surface layer. There is no need of rotation or stirring for swelling. MLVs are obtained on surface of suspension. Separate as a decant and remaining leaving LUVs in solution [13-14].

Nanotechnology

These are solids particles with ideal size range from 1 to 1000nm (1μm). They are comprised of polymers in which medication stacked, typified or entangled. Model - Polyoxyethylene sorbitan monooleate covered nanoparticles containing drug given by I.V. Injection effectively transport over the BBB. Those which low sub-atomic weight can undoubtedly cross BBB when contrasted with high. The arrival of medication from nanoparticle rely on the technique for readiness and structure. Those nanoparticles get ready by surface adjustment might be consumed by synapses and thus, utilized for focusing on BBB. Principle bit of leeway of this to arrive at API upto mind [15-16]. By utilizing nanotechnology we get most extreme bioavailability across BBB in brain.

Microspheres

These are little circular micron size strong particles, going from up to 1 to 1000 μm in size. For the most part these are plan by scattering drug in different kind of polymer e.g.- PMC, Carbopol, Starch, Gelatin etc. These are set up by coacervation stage partition, splash drying, dissolvable vanishing, emulsification, skillet covering, polymerization. More often than not these are utilized for Taste and Odor Masking specialist, for control activity, for security of medication from outer climate [17-18].

Polymeric Micelles and Microemulsions

Micelles are framed by amphiphilic co-polymers with hydrophilic shell and hydrophobic center combinely shaped structure called polymeric micelles. These micelles are artificially and actually stable in hydrophilic media. The ideal size goes from 10 to 100 nm. For Example hydrophobic center polymer poly propylene glycol, poly (D, L-lactide) and so on and a hydrophilic shell polymers e.g., PEG. These are primarily biocompatible and biodegradable. According to audit of mind focusing on contemplated that poloxamer micelles formed with antibodies may expand cerebrum bioavailability of haloperidol, which is a neuroleptic specialist. It demonstrates neuroleptic specialists effectively infiltrate the BBB. On other hand miniature emulsions is a detailing which is utilized to expand dissolvability of

inadequately water solvent medications thus increment the bioavailability of medication. It has globule size range from 1 - 100 nm [19-20].

Dendrimers

Dendrimers are a complex fanned polymer atom which comprise of focal center with branches are joined. The whole shell framed by branches connected with one another. These are of little size contrast with that of nanoparticles and polymeric micelles of little in size. For instance a dendrimer particle, as poly-amidoamine (PAMAM) dendrimer, has a size going from 1.5 - 14.5 nm. Dendrimers utilized as transporter for transportation of anticancer drugaccross the BBB, for the therapy of Central Nervous System carcinomas [21-22].

Peptide-Vector-Mediated Strategy

The other methodology for the conveyance of neuropharmaceuticals is the utilization of little normally determined peptides that cross cell layers effectively, for instance, pegelin and penetratin peptides (18 and 16 amino acids, individually). SynB1 and pegelin (RGGRLSYSRRRFSTSTGR; atomic mass 2099 Da) is gotten from common peptides called protegrins. They have an amphipathic structure in which the emphatically charged and hydrophobic deposits are isolated in the grouping. Substitution of the four cysteine deposits with serine buildups prompts direct peptides (pegelin). The capability of this methodology as a successful conveyance framework for moving medications over the BBB has been shown in creature models [23-24].

Novel Methods

The difficult space of successful brain conveyance has prompted a sharp logical interest and therefore numerous novel techniques have been designed and licensed. In these arrangement, scientists have uncovered the utilization of iontophoresis as an adjuvant for CNS drug conveyance. Iontophoresis has been characterized as the dynamic presentation of ionized particles into tissues by methods for an electric flow. The parent US patent strategy and gadget for conveyance of a naturally dynamic specialist that is shipped by methods for iontophoresis as well as phonophoresis straightforwardly to the CNS utilizing the olfactory pathway to the brain and in this way circum-venting the BBB and is known as transnasal iontophoretic conveyance [25-26].

Atomic Trojan Horses

Endogenous ligands for explicit BBB receptors, otherwise called Trojan ponies, have the ability to carry drugs into the brain. Vasoactive intestinal polypeptide (VIP) takes an interest in the guideline of cerebral blood stream; be that as it may, in vivo considers indicated no neuropharmacological impact because of low vehicle of peptide to the mind, which is owing to the presence of the BBB [27].

Intra-Cerebroventricular Injection

The ICV approach infuses drug into the cerebrospinal liquid (CSF) compartment, which is 140 ml in people. The whole CSF pool in the human mind is turned over each 4–5 h and four to five times each day. CSF exits the cerebrum through mass stream and is consumed into the fringe circulation system at the unrivaled sagittal sinus. Though CSF exits cerebrum by means of mass stream, drug infiltrates into mind parenchyma from the CSF compartment through dissemination. The energy of mass stream is log orders more prominent than the energy of dissemination. For instance, 4 days are required for albumin to diffuse 5 mm in water. Thus, drug leaves the CSF compartment quicker than medication can diffuse into the cerebrum. Following ICV drug organization, the grouping of the medication in brain parenchyma diminishes dramatically with every mm of separation eliminated from the ependymal surface of cerebrum. Medication focus in cerebrum is just 1–2% of the CSF fixation at only 1–2 mm from the surface. The oddity of ICV drug organization is that the medication circulates specially to the blood, as opposed to the cerebrum. Christy and Fishman noted over 40 years back that Ba CSF infusion resembles a moderate intravenous infusion. The portion of barbiturate that prompts pharmacologic movement in cerebrum is a similar whether the medication is given by intravenous or ICV infusion. Following ICV infusion, the medication promptly disperses to the overall course, where

the medication at that point enters cerebrum parenchyma following vehicle over the BBB. The pathway from CSF to brain parenchyma is by means of the circulation system. Certain medications may apply a pharmacologic impact in the brain following ICV organization without initial going through the blood, if the objective receptor of the medication is situated close the ependymal surface of the cerebrum. For instance, narcotic peptides instigate focal absence of pain following ICV organization as the narcotic receptors are situated in the peri-aqueductal dim, just underneath the way of CSF stream [28-30].

Intra-Cerebral Injection

Medication, or quality vector, appropriates into cerebrum by means of dissemination following IC infusion. Diffusion decreases exponentially with the dissemination separation. Subsequently, the brain grouping of a little atom diminishes 90%, comparative with the infusion focus, at only 500 mm eliminated from the infusion site. It is beyond the realm of imagination to expect to accomplish critical dissemination of a medication or quality in the rodent brain, should less in the 1,200 g human brain, following an IC infusion [31].

Convection-Improved Diffusion

The ICV and IC approaches depend on dissemination for drug entrance into the cerebrum, while drug disperses through mass stream on account of CED. A catheter embedded in the brain is associated with a siphon, which drives liquid stream in the brain at an endorsed imbuement rate. The brain is the main organ of the body that does not have a lymphatic framework, which regularly serves to restore extracellular liquid to the overall dissemination. The brain has a B microcirculation, whereby liquid streams along paravascular pathways in corresponding to blood vessel pressure. Notwithstanding, this liquid stream is low, and about 0.1 ml/min in the rodent brain. Interestingly, the pace of CSF stream in the rodent brain is around 3 ml/ min, or 30-overlay quicker than the paravascular pathway. A worry about CED is the extreme astrogliotic response in the district of the liquid stream, which is exhibited by glial fibrillary acidic protein (GFAP) immunocytochemistry in the primate brain following CED.

Trans-nasal Delivery

The trans-nasal organization of a lipid dissolvable little atom, progesterone, was appeared more than 20 years prior to accomplish a higher conveyance in the CSF than in the blood. When the particle crosses the nasal epithelial obstruction and enters the submucous space of the nose, the medication may diffuse over the arachnoid layer and enter the CSF compartment of the olfactory area, and afterward appropriate along the standard CSF stream tracks to be retained into the overall flow. Medications that are lipid dissolvable and have a MW<400 Da, won't just cross the BBB through lipid mediation, yet in addition cross the nasal epithelial hindrance and the arachnoid film. Lipid solvent little particles enter' olfactory CSF from the nose with respect to lipid dissolvability. On the off chance that the medication is water dissolvable, or has a MW>400 Da, at that point the medication crosses natural films inadequately through free dissemination. For this situation, it is important to disturb them epithelial obstruction to accomplish transport. The instillation of a volume of >100 ml/nares in the human is adequate to cause neighborhood injury. Most investigations in rodents that show transport of medication from the nose to the olfactory flap include the instillation of volumes that cause neighborhood injury [32-34].

Olfactory Pathway

An elective CNS drug conveyance methodology that has gotten generally little consideration is the intranasal course. Medication conveyed intranasally are shipped along olfactory tactile neurons to yield critical fixations in the CSF and olfactory bulb. In ongoing investigations, intranasal organization of raw grain agglutinin horseradish peroxidase brought about a mean olfactory bulb focus in the nanomolar range. In principle, this system could be compelling in the conveyance of remedial proteins, for example, Brain Derived Neurotropic Factor (BDNF) to the olfactory bulb as a treatment for Alzheimer's disease. The nasal medication conveyance to the CNS is thought to include either an intraneuronal or extraneuronal pathway. Ongoing proof of direct nose-to-brain transport and direct admittance to CSF of three neuropeptides bypassing the circulatory system has been appeared in human preliminaries,

notwithstanding the natural challenges in conveyance. The challenges that must be defeated incorporate an enzymatically dynamic, low pH nasal epithelium, the chance of mucosal bothering or the chance of huge fluctuation brought about by nasal pathology, for example, normal virus. An undeniable favorable position of this strategy is that it is noninvasive comparative with different systems. Practically speaking, nonetheless, further examination is needed to decide whether remedial medication focuses can be accomplished after intranasal conveyance [35-36].

Nasal Vehicle Courses

After nasal conveyance sedates first arrive at the respiratory epithelium, where mixes can be retained into the fundamental dissemination using similar pathways as some other epithelia in the body: Tran cell and Para cell inactive retention, transporter intervened transport, and assimilation through trancytosis. In spite of the fact that retention over the respiratory epithelium is the significant vehicle pathway for nasally-controlled medications and may speak to a possibly timesaving course for the organization of certain fundamental medications conveyed in cryonics drug conventions (e.g., epinephrine or vasopressin), issue of BBB-intervened prohibition of cerebrum remedial specialists to be of more prominent prompt concern. As needs be, the rest of this article will manage the vehicle of medications to the CNS by method of the olfactory epithelium [37]. At the point when a nasal medication definition is conveyed profound and sufficiently high into the nasal pit, the olfactory mucosa might be ventured and drug transport into the cerebrum as well as CSF by means of the olfactory receptor neurons may happen. The olfactory pathways might be comprehensively characterized into two potential courses: the olfactory nerve pathway (axonal vehicle) and the olfactory epithelial pathway [38]. Axonal vehicle is viewed as a moderate course whereby a specialist enters the olfactory neuron viaendocytotic orpinocytotic instruments and goes to the olfactory bulb by using the same anterograde axonal vehicle systems the cell uses to ship endogenous substances to the brain [86]. Depending on the substance managed, axonal vehicle rates range from 20-400 mm/day to a more slow 0.1-4 mm/day. The epithelial pathway is an essentially quicker course for direct nose-to-cerebrum move, whereby mixes passparacellularly over the olfactory epithelium into the perineural space, which is persistent with the subarachnoid space and in direct contact with the CSF. At that point the particles can diffuse into the cerebrum tissue or will be cleared by the CSF stream into the lymphatic vessels and along these lines into the fundamental course [39-40].

Interstitial Delivery

The most immediate method of dodging the BBB is to convey medicates straightforwardly to the cerebrum interstitium. By coordinating specialists extraordinarily to an intracranial objective, interstitial medication conveyance can hypothetically yield high CNS drug fixations with negligible fundamental introduction and harmfulness. Moreover, with this methodology, intracranial medication fixations can be continued, which is significant in treatment with numerous chemotherapeutic specialists [41].

Infusions, Catheters and Pumps

A few methods have been produced for conveying drugs straightforwardly to the brain interstitium. One such philosophy is the Ommaya repository or implantable siphon as talked about before under intraventricular / intrathecal course. This method, notwithstanding, accomplishes genuinely constant medication conveyance. All the more as of late, a few implantable siphons have been built up that have a few preferences over the Ommaya repository. This can be embedded subcutaneously and topped off by subcutaneous infusion and are fit for conveying drugs as a consistent implantation throughout an all-inclusive timeframe. Besides, the pace of medication conveyance can be differed utilizing outer handheld PC control units. Presently every one of the three unique siphons accessible for interstitial CNS drug conveyance works by an unmistakable instrument. The Infusaid siphon utilizes the fume weight of compacted Freon to convey a medication arrangement at a consistent rate; the Mini Med PIMS framework utilizes a solenoid siphoning instrument, and the Medtronic Synchro Med framework conveys drugs through a peristaltic component. The dissemination of little and enormous medication particles in the cerebrum can be upgraded by keeping up a compel inclination during interstitial medication mixture to produce mass liquid convection through the mind interstitium or by expanding the dispersion angle by amplifying the

grouping of the imbued specialist as an enhancement to straightforward dissemination. Another ongoing examination shows that the epidural (EPI] conveyance of morphine typified in multivesicular liposomes (Depo Foam drug conveyance system] created a supported freedom of morphine and a drawn out absence of pain, and the results recommend that this conveyance framework is without huge neurotic impacts at the portion of 10mg/ml morphine after rehashed epidural conveyance in canines [42-44].

Biodegradable Polymer Wafers, Microspheres and Nanoparticles

In spite of the fact that interstitial medication conveyance to the CNS has had just unassuming clinical effect, its restorative potential may before long be acknowledged utilizing new advances in polymer innovations to adjust the previously mentioned methods. Polymeric or lipid based gadgets that can convey drug particles at characterized rates for explicit timeframes are presently having a gigantic effect in clinical medication. Medication conveyance straightforwardly to the cerebrum interstitium utilizing polyanhydride wafers can go around the BBB and delivery phenomenal degrees of medication legitimately to an intracranial objective in a supported manner for expanded timeframes. The destiny of medication conveyed to the cerebrum interstitium from the biodegradable polymer wafer was anticipated by a numerical model dependent on [a] paces of medication transport by means of dispersion and liquid convection; [b] paces of end from the mind by means of degradation, metabolism and penetration through slender organizations; and [c] paces of nearby official and disguise. Such models are utilized to foresee the intracranial medication focuses that outcome from BCNU-stacked pCPP:SA [1,3 bis-para-carboxyphenoxypropane: sebacic acid] wafers just as other medication polymer mixes, preparing for the sound plan of medications explicitly for intracranial polymeric conveyance. Formation of a polymerically conveyed chemotherapeutic specialist to a water-solvent macromolecule builds drug infiltration into the mind by expanding the time of medication maintenance in cerebrum tissue. In principle, polymeric cytokine conveyance has a few points of interest over conveyance from transducted cells, including obviating the requirement for transfecting cytokine qualities, delivering longer times of cytokine discharge in-vivo and yielding more reproducible cytokine discharge profiles and complete cytokine portion. Microparticles can likewise be effortlessly embedded by stereotaxy in discrete, exact and utilitarian regions of the cerebrum without harming the encompassing tissue. Nanoparticles were discovered to be useful for the treatment of the dispersed and very aggressive brain tumors. Intravenously infused doxorubicin-stacked polysorbate 80-covered nanoparticles had the option to prompt 40% fix in rodents with intracranially relocated glioblastomas. Another Study shows that PEGylated PHDCA (n-hexadecylcyanoacrylate] nanoparticles made by PEGyalated amphiphilic copolymer enter into the brain to a bigger degree than the wide range of various tried nanoparticle details, without prompting any change of the BBB penetrability. What's more, the outcome characterizes two significant necessities to consider in the plan of satisfactory brain conveyance frameworks, long-coursing properties of the transporter and fitting surface attributes to allow communications with endothelial cells. Valproic corrosive stacked nanoparticles demonstrated diminished harmful results of valproate treatment, not by decreasing the remedially important measurement but rather by restraint of development of poisonous metabolites. All in all, the limit of the biodegradable polymer conveyance procedure to convey medicates straightforwardly to the cerebrum interstitium is immense [45-47].

Ongoing Advances in Nanotechnology

The examination group of University of Michigan has built up an apparatus to analyze and treat the most harmful types of mind malignancy. That is 20 to 200 nanometer diameter nanoparticles; they named Probes Encapsulated by Biologically Localized Embedding [pebbles]. They planned the stones to convey an assortment of specialists on their surface, each with an exceptional capacity. The significant likely favorable position of utilizing these nanoparticles to treat malignant growth is of multifunctional. One objective particle immobilized on a superficial level could be utilized to help picture the objective utilizing Magnified Resonace Imaging (MRI], while a third specialist joined to the PEBBLE could convey a damaging portion of medication or poison to close by disease cells. Each of the three capacities can be consolidated in a solitary minuscule polymerspere to make a strong weapon against cancer. Kopel man presented the regular MRI contrast component gadolinium to the stones. When infused into the circulation system, then a no particles travel their way through the circulatory system. Also, in light of the fact that they can

cross over they have a focusing on specialist connected, the rocks gather in the mind tumor empowering a reasonable MRI picture inside only a couple hours [48-49]. Analysts fused a medication called Photofrin alongside iron oxide into nanoparticles that would target harmful mind tumors. Photofrin is a sort of photodynamic treatment [PDT], in which the medication is drawn through the circulation system to tumors cells; a unique kind of laser light initiates the medication to assault the tumor. Iron oxide is a differentiation specialist used to improve attractive reverberation imaging (MRI) [50].

Fanciful Peptide Innovation

Fanciful peptides are framed when a medication that is typically not shipped through the BBB is formed to a cerebrum drug-focusing on vector. The last is an endogenous peptide, altered protein, or peptidomimetic monoclonal counter acting agent (mab] that goes through RMT(Rapid metabolic transfer] through the BBB on endogenous receptor frameworks, for example, the insulin receptor or the tfr. Peptidomimeticmabs tie to exofacial epitopes on the BBB receptor that are taken out from the endogenous ligand restricting site and piggyback over the BBB on the endogenous RMT framework inside the BBB, In this , a medication is monobiotinylated in corresponding with the creation of a vector/energetic in or a vector/ streptavidin (SA)combination protein. The biotinylated drug is created in one vial and the vector/eager in combination protein is delivered in another vial, and the 2 vials are blended before organization [51-52]. Inferable from the incredibly high fondness of enthusiastic in or SA official of biotin, there is prompt catch of the biotinylated neurotherapeutic specialist by the vector/ardent in or vector/SA combination protein. Monoclonal immunizer/eager in and mab/SA combination qualities and combination proteins are delivered with hereditary designing. Cerebrum drug conveyance in rodents is conceivable with the OX26 mouse mab to the rodent tfr. Cerebrum drug conveyance in people is conceivable with the hereditarily engineered chimeric HIRmab. The action of the hereditarily engineered chimeric HIRmab is indistinguishable from that of the original murine HIRmab and the chimeric neutralizer is enthusiastically taken up by the primate cerebrum. The brain take-up of the HIRmab in the rhesus monkey is 2% to 4% of the infused portion which is a degree of cerebrum take-up that is 1 to 2 log orders more prominent than the mind take-up of a neuroactive little atom, for example, morphine [53-54].

Neuroprotection with Peptide Radiopharmaceuticals

The act of mind imaging utilizes little particle radio synthetic substances that predicament to monoamine or amino corrosive synapse frameworks. While there are not exactly a dozen monoaminergic or aminoacidergic synapse frameworks, there are hundreds of peptidergic neurotransmission frameworks. Therefore, the utilization of peptide radiopharmaceuticals could significantly expand the demonstrative potential of neuroimaging innovation. Potential up-and-comers for neuroimaging incorporate Epidermal Growth factor [EGF] peptide radiopharmaceuticals for the early location of brain tumors and A_ peptide radiopharmaceuticals as a demonstrative brain examine for Alzheimer diseases. Numerous malignant gliomas over express the EGF receptor [EGF-R] and EGF are a potential peptide radiopharmaceutical for the imaging of cerebrum tumors [55].

Protein Neurotherapeutic Specialist and Neuroprotection in Stroke

Practically all little molecule neuroprotective specialists have fizzled in clinical stroke preliminaries on the grounds that either [a] these atoms have horrible wellbeing profiles or [b] the medications don't cross the BBB. The restorative window for neuroprotection is the initial 3 hours after stroke, and during this time, the BBB is unblemished. The BBB is disturbed in later stages following stroke, however as of now, chances for neuroprotection have been lost. Consequently, if compelling neuroprotective specialists for stroke are to be created, these particles must have ideal security profiles and should have the option to cross the BBB. A model neurotrophin, cerebrum determined neurotrophic factor [BDNF], was reformulated to empower BBB transport, and the BDNF illusory peptide is neuroprotective after postponed intravenous organization in either provincial or worldwide mind ischemia [56].

Medication Delivery from Biological Tissues

Another technique to accomplish interstitial medication conveyance includes delivering drugs from organic tissues. The simple way to deal with this method is to embed into the mind a tissue that normally secretes an ideal remedial specialist. This methodology has been most broadly applied to the treatment of Parkinson's disease. Relocated tissue regularly didn't endure attributable to an absence of neurovascular innervation. As of late the upgraded vascularization and microvascular porousness in cell-suspension undeveloped neural unions comparative with strong unions has been exhibited. An elective augmentation of this strategy is to utilize quality treatment to create improved organic tissue for interstitial medication conveyance. Before implantation, cells can be hereditarily adjusted to combine and delivery explicit restorative specialists. The remedial capability of this procedure in the treatment of mind tumor was illustrated. The utilization of nonneuronal cells for helpful protein conveyance to the CNS has as of late been surveyed. The endurance of unfamiliar tissue unions might be improved by progressions in methods for refined unmistakable cell types. Co-joined cells designed to deliver neurotropic elements with cells designed to deliver helpful proteins may improve the endurance and advancement of unfamiliar tissue. Preferably it is conceivable to act in-vivo hereditary designing to cause explicit endogenous brain tissue to communicate an ideal protein, dodging the ischemic and immunogenic difficulties experienced with the implantation of unfamiliar tissue unites. One such strategy that has been effectively utilized for the treatment of CNS malignancies includes in-vivo tumor transduction with the herpes simplex thymidine kinase [HS-tk] quality followed by treatment with hostile to herpes drug ganciclovir was accomplished by intra-tumoral infusion of retroviral vector-creating cells containing the HS-tk quality, delivering the transfected tumor cells vulnerable to treatment with ganciclovir. Other vector frameworks utilized in CNS quality exchange examines incorporate retroviruses, adenoviruses, adeno-related infections, encapluation of plasmid DNA into cationic liposomes and impartial and oligodendrial undifferentiated cells. Despite the fact that this methodology holds exceptional helpful potential in the treatment of CNS illnesses, its adequacy has hitherto been blocked by various deterrents: confined conveyance of vector frameworks over the BBB, wasteful transfection of host cells, and nonselective articulation of the transgene and pernicious guideline of the transgene by the host [57-59].

Hereditary Designing

Embedding inside the brain a living tissue that communicates and secretes the restorative atom has been tried, and positive outcomes were gotten in PD treatment. As of late, neural immature microorganisms (NSC) were embedded into the hippocampus of an Alzheimer's sickness and down condition model mice to change the levels of tau/reelin-positive granules. Another exemple is the neurotransplantation in mice core accumbens of undifferentiated organisms which communicated the human dopamine receptor to change liquor utilization. In any case, embedded cells don't endure limitlessly since there is no revascularization around the tissue. Promising arrangements from hereditary designing were evaluated to acquire distinctive cell types that emit the ideal specialist with a longer endurance rate, for example, the co-joining designed neurotrophic factor-delivering cells with designed helpful specialist delivering cells. More as of late another examination attempted to tackle that issue doing a co-transplantation with partner cells to offer trophic help [60-61].

Directed Medication Conveyance

A proficient medication focused on conveyance can decrease significantly the portion of medication required and in result improve the wellbeing in vivo, which is the significant point in medication's commercialization [62]. The technique comprises of connecting ligands that are explicitly perceived by BCECs receptors, for example, sugar buildups, folic corrosive or even designed mAbs. Focusing on moieties can be connected legitimately on the outside of the transporter framework or on the outer finish of the PEG in the event that it is PEGylated, framing what is known as third era transporters. Every nanotechnology-based conveyance frameworks can likewise be subclassified into first, second or third era relying carefully upon their surface changes. LPs, NPs furthermore, SLNs that has not gone through any surface changes are viewed as the first age [63]. In the event that they do introduce adjustments, for example, PEGylation or other settling strategies to lessen plasmatic leeway then they are known as second era or

too named secrecy transporter. The last section floods from the capacity of alterations for example, PEGylation that allows the conveyance framework to avoid macrophages of the RES. Finally, third era or focused on sterically settled conveyance frameworks separated from improved PK from the subsequent age can likewise target explicit organs or cell-types [64]. Preclinical information in mind disease models show that PEGylated liposomal doxorubicin with an extra glutathione (GSH) covering can essentially decrease cerebrum tumor development. Other technique could be the plan of glucosyl liposome ligands, which can cross BBB by GLUT1 as medication transporters in focusing on conveyance framework. Glucosyl changed liposomes demonstrated likely application with brain focusing on, high exchange proficiency, great in vivo cycling soundness and simple readiness. Surface adjustment of liposomes with CPPs encourages endosomal getaway and expands their cell conveyance. There are examines joining CPPs with Transferrin (Tf)- liposomes which bring about biocompatible details prompting proficient movement of doxorubicina across cell and cerebrum endothelial boundaries both in vitro and in vivo . Lactoferrin (Lf), a solitary chain iron-restricting glycoprotein, is important for Tf family which can enter the BBB by means of receptor-interceded transcytosis [65].

A few investigations built up a doxorubicin-stacked lactoferrin-changed procationic liposome conveyance framework and assessed its restorative impact for glioma. Different investigations zeroed in on creating double focusing on daunorubicin PEG-liposomes by forming vesicles with p-aminophenyla-D-manno-pyranoside (MAN) and transferrin (TF). The ligand MAN assumes a significant job in shipping the liposomes over the BBB while TF goes about as a fundamental function in focusing on cerebrum glioma cells. Despite the fact that the principle point of focused LPs and NPs is to offer an organ-explicit take-up numerous endeavors have gone past and given medication transporters the capacity to point an extra objective once inside the CNS. The optional objective is accomplished by 3 procedures: double focusing on, consecutive focusing on and particular activity focusing on. The principal, double focusing on approach utilizes a solitary focusing on moiety that has its receptor communicated at the BBB and at the ideal site of activity. Besides, successive focusing on applies its work through the blend of two focusing on ligands. The first coordinates the compound to the brain while the other one is the liable for cell explicitness inside the brain. Finally, particular activity focusing on is utilized to accomplish cell-type explicitness. This focusing on technique imparts regular highlights to the supportive of medication approach. The conveyed drug, when arrive at the brain isn't dynamic until a cell-type explicit catalyst separates and discharges the medication. Since the catalysts are limited to a specific subpopulation of cells just these cells will be under the impact of the helpful compound. Furthermore, nanoparticulate vectors of medications likewise can be artificially altered to increment cell take-up and the expected conveyance in various cell compartments. Hostile to malignancy specialists have been stacked in nanocontainers formed with ligands focusing on the BBB to improve selectivity for mind tumors [66].

CONCLUSION

Presently a day, numerous youthful scientists are pulled in toward brain targeting because of its colossal application in the treatment of different CNS sicknesses in light of the fact that generally tranquilizes can't cross the Blood brain barrier. This short survey examine the one of the novel innovation that has been created to focus on the brain and have different clinical advantages, for example, diminished medication portion, less results, non obtrusive courses, and better patient consistence.

REFERENCES

1. Sandipan Roy; Strategic Drug Delivery Targeted to the Brain: A Review; Der Pharmacia Sinica, 2012, 3 (1):76-92
2. Luca Anna Bors and Franciska Erd″o; Overcoming the Blood–Brain Barrier. Challenges and Tricks for CNS Drug Delivery; Scientia Pharmaceutica; 2019,87,6.

3. Avhad P.S , Patil P.B., Jain N.P., Laware S.G.; A Review on Different Techniques for Brain Targeting ; International Journal of Pharmaceutical Chemistry and Analysis; July - September 2015;2(3):143-147
4. Ambikanandan Misra, Ganesh S., Shrenik P. Shah; Drug delivery to the central nervous system: a review; J Pharm Pharmaceut Sci (www.ualberta.ca/~csps) 6(2):252-273, 2003
5. Yasir Mehmood, Ayesha Tariq, Faheem Ahmad Siddiqui; Brain targeting Drug Delivery System: A Review; International Journal of Basic Medical Sciences and Pharmacy (IJBMSP) Vol. 5, No. 1, June 2015, ISSN: 2049-4963
6. Kalpana Nagpal, Shailendra Kumar Singh† & Dina Nath Mishra; Drug targeting to brain: a systematic approach to study the factors, parameters and approaches for prediction of permeability of drugs across BBB; Expert Opin. Drug Deliv.
7. Daljeet Sharma, Narendra Nager, Ravindra Pal Sing, Ashish Kumar Sharma; Drug Targeting To Brain : A Review; Ijprd/2011/Pub/Arti/Vov-3/Issue-1/March/004, Issn 0974 – 9446
8. Swatantra Bahadur Singh; Novel Approaches for Brain Drug Delivery System-Review; International Journal of Pharma Research & Review, June 2013; 2(6):36-44 ISSN: 2278-6074
9. Upasana Sharma, Pragya Nand Badyal, Swati Gupta; Polymeric Nanoparticles Drug Delivery to Brain: A Review; International Journal of Pharmacology and Pharmaceutical Sciences 2015; Vol: 2, Issue: 5, 60-69
10. Keyanpour Rad Mansoor, Kianpour Rad Sima, Kianpour Rad Soheila; J Nanomed Res. 2019;8(1):1–4.
11. Aishwarya V, Sumathi T; Enhanced Blood–Brain Barrier Transmigration Using the Novel Chrysin Embedded Solid Lipid Nanoformulation: A Salient Approach on Physico-Chemical Characterization, Pharmacokinetics and Biodistribution Studies ; International Journal of Pharmaceutical and Clinical Research 2016; 8(12): 1574-1582
12. Md. Didaruzzaman Sohel, Tania Sultana and Md. Hassan Kawsar; Largest Obstacle of Drug Delivery to the Blood Brain Barrier and Current Approach to Solve this Problem: Recent Comprehensive Review; Glob J Nanomed 4(1): GJO.MS.ID.555633 (2018)
13. Barbara Pavan 1,*, Alessandro Dalpiaz 2, Nunzia Ciliberti 2, Carla Biondi 1, Stefano Manfredini 2 and Silvia Vertuani; Progress in Drug Delivery to the Central Nervous System by the Prodrug Approach; Molecules 2008, 13, 1035-1065; DOI: 10.3390/molecules13051035
14. Nishikant D1*, Shiva S2, Rishu T3, and Vikrant C4; Nanotechnology as a Targeted Drug Delivery System to Brain; Nanoscience and Nanotechnology: An Indian Journal Research | Vol 10 Iss 5; www.tsijournals.com | September-2016
15. Emil Joseph, Ranendra Narayan Saha; Advances in Brain Targeted Drug Delivery: Nanoparticulate Systems; Journal of PharmaSciTech; http://www .pharmascitech.in, Volume 3 (Issue 1)2013.
16. Luca Anna Bors and Franciska Erdo; Overcoming the Blood–Brain Barrier. Challenges and Tricks for CNS Drug Delivery; Sci. Pharm. 2019, 87, 6; doi:10.3390/scipharm87010006 www.mdpi.com/journal/scipharm
17. William M. Pardridge; Drug Targeting to the Brain; Pharmaceutical Research, Vol. 24, No. 9, September 2007 (# 2007) DOI: 10.1007/s11095-007-9324-2
18. Swatantra Bahadur Singh; Novel Approaches for Brain Drug Delivery System-Review; International Journal of Pharma Research & Review, June 2013; 2(6):36-44 ISSN: 2278-6074
19. Pardridge, W.M., Peptide drug delivery to the brain. Raven Press, New York, U.S.A., 1991.
20. Pardridge, W. M. The blood-brain barrier: bottleneck in brain drug development. NeuroRx 2005, 2, 3-14.
21. Brightman, M.W.; Reese, T.S., J. Cell. Biol. 1969, 40 (3), 648-677
22. Pardridge, W.M., J. Cereb. Blood Flow Metab. 1997, 17 (7], 713-31
23. Cornford, E.M., Braun, L.D., Oldendorf, W.H. and Hill, M.A., Am J Physiol, 1982, 243:C161–C168.
24. Siegal, T. and zylber-Katz, E., Clin Pharmacokinet, 2002, 41:171-186.
25. Illum, L., J. Control. Release. 2003, 87, 187-198.
26. Cole, S.P.C., Bhardwaj, G., Gerlach, J.H., McKemzie, J.G., Grant, C.E., Almquist, K.C., Stewart, A.J., Kurz, E.U., Duncan, A.M.V. and Deeley, R.G., Science, 1992, 258:1650-1654.
27. Taylor, E.M., Clin Pharmacokinet, 2002, 41, 81-92. [10] Sadeque, A.J., Wandel, C., He, H., Shah, S., and Wood. A.J., Clin Pharmacol Ther, 2000, 68, 231-237.

28. Salvolainen, J., Edwards, J.E., Morgan, M.E., McNamara, P.J., and Anderson, B.D., Drug Metab Dispos, 2002, 30, 479-482. [29] Fellner, S., Bauer, B., Miller, D.S., Schaffrik, M., Fankhanel, M., Spruss, T., Bernhardt, G., Graeff, C., Farber, L., Gschaidmeier, H., Buschauer, A., and Fricker, G., J Clin Invest, 2002, 110:1309-1318.
29. Chow, H. N. S.; Chen, Z.; Matsuura, G. T., J. Pharm. Sci. 1999, 88, 754-758.
30. Sakane, T.; Akizuki, M.; Yoshida, M.; Yamashita, S.; Naday, T.; Hashida, M.; Sezaki, H. J., Pharm.Pharmacol. 1991, 43, 449-451.
31. Kern, W.; Born, J.; Schreiber, H.; Fehm, H.L., Diabetes 1999, 48, 557-563.
32. Cosolo, W. C.; Martinello, P.; Louis, W. J.; Christophidis, N., Am. J. Physiol. 1989, 256, R443-447.
33. Sanovich, E.; Bartus, R.T., ; Friden, P.M.; Dean, R. L.; Le, H.Q.; Brightman, M. W., Brain Res. 1995, 705, 125-135.
34. Erdlenbruch, B.A.M.; Fricker, G.; Miller, D.S.; Kugler, W.; Eibl, H.; Lakomek, M., Br. J. Pharm. 2003, 140, 1201-1210.
35. Audus, K. L.; Chikhale, P.J.; Miller, D. W.; Thompson, S.E.; Borchardt, R. T., Adv. Drug Res. 1992, 23, 3-53.
36. Tamai, I.; Tsuji, A., J. Pharm. Sci. 2000, 89, 1371-1388.
37. Wade, L. A.; Katzman, R., J. Neurochem. 1975, 25, 837-842. [22] Negri, L.; Lattanzi, R.; Tabacco, F.; Scolaro, B.; Rocchi, R., Br.J. Pharmacol. 1998, 124, 1516-1522.
38. Saheki, A. T. T.; Tamai, I.; Tsuji, A., Pharm. Res. 1994, 11, 305-311.
39. Mackey, J.; Mani, R.; Selner, M.; Mowels, D.; Young, J.; Belt, J.; Crawford, C.; Cass, C., Cancer Res. 1998, 58, 4349-4357.
40. Yao, S.; Cass, C.; Young, J., Mol. Pharmacol. 1996, 50, 388-393.
41. Kannan, R.; Kuhlenkamp, J.; Ookhtens, M.; Kaplowitz, N., J. Pharmacol. Exp. Ther. 1992, 263, 964-970.
42. Zlokovic, B.; Hyman, S.; McComb, J.; Lipovac, M.; Tang, G.; Davson, H., Biochem. Biophys. Acta 1990, 1025, 191-198.
43. Pan, W.; Kastin, A., Peptides 1999, 20, 1091-1098.
44. Friden, P.M.; Walus, L.R.; Musso, G.F.; Taylor, M. A.; Malfroy, B.; Starzyk, R. M., Proc. Natl. Acad. Sci. USA 1991, 88, 4771-4775.
45. Friden, P.M.; Walus, L.R., Adv. Exp. Med. Biol. 1993, 331, 129-136.
46. Fukuta, M.; Okada, H.; Iinuma, S.; Yanai, S.; Toguchi, H., Pharm. Res. 1994, 11, 1681-1688.
47. Oldendorf, W.H., Brain Res, 1970, 24, 1629–1639.
48. Kakee, A., Terasaki, T. and Sugiyama, Y., J Pharmacol Exp Ther, 1996, 277:1550-1559.
49. Begley, D.J., Squires, L.K., Zlokovic, B.V., Mitrovic, D.M., Hughes, C.C.W., Revest, P.A. and Greenwood, J., J Neurochem, 1998, 55:1222- 1230
50. Jaehde, U., Langemeijer, M.W.E., de Boer, A.G. and Breimer, D.D., J Pharmacol Exp Ther, 1992, 263(3], 1140-1146.
51. De lange, E.C.M., de Boer, A.G. and Breimer, D.D., STP Pharm Sci, 1997, 7(1), 17- 28.
52. Terasaki, T., Deguchi, Y., Kasama, Y., Pardridge, W.M. and Tsuji, A., Int. J Pharm, 1992, 81, 143–152
53. Menacherry, S., Hubert, W. and Justice, J.B., Anal Chem, 1992, 64, 577–583.
54. De Boer AG: Sutanto W (eds], Tramsport across the blood-brain barrier: In-vitro and in-vivo techniques, Academic publishers, Amsterdam, Harwood, 1997, pp 91-97.
55. Gath, U., Hakvoort, A., Wegener, J., Decker, S. and Galla, H.J., Eur J Cell Biol, 1997, 74, 68-78.
56. Erben, M., Decker, S., Franke, H. and Galla, H.J., J Biochem Biophys Methods, 1995, 30, 227-238.
57. Wegener, J., Sieber, M. and Galla, H.L., J Biochem Biophys Methods, 1996, 32, 151-170.
58. Dehauck, M.P., Dehouck, B., Schluep, C., Lemaire, M. and Cecchelli, R., Eur J Pharm Sci, 1995, 3, 357-365.
59. Pardridge, W.M., Annu Rev Pharmacol Toxicol, 1988, 28, 25-39.
60. Cornford, E.M., Braun, L.D., Oldendorf, W.H. and Hill, M.A., Am J Physiol, 1982, 243, C161–C168.
61. Siegal, T. and zylber-Katz, E., Clin Pharmacokinet, 2002, 41, 171-186.
62. Cole, S.P.C., Bhardwaj, G., Gerlach, J.H., McKemzie, J.G., Grant, C.E., Almquist, K.C., Stewart, A.J., Kurz, E.U., Duncan, A.M.V. and Deeley, R.G., Science,1992, 258:1650-1654.
63. Taylor, E.M., Clin Pharmacokinet, 2002, 41:81-92.
64. Sadeque, A.J., Wandel, C., He, H., Shah, S., and Wood. A.J., Clin Pharmacol Ther, 2000, 68, 231-237.

65. Salvolainen, J., Edwards, J.E., Morgan, M.E., McNamara, P.J., and Anderson, B.D., Drug Metab Dispos, 2002, 30, 479-482.
66. Fellner, S., Bauer, B., Miller, D.S., Schaffrik, M., Fankhanel, M., Spruss, T., Bernhardt, G., Graeff, C., Farber, L., Gschaidmeier, H., Buschauer, A., and Fricker, G., J Clin Invest, 2002, 110, 1309-1318.

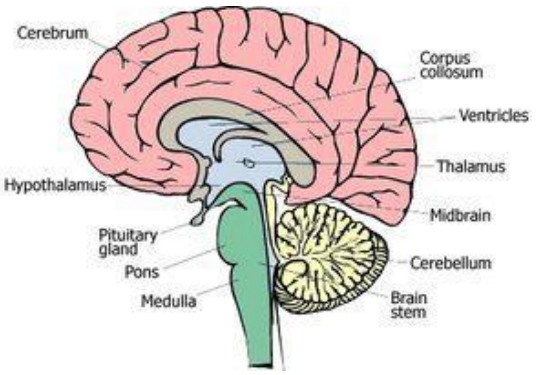


Figure 1: Anatomy of Brain

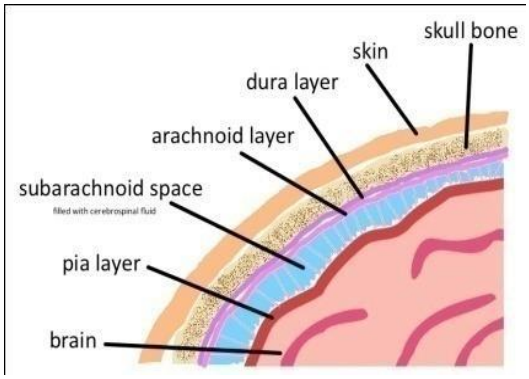


Figure 2: Diagram showing meninges and cerebrospinal fluid

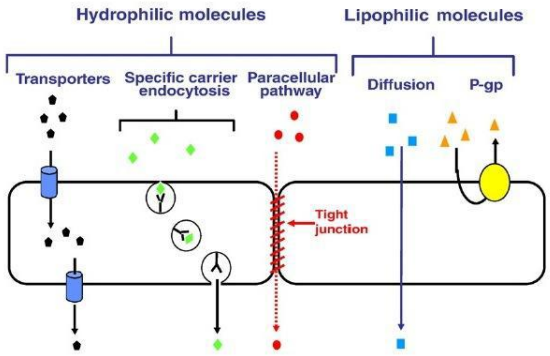


Figure 3: Schematic representation of the transport of molecules across the BBB

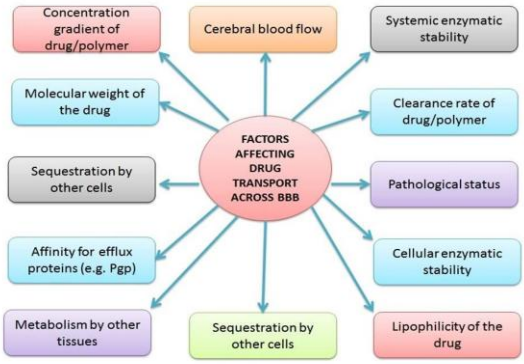


Figure 4: Schematic representation of the factors affecting drug transport across the BBB

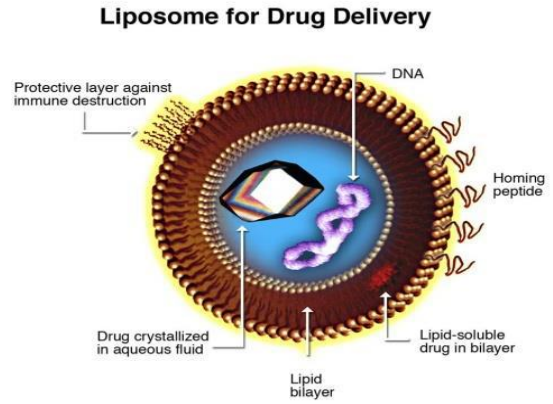


Figure 5 : Brain drug Delivery-liposomal Approach

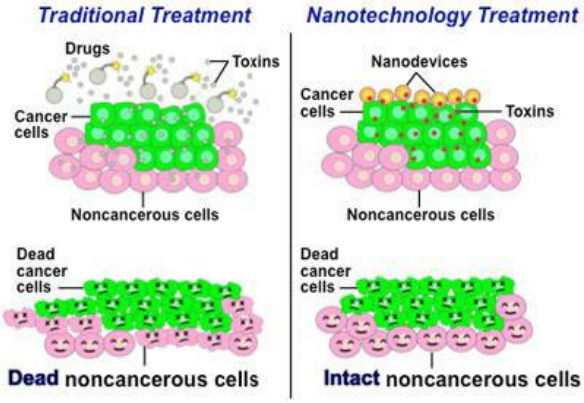


Figure 6: Brain Drug Delivery:-Nanotechnology Approaches

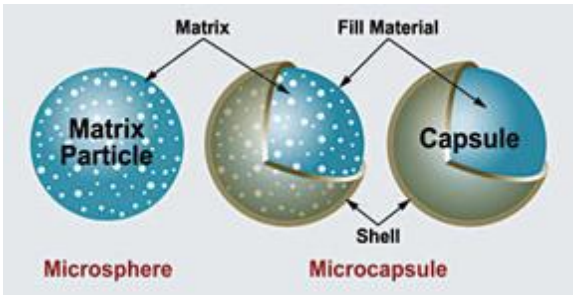


Figure 7: Brain Drug Delivery: Microsphere Approach

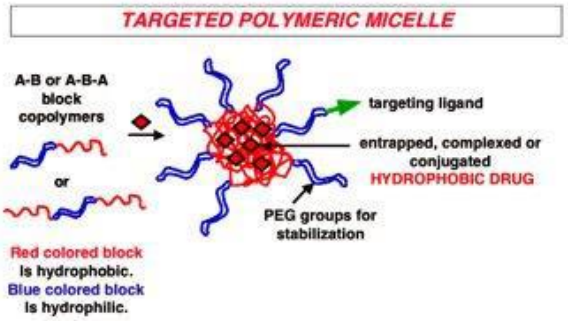


Figure 8: Brain Drug Delivery:-Micellar Approach

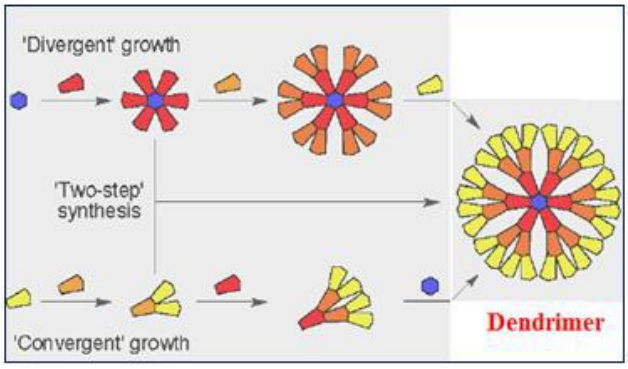


Figure 9: Brain Drug Delivery:-Dendrimers Approach

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Current Trends in the Pharmacotherapy of Peptic Ulcer

S. Nithya, R. Kothai and B. Arul*

Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

Received: 24 Nov 2020 Revised: 07 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
B. Arul
Department of Pharmacy Practice,
Vinayaka Mission's College of Pharmacy,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.
Email: arul1971@yahoo.com

ABSTRACT

Peptic ulcer is a chronic disease. It is caused by acid-induced in the stomach. The ulcer is produced by infection with a type of bacteria called *Helicobacter pylori*. There are different types of ulcers, among these most common are peptic ulcer, aphthous ulcer, and esophageal ulcer. *H.pylori* is the main cause of stomach ulcers. The acid secretion is controlled by the action of three potent stimulants: histamines, acetylcholine, and gastrin. Endoscopy is no doubt the chosen method for the diagnosis of primary peptic ulcers. Urease test has high sensitivity and specificity and it uses several staining methods, it has good diagnostic accuracy. The main non-invasive methods are serology and respiratory test with urea marked my Carbon. Treatment of *Helicobacter pylori* infection is important for the management of gastrointestinal disorders such as peptic ulcer and gastric cancer. We reviewed both bacterial and host factors involved in the therapeutic management of the *H.pylori* infection. In addition, data on the most successful therapy regimens - sequential and concomitant therapies - currently available for *H.pylori* eradication were evaluated. The main aim of this review article is to summarize the etiology, pathogenesis, diagnosis, treatment, mechanism of action of the drugs.

Keywords: Peptic ulcer disease, *Helicobacter pylori* infection, Gastric ulcer, NSAID's, Gastric acid secretion

INTRODUCTION

A peptic ulcer is a chronic disease that is caused by acid-induced in the stomach [1]. The symptoms included burning sensation and hunger. Four major complications of peptic ulcers include bleeding, perforation, penetration, and obstruction. There is an imbalance between aggressive factors of (*Helicobacter pylori*, NSAID'S, gastric acid). It affects the age group of patients from 20-70 years. The major symptoms of peptic ulcer include weight loss, poor appetite,

Bloating nausea, and vomiting. It affects people of all ages. Nearly, 4% of people are affected by peptic ulcers. An ulcer is produced by infection with a type of bacteria called *H.pylori* [2]. The role of *Helicobacter pylori* in the pathogenesis of peptic ulcer disease and chronic antral gastritis is being increasingly recognized. Although its relationship to functional dyspepsia and gastric adenocarcinoma is still uncertain, several studies have shown that relapse or remission of duodenal ulcer is strongly associated with the presence or absence of *H.pylori* colonization of the gastric mucosa. The basic treatment of peptic ulcer was revolutionized by the discovery of H2-receptor antagonist that led to the principle of acid conceal therapy for duodenal ulcer [3].

Type of peptic ulcer

There are different types of ulcers of which, most common is a peptic ulcer which includes ulcers of the digestive tract in the stomach or the duodenum[4]. The most important include some of the bacterial infection, various drugs & chemicals, gastric secretion, lipid metabolites, neuropeptides, inflammatory mediators, and reactive free radicals. The causative agent is *H.pylori* or reaction to certain medicines like a non-steroidal anti-inflammatory drug (NSAID).

Aphthous ulcers

The mouth ulcer is common and usually due to trauma such as an ill-fitting denture, fractured teeth, or fillings [5]. Anemia, measles, viral infection, oral candidiasis, chronic infection, throat cancer mouth cancer, and vitamin B deficiency are some of the common causes of ulcers or sores in the mouth. The oral ulcerative disease affects 15% - 20% of the population worldwide [6,7].

Esophageal ulcer

An esophageal ulcer is a form of lesions that occur in the food pipe [8]. These are most commonly formed at the end of the esophageal and it produces pain in the right side below the breast bone in the same area where symptoms of heartburn are felt [9], prolonged use of drugs like NSAIDs, and smoking.

Etiology and pathogenesis of ulcer *H.pylori*

H.pylori is the main cause of stomach ulcer, it was 1st identified by the two Australian scientists in 1982, *H.pylori* is a gram-negative bacillus it is spinal shaped bacteria[10]. The acid secretion is controlled by the action of three potent stimulants: histamines, acetylcholine, and gastrin, they are acting through an outflow of events and its activation of the H^+K^+ATPase pump, which secrets hydrogen ions in exchange with potassium. Pepsin is a proteolytic enzyme that is secreted in cholinergic stimulation under the form of pepsinogen I and II [11]. *H.pylori* causes increased expression of cytokines such as TNF-α in gastritis. Further, IL- 1β causes further induction of *H.pylori* induced gastritis. *H.pylori* infected gastric mucosa shows infiltration of intraepithelial cells causes severe neutrophil infiltration [12]. Prostaglandins increase mucous resistance through an increased blood flow and increase the stimulation of mucosa and bicarbonate secretion, decrease in acid secretion [13]. The most probable hypotheses regarding the etiopathogenesis of ulcerous diseases are due to genetic factors, pathophysiologic disturbances, and environmental factors [14].

Gastric Mucosa

Gastric mucosal layers act as a barrier that restricts the exposure of the gastric cells to various injurious agents of both exogenous and endogenous origin [15]. Gastric acid plays a stringent role in gastric defense. It is the first line of mucosal defense to present bacterial colonization and reduce their ability to enter into the mucosal layer. Secretion of mucus is an obvious contributor in improving epithelial recovery after acute injury through the formation of a mucoid cover beneath initializing re-epithelization [16].

NSAIDs

NSAIDs are widely used for their analgesic, anti-inflammatory, and antipyretic effects. NSID use is known to increase the risk of PPU (Perforated Peptic Ulcer)[17]. Nearly 25% of chronic users of these drugs develop gastric ulcer disease. It is now well – recognized that ulceration induced by NSAIDs is mediated by suppression of the cyclooxygenase (cox)- dependent pathway and subsequently blocking the synthesis of PG. different factors, such as leukocyte endothelium interaction, neutrophils infiltration, cytokine imbalance, and oxidative mucosal stress, contribute to the pathogenesis of gastric mucosal injury initiated by NSAIDs[18]. Further, NSAID also causes a marked reduction in mucosal blood flow, mucus bicarbonate secretions, impaired platelet aggregation, reduced epithelial cell renewal, and increased leukocyte adherence that is responsible for the pathogenesis of ulceration [19]. Gastric acid worsens the NSAID effects by deepening superficial lesions, interfering with platelet aggregation, and impairing the ulcer healing process [20,21].

Diagnosis

Diagnosis begins with clinical suspicion when patients present with symptoms such as epigastric abdominal pain, burning, postprandial fullness, or early satiety. Classically, patients with duodenal ulcers complain of worsening abdominal pain on an empty stomach and describe hunger or abdominal pain two to three hours after meals or at night. In contrast, patients with gastric ulcers report nausea, vomiting, weight loss, and post-prandial abdominal pain. Elderly patients are often minimally symptomatic and some patients with untreated PUD may have intermittent symptoms due to spontaneous healing and then relapse due to persistence of risk factors, such as continued NSAIDs use or *H.pylori* infection [22].

Diagnosis of infection

Several methods are currently available to detect the presence of *H.pylori*, each with its advantages, disadvantages, and limitations. A classic way to categorize the methods is according to whether or not an endoscopy is necessary. Biopsy-based tests include histological evaluation, culture, polymerase chain reaction (PCR), and the rapid urease test (RUT), all of which are performed on tissue obtained during endoscopy. Alternatively, the urea breath test (UBT), serology, and stool antigen test (SAT) can be performed as non-invasive procedures. A second way to classify these tests is according to whether they are used before or after *H.pylori* eradication treatment. For this review, we will classify diagnostic tests in this manner because this classification may be more useful to a clinician [23].There are several methods, invasive or not, for detecting the infection. Invasive tests include urease test, culture, histology, and polymerase chain reaction (PCR), which are carried out in fragments of gastric biopsies collected by endoscopy. Culture is the gold standard; it permits the typing of strains and the performance of antibiograms. However, it is expensive and available only in research centers. Its sensitivity varies according to the culture medium used and to the experience of each laboratory with a determined medium. The urease test is based on the bacterium's potent urease activity. It uses to substrate agar, urea, and phenol red (pH indicator). If the biopsy fragment immersed in the medium contains the microorganism, then hydrolysis of urea into ammonia and carbon dioxide will take place, with an increase in pH and consequent change of the agar yellowish color to rose, within 24 hat the most. Urease test has high sensitivity and specificity, and it is the most widely used method in the ambit of endoscopy. Histologic research may be carried out with several staining's, and it has a good diagnostic accuracy, which will depend on the laboratory experience. It permits, in addition to research about the bacterium, histopathologic study.

The main noninvasive methods are serology and respiratory test with urea marked by carbon (13C or 14C). Serologic tests commercially available, although presenting excellent accuracy in the diagnosis of infection among patients older than 12 years of age, do not have the necessary sensitivity and specificity among children younger than 12 years. Serology is the most common technique employed in epidemiological studies, but it is not indicated to the diagnosis of infection in a single patient, neither before nor after antimicrobial treatment. The reduction of antibody levels is done very slowly, after the bacterium eradication; they may persist for over a year. The respiratory test with urea marked with 13C or 14C is based on the same principle as the urease test. In children, only 13C is used, for being nonradioactive. Samples of expired air are collected 30 minutes before and after the ingestion of marked urea,

and the difference of CO2 excretion in both samples is determined. The cutoff to be considered as positive will depend on the spectrometer used. For the infrared spectrometer, the delta considered as positive is higher than 4 per thousand. The respiratory test is highly specific and sensitive, being the chosen one for the eradication control. Any test that is done with this purpose, except for serology, should be carried out with an interval of at least 4 weeks after the antimicrobial treatment.

Diagnosis of the ulcerated lesion

Endoscopy is no doubt the chosen method for the diagnosis of primary peptic ulcers. It permits to check the lesion characterization if it is cicatrized or in activity, the presence of bleeding, and the application of endoscopic techniques for the control of massive hemorrhages, in addition to the collection of biopsies for the diagnosis of *H.pylori* and histopathologic study. Usually, among children who evolve in an asymptomatic way, the repetition of endoscopy to validate cicatrization after treatment is not necessary, except for special cases, such as giant, very deep and recidivous primary ulcers, or those which perforated or which were accompanied by important hemorrhage [24].

Treatment

Treatment of *Helicobacter pylori* infection is important for the management of gastrointestinal disorders such as peptic ulcer and gastric cancer. Due to the increase in the prevalence of *H.pylori* resistance to antibiotics, triple therapy with clarithromycin is no longer the best treatment for *H.pylori*. It's a common bacteria infecting about half of the world's population, with higher prevalence in developing countries, where *H.pylori* could infect up to 80% of the population [25], than in developed ones. For the last two decades, the recommended treatment for *H.pylori* eradication is the standard triple therapy [26], using a proton pump inhibitor or ranitidine bismuth citrate, combined with clarithromycin and amoxicillin or metronidazole. The efficacy of these triple regimens has decreased lately to rates lower than 70%, due to *H.pylori* resistance to key antibiotics, mainly clarithromycin, but also metronidazole and levofloxacin [27,28]. These low rates of successful treatment are not acceptable under the Maastricht consensus which points out that rates consistently below 80% by intention-to-treat are not acceptable for treating *H.pylori*[29]. Information about local resistance to antibiotics should be taken into account before establishing a treatment plan for the patient to avoid repeated treatments. Several expositions to antibiotic treatments could result in more side effects and a decrease in the percentage of antibiotic resistance. For this reason, this review is an overview of *H.pylori* eradication focused on second-line therapies that are used such as sequential therapy and quadruple therapy.

H.pylori resistance to antibiotics

Classical treatment

During the 90s, the standard triple therapy was the gold standard in the treatment of *H.pylori* infections. The standard triple therapies are based on a proton pump inhibitor, clarithromycin, and amoxicillin or metronidazole. In a recent systematic review, the global incidence of primary *H.pylori* resistance to clarithromycin has been reported to be as high as 17.2%, showing an increase worldwide [30]. The prevalence of *H.pylori* resistance to clarithromycin varies among different countries, such as 10.6 to 25% in North America, 16% in Japan, and 1.7 to 23.4% in Europe [31, 32]. Metronidazole is a key component included in the triple therapies, which is associated with a high level of resistance[33]. The prevalence of metronidazole resistance has been estimated to be from 17 to 44% for Europe and America, respectively [34,35]. The highest level of resistance to this antibiotic in Europe has been reported in Western Europe, where 20 to 45% of the *H.pylori* isolates are metronidazole-resistant [36]. Due to the high level of resistance to the two key antibiotics of standard triple therapies, clarithromycin and metronidazole, and the different patterns of resistance in different populations, standard triple therapies should be adapted to the local resistance pattern, and when possible, treatment should be based on susceptibility data obtained by testing the strain after culture.

Standard triple therapies

Although 7-d triple therapies (PPI, clarithromycin plus amoxicillin or metronidazole) have been the most used treatment in the management of *H.pylori* infection, their efficacy has decreased gradually worldwide during the last decade. This has been largely related to a worldwide increase of bacterial resistance, particularly against clarithromycin, the key antibiotic in the *H.pylori* treatment. In addition, bismuth salts are no longer available in several western countries, so that quadruple therapy is not feasible worldwide. Based on this evidence, different various attempts have been performed aiming made to increase the efficacy of first-line therapies, such as these include the use of new antibiotics (i.e., such as quinolones-based therapy (or and switching to different antibiotic combinations regimens, such as the "sequential" therapy and the "concomitant" therapy.

Bismuth quadruple therapy

This therapy contains two antibiotics, tetracycline, and metronidazole, plus bismuth and PPI for 14 days [37]. This therapy is preferred as a first-line treatment option for areas with a high incidence of clarithromycin resistance and also as second-line therapy when the first treatment with the classical triple therapy against *H.pylori* was failed [26]. This therapy works independently of clarithromycin, the most problematic antibiotic in terms of resistance. Related to metronidazole, the use of high doses and prolonged treatment duration allows minimizing the impact on metronidazole-resistant strains, providing high eradication rates even in areas with a high level of resistance to this antibiotic [38].

Sequential therapy

Sequential therapy uses the same antibiotics as standard triple therapy, but they are given them sequentially: 5 days with a PPI plus amoxicillin, followed by 5 days of PPI plus clarithromycin and amoxicillin [30].Most studies have shown that sequential therapy and bismuth-based quadruple therapy have equivalent success in first-line therapy. Sequential therapy was evaluated in a pediatric population with iron deficiency. Children aged 12 to 15 years with an active *H.pylori* infection were evaluated for serum ferritin and then were randomized into 2 groups to receive either standard or sequential eradication therapy.

Hybrid therapy

This therapy is based on 7 days of therapy with PPI and amoxicillin, followed by 7 days of quadruple therapy with a PPI, amoxicillin, clarithromycin, and metronidazole. There are not much data in the literature, comparing this therapy with others, the standard or sequential therapies, but the results do not indicate that hybrid therapy will be superior to sequential Therapy [26].

Levofloxacin-based therapies

Due to the increase of clarithromycin resistance, levofloxacin, a broad spectrum quinolone, is used for the *H.pylori* eradication in order to substitute clarithromycin in triple or sequential regimens. The eradication rate of therapies containing levofloxacin could be more than 90%, especially in areas where the local resistance to levofloxacin is low [less than 10%]. As for clarithromycin and metronidazole, an increase of levofloxacin resistance is being found, due to the fact that quinolones are often used for the treatment of urinary infections. The resistance to quinolones is around 20% in Europe, 15% in America, and 10% in Asia [39].

Non-bismuth quadruple therapy

It is another valid therapy in countries with a high incidence of clarithromycin resistance. This therapy contains PPI [but without bismuth], clarithromycin, amoxicillin, and metronidazole for 10 days. The main disadvantage of this treatment is a large number of pills in comparison with other therapies [26].

Mechanism of action

Non-immunological mechanism

The first line of defense against pathogenic bacteria is the acidity of the stomach and the gastric mucosa barrier. It was suggested that, by taking probiotics, this first line of defense could be stronger due to the production of antimicrobial substances competing with *H.pylori* for adhesion receptors, stimulating mucin production, and stabilizing the gut mucosal barrier.

Antimicrobial substances

Probiotics may inhibit *H.pylori* growth by secreting short-chain fatty acids and antibacterial substances. Short-chain fatty acids such as acetic, propionic, and lactic acids are produced during the carbohydrates metabolism by probiotics and as consequence, a pH reduction is found. In 1989, the first group was observed an antagonistic effect of a Lactobacillus strain against *H.pylori* related to short-chain fatty acids [40]. Also, the antimicrobial activity could be due to the inhibition of urease activity of *H.pylori* as has been shown in other publications [41].

Competition for adhesion

There are several possible mechanisms by which probiotic bacteria can inhibit the adhesion of *H.pylori*. The adhesion of *H.pylori* to epithelial cells is important in determining the outcome in *H.pylori*-associated diseases; the ability of the bacteria to establish physical contact with the gastric epithelium is affected by the influence of the epithelial mucosa, receptors associated with the adhesion of *H.pylori* to the epithelium, and immune cells [42].

Mucosal barrier

Mucosal surfaces have protective strategies to defend against noxious substances and pathogens found within the intestinal lumen. Some strategies, such as mucins, large complex glycoproteins that protect intestinal mucosal surfaces from microbial pathogens by limiting access of environmental matter to their epithelial cells [43]. Several mucins have been identified. *H.pylori* is known to suppress MUCI and MUC5 gene expression in a human gastric cell line [44].

Immunologic mechanisms

The inflammatory response to gastric *H.pylori* infection is characterized by the release of various inflammatory mediators such as chemokines and cytokines. Probiotics could modify the immunologic response by the modulation of anti-inflammatory cytokines secretion, which would result in a reduction of gastric activity and inflammation [45].

CONCLUSION

A peptic ulcer is an acid-induced lesion of the digestive tract that is usually located in the stomach or proximal duodenum and is characterized by denuded mucosa with the defect extending into the submucosa or muscularis propria. Common causes include the bacteria *H.pylori* and anti-inflammatory pain relievers including aspirin. Upper abdominal pain is a common symptom. Treatment usually includes medication to decrease stomach acid production. If it is caused by bacteria, antibiotics may be required. The efficacy of standard triple therapies is decreasing worldwide, mainly due to an increasing prevalence of primary antibiotic resistance. Therefore, to cure *H.pylori* in clinical practice is becoming progressively more difficult, and some patients require more than two consecutive therapeutic regimens. It may be concluded that first-line therapy like novel molecules and vaccines could be considered.

REFERENCES

1. Kuna L, Jakab J, Smolic R, Raguz-Lucic N, Vcev A, Smolic M. Peptic Ulcer Disease: A Brief Review of Conventional Therapy and Herbal Treatment Options. J Clin Med [Internet]. 2019 Feb 3;8(2):179.
2. Amandeep K, Robin S, Sharma R, Sunil K. Peptic ulcer: A review on etiology and pathogenesis. IRJP. 2012 Jun 1;3:34–8.
3. Kusters JG, van Vliet AHM, Kuipers EJ. Pathogenesis of Helicobacter pylori infection. Clin Microbiol Rev [Internet]. 2006 Jul;19(3):449–90.
4. Bandyopadhyay D, Biswas K, Bhattacharyya M, Reiter RJ, Banerjee RK. Gastric toxicity and mucosal ulceration induced by oxygen-derived reactive species: protection by melatonin. Curr Mol Med. 2001 Sep;1(4):501–13.
5. Scully C, Shotts R. ABC of oral health. Mouth ulcers and other causes of orofacial soreness and pain. BMJ [Internet]. 2000 Jul 15;321(7254):162–5.
6. Tilliss T, McDowell J. "Differential Diagnosis: Is It Herpes or Aphthous?" J Contemp Dent Pract. 2002 Mar 1;3:1–15.
7. Scully C, Porter S. Recurrent aphthous stomatitis: current concepts of etiology, pathogenesis and management. J oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol. 1989 Jan;18(1):21–7.
8. Al-Mofarreh MA, Al Mofleh IA. Esophageal ulceration complicating doxycycline therapy. World J Gastroenterol [Internet]. 2003 Mar;9(3):609–11.
9. Majumdar D, Bebb J, Atherton J. Helicobacter pylori infection and peptic ulcers. Medicine (Baltimore). 2011 Mar 1;39:154–161.
10. Ahmed N. 23 years of the discovery of Helicobacter pylori: is the debate over? Ann Clin Microbiol Antimicrob [Internet]. 2005 Oct 31;4:17.
11. Prinz C, Kajimura M, Scott D, Helander H, Shin J, Besancon M, et al. Acid secretion and the H,K ATPase of stomach. Yale J Biol Med [Internet]. 1992;65(6):577–96.
12. White JR, Winter JA, Robinson K. Differential inflammatory response to Helicobacter pylori infection: etiology and clinical outcomes. J Inflamm Res [Internet]. 2015 Aug 13;8:137–47.
13. Cohen MM. Role of endogenous prostaglandins in gastric secretion and mucosal defense. Clin Invest Med. 1987 May;10(3):226–31.
14. Hendrickson BA, Gokhale R, Cho JH. Clinical aspects and pathophysiology of inflammatory bowel disease. Clin Microbiol Rev [Internet]. 2002 Jan;15(1):79–94.
15. Arakawa T, Watanabe T, Tanigawa T, Tominaga K, Fujiwara Y, Morimoto K. Quality of ulcer healing in gastrointestinal tract: its pathophysiology and clinical relevance. World J Gastroenterol [Internet]. 2012 Sep 21;18(35):4811–22.
16. Aihara T, Nakamura E, Amagase K, Tomita K, Fujishita T, Furutani K, et al. Pharmacological control of gastric acid secretion for the treatment of acid-related peptic disease: past, present, and future. Pharmacol Ther. 2003 Apr;98(1):109–27.
17. García Rodríguez LA, Jick H. Risk of upper gastrointestinal bleeding and perforation associated with individual non-steroidal anti-inflammatory drugs. Lancet (London, England). 1994 Mar;343(8900):769–72.
18. Griffin MR, Piper JM, Daugherty JR, Snowden M, Ray WA. Nonsteroidal anti-inflammatory drug use and increased risk for peptic ulcer disease in elderly persons. Ann Intern Med. 1991 Feb;114(4):257–63.
19. Farzaei MH, Abdollahi M, Rahimi R. Role of dietary polyphenols in the management of peptic ulcer. World J Gastroenterol. 2015 Jun;21(21):6499–517.
20. Valkhoff VE, Sturkenboom MCJM, Kuipers EJ. Risk factors for gastrointestinal bleeding associated with low-dose aspirin. Best Pract Res Clin Gastroenterol. 2012 Apr;26(2):125–40.
21. Malfertheiner P, Chan FKL, McColl KEL. Peptic ulcer disease. Lancet (London, England). 2009 Oct;374(9699):1449–61.
22. Narayanan M, Reddy KM, Marsicano E. Peptic Ulcer Disease and Helicobacter pylori infection. Mo Med [Internet]. 2018;115(3):219–24.

23. Garza-González E, Perez-Perez GI, Maldonado-Garza HJ, Bosques-Padilla FJ. A review of Helicobacter pylori diagnosis, treatment, and methods to detect eradication. World J Gastroenterol [Internet]. 2014 Feb 14;20(6):1438–49.
24. Cosgun Y, Yildirim A, Yucel M, Karakoc AE, Koca G, Gonultas A, et al. Evaluation of Invasive and Noninvasive Methods for the Diagnosis of Helicobacter Pylori Infection. Asian Pac J Cancer Prev [Internet]. 2016 Dec 1;17(12):5265–72.
25. Moayyedi P, Hunt RH. Helicobacter pylori public health implications. Helicobacter. 2004;9 Suppl 1:67–72.
26. Papastergiou V, Georgopoulos SD, Karatapanis S. Treatment of Helicobacter pylori infection: Past, present and future. World J Gastrointest Pathophysiol [Internet]. 2014 Nov 15;5(4):392–9.
27. Agudo S, Pérez-Pérez G, Alarcón T, López-Brea M. Rapid detection of clarithromycin resistant Helicobacter pylori strains in Spanish patients by polymerase chain reaction-restriction fragment length polymorphism. Rev Esp Quimioter [Internet]. 2011 Mar;24(1):32–6.
28. Nishad KM, Arul B RS. Design and comparative evaluation of clarithromycin gastric bioadhesive tablets by ex vivo and in vivo methods. Asian J Pharm Clin Res. 2018;11(4):248–56.
29. Graham DY, Lu H, Yamaoka Y. A report card to grade Helicobacter pylori therapy. Vol. 12, Helicobacter. England; 2007. p. 275–8.
30. Kuipers EJ. Helicobacter pylori and the risk and management of associated diseases: gastritis, ulcer disease, atrophic gastritis and gastric cancer. Aliment Pharmacol Ther. 1997 Apr;11 Suppl 1:71–88.
31. Elitsur Y, Lawrence Z, Rüssmann H, Koletzko S. Primary clarithromycin resistance to Helicobacter pylori and therapy failure in children: the experience in West Virginia. Vol. 42, Journal of pediatric gastroenterology and nutrition. United States; 2006. p. 327–8.
32. Horiki N, Omata F, Uemura M, Suzuki S, Ishii N, Fukuda K, et al. Risk for local recurrence of early gastric cancer treated with piecemeal endoscopic mucosal resection during a 10-year follow-up period. Surg Endosc. 2012 Jan;26(1):72–8.
33. Malfertheiner P, Megraud F, O'Morain C, Bazzoli F, El-Omar E, Graham D, et al. Current concepts in the management of Helicobacter pylori infection: the Maastricht III Consensus Report. Gut. 2007 Jun;56(6):772–81.
34. Ogata SK, Godoy APO, da Silva Patricio FR, Kawakami E. High Helicobacter pylori resistance to metronidazole and clarithromycin in Brazilian children and adolescents. J Pediatr Gastroenterol Nutr [Internet]. 2013;56(6):645–8.
35. De Francesco V, Zullo A, Ierardi E, Vaira D. Minimal inhibitory concentration (MIC) values and different point mutations in the 23S rRNA gene for clarithromycin resistance in Helicobacter pylori. Vol. 41, Digestive and liver disease : official journal of the Italian Society of Gastroenterology and the Italian Association for the Study of the Liver. Netherlands; 2009. p. 610–1.
36. López-Brea M, Martínez MJ, Domingo D, Alarcón T. A 9 year study of clarithromycin and metronidazole resistance in Helicobacter pylori from Spanish children. J Antimicrob Chemother. 2001 Aug;48(2):295–7.
37. Harb AH, El Reda ZD, Sarkis FS, Chaar HF, Sharara AI. Efficacy of reduced-dose regimen of a capsule containing bismuth subcitrate, metronidazole, and tetracycline given with amoxicillin and esomeprazole in the treatment of Helicobacter Pylori infection. United Eur Gastroenterol J. 2015 Feb;3(1):95–6.
38. Lee ST, Lee DH, Lim JH, Kim N, Park YS, Shin CM, et al. Efficacy of 7-Day and 14-Day Bismuth-Containing Quadruple Therapy and 7-Day and 14-Day Moxifloxacin-Based Triple Therapy as Second-Line Eradication for Helicobacter pylori Infection. Gut Liver [Internet]. 2015 Jul;9(4):478–85.
39. Liang C-M, Cheng J-W, Kuo C-M, Chang K-C, Wu K-L, Tai W-C, et al. Levofloxacin-containing second-line anti-Helicobacter pylori eradication in Taiwanese real-world practice. Biomed J. 2014;37(5):326–30.
40. Bhatia SJ, Kochar N, Abraham P, Nair NG, Mehta AP. Lactobacillus acidophilus inhibits growth of Campylobacter pylori in vitro. J Clin Microbiol [Internet]. 1989 Oct;27(10):2328–30.
41. Sgouras D, Maragkoudakis P, Petraki K, Martinez-Gonzalez B, Eriotou E, Michopoulos S, et al. In vitro and in vivo inhibition of Helicobacter pylori by Lactobacillus casei strain Shirota. Appl Environ Microbiol. 2004 Jan;70(1):518–26.
42. Zhang C, Zhang H, Yu L, Cao Y. Helicobacter pylori dwelling on the apical surface of gastrointestinal epithelium damages the mucosal barrier through direct contact. Helicobacter. 2014 Oct;19(5):330–42.

43. Mack DR, Ahrne S, Hyde L, Wei S, Hollingsworth MA. Extracellular MUC3 mucin secretion follows adherence of Lactobacillus strains to intestinal epithelial cells in vitro. Gut. 2003 Jun;52(6):827–33.
44. Hanisch F-G, Bonar D, Schloerer N, Schroten H. Human trefoil factor 2 is a lectin that binds α-GlcNAc-capped mucin glycans with antibiotic activity against Helicobacter pylori. J Biol Chem. 2014 Oct;289(40):27363–75.
45. Wiese M, Eljaszewicz A, Andryszczyk M, Gronek S, Gackowska L, Kubiszewska I, et al. Immunomodulatory effects of Lactobacillous plantarum and Helicobacter pylori $CagA^+$ on the expression of selected superficial molecules on monocyte and lymphocyte and the synthesis of cytokines in whole blood culture. J Physiol Pharmacol an Off J Polish Physiol Soc. 2012 Jun;63(3):217–24.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Evaluating the Acute Toxicity Effect of Benzophenone-3, and it's Environmentally Relevant Concentration on Behavioral Changes and Growth Performance in Zebrafish

S. Velanganni and S. Miltonprabu*

Department of Zoology, Annamalai University, Annamalainagar, Tamil Nadu, India.

Received: 01 Dec 2020 Revised: 14 Dec 2020 Accepted: 21 Dec 2020

***Address for Correspondence**
S. Miltonprabu
Department of Zoology,
Annamalai University,
Annamalainagar, Tamil Nadu, India.
Email: smprabu73@gmail.com

ABSTRACT

Benzophenone-3 (BP-3) is widely used in sunscreens and other personal care products (PCPs), cosmetics, etc. The widespread use of BP-3 has resulted in its release into the aquatic environment, and hence it's impact on the aquatic ecosystem is of grave concern. Therefore, the aim of this study was to determine the LC50 of BP3 and growth performance and behaviour study at environmentally relevant concentration (44 μg/L in river) on Zebrafish. In acute toxicity analysis (LC50), adult Zebrafish was exposure to different concentrations of BP3 (2, 4, 6, 8 and 10 mgL-1) for 24, 48, 72 and 96 h. In growth performance analysis, fish were exposed to BP3 at environmentally relevant concentration for 45 days as well as behaviour study fish were noticed at the environmentally relevant concentration for 24 hrs. The results revealed that the 24, 48, 72 and 96 hours LC50 mortality of BP3 in Zebrafish were 4.468, 3.998, 3.25 and 2.549 mgL-1, respectively. Further, the BP3 exposed fishes displayed a significant decline in the levels of body weight, net weight gain, length gain, specific growth rate and condition factor. In contrast, the feed conversion ratio and feed efficiency ratio was remarkably increased when compared to control fish. The behavioural changes also increased in the environmentally relevant concentration of BP3 (44 μg/L) exposed fishes. The present results display that at an environmentally relevant concentration, BP3 can induce abnormal changes in Zebrafish.

Keywords: Acute toxicity, benzophenone-3, growth performance and Zebrafish.

INTRODUCTION

In recent times, production and use of sunscreen have continuously increased due to the rise in awareness of protecting the skin against sunlight exposure damage and thus reducing the risk of skin cancer (Waldman and

Grant-Kels, 2019). From the vast array of chemical compounds contained in the sunscreens, among them, the main active ingredients are the ultraviolet (UV) filters. The main purpose of UV filters is to absorb or reflect UVA and/or UVB radiations that range between 280 to 400 nm (Sanchez-Quiles and Tovar-Sanchez, 2015).Among the many UV filters, Benzophenone-3 (BP-3) is one of the most popular BP-type of UV filters that have been used for over 40 years as one of the sunscreen ingredients (Kim and Choi, 2014). BP-3 can get absorbed through the mouth as well as the skin of humans as it is lipophilic by nature, and highly stable in light, thus leading to bioaccumulation (Du *et al*., 2017). In the environment, BP3 ranges from ng to μg/L. The highest concentrations detected are 44 μg/L in the river, 34.3 μg/L in the seawater, 10.4 μg/L in the wastewater influent, 4.5 μg/L in the swimming pool, 0.45 μg/L in the tap water, 0.2 μg/L in the lake, and 0.034 μg/L in the ground water (Kim and Choi, 2014; Ramos *et al*., 2015). Previously, there had been some limited reports about the toxic nature of BP3. It has been reported to reduce the levels of thyroid hormone T3 (Lee *et al*., 2018), caused a decline in the reproductivity (Santamaría *et al*. 2019), lead to endocrine dysfunction (Karin *et al*., 2015) and can induce dysregulated expression of neurogenesis and neurotransmitter-related genes (Wnuk *et al*., 2018b). Moreover, under mid-altitude conditions, the half-life of BP-3 on the surface water was found to be couple of weeks in summer, whereas, in winter, over 3 months (Vione *et al*., 2013), suggesting a very slow degradation in the aquatic environment. This increases its chances of persistence as well as bioaccumulation and biomagnification in the aquatic ecosystem. However, fewer studies have explored its adverse effects elicited in aquatic organisms at concentrations that are environmentally relevant (Tao *et al*., 2020). Additionally, there was no proper acute toxicity, and its impact on growth performance for BP3 reported on aquatic organisms. Thus, our present study was designed to evaluate the LC_{50} of BP3, and its impact on growth performance at an environmentally relevant concentration (44 μg/L in river) on Zebrafish.

MATERIALS AND METHODS

Chemicals

Benzophenone-3 (98% purity) was procured from Sigma Aldrich, USA Reaming other chemicals and reagents used were of analytical grade and obtained from Merck, Himedia, Mumbai, India.

Experimental animal and test conditions

Adult Zebrafish (*Danio rerio,* wild-type, AB strain) of both genders (0.5 ± 0.3 g; 3.1 ± 0.4 cm length) were obtained from the Red hills fish farm (Chennai, Tamil Nadu, India). Fish were acclimatized to laboratory conditions in continuously aerated dechlorinated tap water and maintained under a photoperiod of 12-h/12-h light–dark cycle. During the acclimatization period, fish were fed twice a day with commercial pellets, and the residues and metabolic wastes were removed daily. The water quality parameters of the dechlorinated tap water used for fish maintenance were determined. Fish specimens were subjected to prophylactic treatment by bathing them in 0.05% (w/v) potassium permanganate (KMnO4) solution for 2 min. The fish were then acclimatized for 15 days under laboratory conditions prior to cadmium exposure.

Stock solution preparation

The BP3 stock solution (1000 mg/L) was prepared using dimethyl sulfoxide (DMSO) and was stored at −20°C. The working solutions were later prepared by diluting the stock solution immediately prior to the experiments. The standard solution was added to the experimental vessels with test fish to obtain the environmentally relevant concentration of BP3 (44 μg/L).

Acute toxicity (LC50)

Before the commencement of the experiment, the fish were not fed for 24 hours. The next day, the stock solutions were prepared and added to the water at different concentrations BP3 (2, 4, 6, 8 and 10 $\mathbf{mgL^{-1}}$) (Walum, 1998). For each concentration, ten fish were utilised. The % mortality of fish in different concentrations was noted after exposure periods of 24, 48, 72 and 96 hours. The LC_{50} values were different for different exposure periods and were

obtained after computing probit analysis. In the probit analysis, the concentrations were converted into the log concentrations, and percent mortality values were converted into probit scale. Additionally, using regression analysis, graphs were drawn to obtain straight lines and derive the LC_{50} values for each of the exposed concentrations. Finally, the Chi-square tests were carried out to test the goodness of fit for comparing the observed Y-values as well as the expected Y-values (Finney, 1964).

Experimental design of chronic analysis on environmentally relevant concentration of BP3

The adult Zebrafish were exposed to environmentally relevant concentration of BP3 (44 μg/L) for a period of 45 days. A group of 100 health fish of the same size were exposed to the selected concentration. In parallel to this, a control group was also maintained. Three replicates were maintained for each concentration and control groups. The medium and the test solutions were renewed at the end of 24 h till 45 days. At the end of 15, 30 and 45 days of exposure periods the fish from control and treated groups were caught from the tank using hand net to evaluate effects of BP3 on growth of Zebrafish. The behavioral changes were noticed at different duration like 8, 16 and 24 hours at environmentally relevant concentration of BP3 (44 μg/L).

Growth performance and food utilization were determined using the methods of Sveier et al. (2000). The net weight gain (NWG), specific growth rate (SGR), feed conversion ratio (FCR), feed efficiency ratio (FER) and condition factor (k) were calculated according to the following formulae:

1. Net Weight gain (NWG) = Average final weight (g) - Average initial weight (g).
2. Specific growth rate (SGR) = Final weight-Initial Weight (g) × 100/ days
3. Feed conversion ratio (FCR) = Dry feed given (g)/ Weight gain of fish (g)
4. Feed efficiency ratio (FER) = Weight (g)/ Feed intake (g).
5. Condition factor (K) = 100 W/L3

Where, W is the wet body weight (g) and L is the total length (cm).

Statistics

All the results were presented as mean ± SD of ten fish in each group. The value of $p< 0.05$ was considered as statistically significant by one-way analysis of variance (ANOVA) followed by Duncan's Multiple Range test (IBM SPSS Statistics for Windows, version 15).

RESULTS

Acute toxicity (LC50)

The mortality of *Danioraria* on exposure to different concentrations of BP3 (2, 4, 6, 8 and 10 **mgL^{-1}**) at 24, 48, 72 and 96 h of duration are presented in Table 1. During the experimental period, the fish mortality rate significantly increased with increase in the concentration of BP3 exposure and duration. The lowest fish mortality rate was 21. 875 noticed at 2 **mgL^{-1} of BP3 exposure on 24hrs, while 100% mortality was present at 8 mgL^{-1} of BP3 exposure on 96 hrs.** The 24, 48, 72 and 96 hours LC_{50} mortality of BP3 in Zebrafish were found to be **4.468, 3.998, 3.25 and 2.549 mgL^{-1},** respectively. The chi-square values were found to be 0.312, 0.531, 0.358 and 0.276, which revealed the mortality rate of BP3 exposed to Zebrafish was significant at $p<0.05$ (Table 2).The increased concentration of BP3 resulted in increased mortality of fish, while the increase in exposure duration resulted in increased mortality at lower concentrations of BP3. The LC_{50} values were determined by using Probit analysis method (Finny-1971)(Figure 1 - 4).

Behavioral changes

The behavioral changes were noticed at different duration like 8, 16 and 24 hours (Table 3). The control fishes did not exhibit any unusual behavioral responses like hyper excitability, opercular movement, dyspigmentation, mucus secretion and imbalanced swimming for 24 hrs. The fish, *Daniorerio,* exposed to environmentally relevant concentration of BP3 (44 μg/L) for 24 hours showed changes in behavioral responses when compare to control fish.

During the 0-8 hours interval, BP3 exposed fishes showed a mild rapid fin movement, faster opercular activity and hyper excitability, whereas, no mucus secretion, dyspigmentation and imbalanced swimming was observed in BP3 treated fish. During 8-16 hours exposure intervals, BP3 exposed fishes exhibited moderate rapid fin movement, hyper excitability and opercular movement, along with mild dyspigmentation, mucus secretion and imbalanced swimming. In interval of 16 – 24 hrs, environmentally relevant concentration of BP3 (44 µg/L) exposed fishes displayed high rapid fin movement, opercular movement and hyper excitability, along with moderate dyspigmentation, mucus secretion and imbalanced swimming. No mortality was noticed during the experimental period (24 hrs). When the duration was increase, the behavioural changes also increased in environmentally relevant concentration of BP3 (44 µg/L) exposed fishes.

Growth performance

Figure 5 and 6 displays the control and environmental relevant concentration of BP3 exposed Zebrafish. In growth performance analysis, body weight, length gain, net weight gain, specific growth rate, feed conversion ratio, feed efficiency ratio and condition factor were evaluated at different interval like 15, 30 and 45 days in BP3 exposed Zebrafish. The BP3 exposed fishes displayed a significant decline in the level of body weight, net weight gain, length gain, specific growth rate and condition factor, whereas feed conversion ratio and feed efficiency ratio were remarkably increased when compared to control fish. A significant change was noticed at duration of 15, 30 and 45 days in all the growth performance parameters when compared to control fish.

DISCUSSION

A toxicant is an agent or substance that can elicit numerous adverse effects in a biological system, seriously damaging its structure or function or lead to death. In fish and other aquatic organisms, effects that occur within a few hours or days or weeks are considered acute (Zaki*et al*., 2014; Mahnaz *et al*., 2018). Generally, acute effects are relatively severe. A chemical is considered as acutely toxic if by its direct action kills 50% or more of the exposed population of test organisms in a relatively short period of time, such as 24, 48, 72 and 96 hrs (Adebayo *et al*., 2013; Saganuwa, 2016). In the present study, the LC_{50} concentration of BP3 were **4.468, 3.998, 3.25 and 2.549 mgL^{-1}, in** Zebrafish at 24, 48, 72 and 96 hrs, respectively. The mortality rate was notably increased along with the increase in concentration and duration of exposure of BP3. Thus, the above result indicated that BP3 (UV filter) can induce mortality in aquatic organisms, and may be due to the disruption of endocrine function as well as neurotoxicity (Chen *et al*., 2016). The above obtained result was also supported by Du et al. (2017), who reported that, the 96 h-EC_{50} value of *Chlorella vulgaris* was 2.98 mg/L, the 48 h-LC50 value of *Daphnia magna* was 1.09 mg/L, and the 96 h-LC50 value of *Brachydanio rerio* was 3.89 mg/L. This result indicated that Zebrafish is showing low susceptibility to BP3 than *Brachydanio rerio.* Further, UV filter BP3 is a more toxic than BP4, was confirmed by Chen *et al*., 2016, who stated that the 96 h-EC50 value of *C. vulgaris* was 201.00 mg/L, the 48 h-LC50 value of *D. magna* was 47.47 mg/L, and the 96 h-LC50 value of *B. rerio* was 633.00 mg/L. The studies on fish behaviours provide a lot of knowledge and information because any behaviour alteration can be directly related to physiological biomarker in aquatic species. For example, the monitoring of behavioural response becomes an impending option to environmental change, disease, stress and the presence of toxic compound in water, which initiates the variation of fish behaviour (Zarantoniello *et al*., 2020). In BP3 exposed fish, the fish exhibited high rapid fin movement, opercular movement and hyper excitability. Additionally, moderate dyspigmentation, mucus secretion and imbalance in swimming during the end of experimental period was also observed when compared to control fish. The rapid fin movements, faster opercular movements and the hyper excitability were developed in BP3 exposed fish, and may be due to accumulation of BP3 in fish body, which induces ionic disturbances in blood as well as in tissues. The imbalance in swimming was developed in BP3 exposed fish, which might be due to inactivation of the acetylcholinesterase, and leading to the accumulation of acetylcholine at synaptic junctions. These results were further supported by Kelley et al. (2019), who reported that BP-3 interferes with social behaviour in the male Siamese fighting fish.

The growth of fish can be quantified by utilizing some parameters such as body weight, length gain, net weight gain, specific growth rate, feed conversion rate, feed efficiency ratio and condition factor (Zebral *et al*., 2018). The current results demonstrated that abnormal level of growth parameters in Zebrafish when exposed to environmental relevant concentration of UV filter BP3 (44 µg/L) than control Zebrafish. These abnormal changes occurred in fish growth, at the environmentally relevant concentration of BP3 (44 µg/L), may be due to the harmful effect induced in the Zebrafish, which leads to an imbalance in the normal physiology leading to reduced growth of fish. A healthy body weight and length are generally considered to be an indicator of good health of organism. Earlier, Zebral et al. (2018) stated that body weight and length inhibition is also a prominent effect of toxicant accumulation following chronic exposure (Zebral *et al*. 2018). In the present analysis, the level of body weight, length gain, net weight gain and specific growth rate were notably reduced in BP3 exposed fish when compared to control fish at different intervals (15, 30 and 45 days). This result clearly displayed that the chronic exposure of BP3 (44 µg/L) can be toxicant to fish, due to accumulation of BP3 in tissues of fish. These results were support by Teoh et al. (2020), who reported that the highest concentration of oxybenzone (300 and 400 mgL−1) had adverse effects on growth rate and biomass of microalgae. Further, Santamaria et al. (2020) concluded that the dermal exposure to the UV filter benzophenone-3 affects fetal growth of the progeny in mice during early pregnancy. These supporting studies further confirm the toxic effects of BP3 on living organisms.

Feed is a key role to increasing production. Improved nutritional value in feed may provide better growth. Utilization of nutrients in an efficient diet is an important factor to improve the growth. The FCR and FER important parameters help to analyse the feed intake of organism (Dvergedal *et al*., 2019). The decreases food intake and assimilation and this may lead to the reduction in the growth rate in fish (Mengistu *et al*., 2017). In BP3 exposed fishes showed a significant increase level of FCR and FER when compared to control at different intervals. This effect may be due to the detrimental effects of the environmentally relevant concentration of BP3 (44 µg/L) in Zebrafish, which leads to an imbalance in the normal physiology leading to reduced feed uptake and growth in fish. The condition factors (K) is used as an indicator reflecting the wellbeing of an organism, as it integrates various levels of organizational processes. A decrease in condition factors is considered as a reflection of energy reserves depletion as these are positively related to muscle and livers energy content (Mazumder *et al*., 2016). In the present experiment, the condition factor was declined in BP3 exposed fish when compared to control fish. This may be due to BP3 that might have affected the olfactory systems, which in turn might have disrupted the feeding behaviour, resulting in alterations of metabolic activities as well as energy allocations in the system of the fish.

CONCLUSION

The current study presented that the 96 hrs LC_{50} value of UV filter BP3 was **2.549 mg/L, which is nearly fifty-eight times the environmental concentration (**44 µg/L) reported in the river. Further, the chronic exposure of environmentally relevant concentration of BP-3 also brought about changes in the behaviour as well as inhibited the growth of the Zebra fish.

CONFLICT OF INTEREST

All authors declare that there were no conflicts of interest concerning this publication

REFERENCES

1. Waldman RA. Grant-Kels JM. The role of sunscreen in the prevention of cutaneous melanoma and non melanoma skin cancer. J. Am. Acad. Dermatol 2019; 80:574–576.

2. Sanchez-Quiles D, Tovar-Sánchez A. Are sunscreens a new environmental risk associated with coastal tourism? Environ. Int 2015; 83:158–170.
3. Kim S, Choi K. Occurrences, toxicities, and ecological risks of benzophenone-3, a common component of organic sunscreen products: A mini-review. Environ. Int. 2014;70:143–157.
4. Du Y, Wang WQ, Pei ZT, et al. Acute Toxicity and Ecological Risk Assessment of Benzophenone-3 (BP-3) and Benzophenone-4 (BP-4) in Ultraviolet (UV)-Filters. Int J Environ Res Public Health 2017;14(11):1414.
5. ECHA Oxybenzone—Substance Information—ECHA. [(accessed on 3 August 2017)]; Available online: https://echa.europa.eu/substance-information/-/substanceinfo/100.004.575#TRADE_NAMEScontainer.
6. Ramos S, Homem V, Alves A, Santos L. Advances in analytical methods and occurrence of organic UV-filters in the environment—a review. Sci. Total Environ 2015;526: 278–311.
7. Lee J, Kim S, Park YJ, Moon HB, Choi K. Thyroid hormone-disrupting potentials of major Benzophenones in two cell lines (GH3 and FRTL-5) and embryo-larval zebrafish. Environ. Sci. Technol 2018; 52 (15): 8858–8865.
8. Karin KP, Albrektsen G, Minghlani M, Awad M, et al. Endocrine disrupting effect of the UV filter benzophenone-3 in zebrafish, Daniorerio. Environmental toxicology and chemistry / SETAC 2015:34.
9. Wnuk A, Rzemieniec J, Litwa E. The crucial involvement of retinoid X receptors in DDE neurotoxicity. Neurotox. Res 2016;29(1):155–172.
10. Vione D, Caringella R, De Laurentiis E, Pazzi M, Minero C. Phototransformation of the sunlight filter benzophenone-3 (2- hydroxy-4-methoxybenzophenone) under conditions relevant to surface waters. Sci Total Environ 2013;463–464:243–251.
11. Tao J, Bai C, Chen Y, Zhou H. Environmental relevant concentrations of benzophenone-3 induced developmental neurotoxicity in zebrafish. Science of the Total Environment 2020; 721:137686.
12. Walum E. Acute oral toxicity. Environ Health Perspect 1998;106:497-503.
13. Finney DJ, 1964. Probit analysis. Second edition: Cambridge University Press, London.
14. Sveier H, Raae AJ, Lied E. Growth and protein turnover in Atlantic salmon (Samosalar L.); the effect of dietary protein level and protein particle size. Aquaculture 2000;185:101-120.
15. Mahnaz SS, Sadegh P. Evaluation of toxicity and lethal concentration (LC50) of silver and selenium nanoparticle in different life stages of the fish Tenualosailish (Hamilton 1822). Oceanogr Fish Open Access J 2018;7(5): 555722.
16. Zaki MS, Authman MMN, Hammam AMM, Shalaby SI. Aquatic environmental pollution in the Egyptian countryside and its effect on fish production (Review). Life Sci J. 2014;11:1024-1029.
17. Adebayo IA, Akin-Obasola BJ and Bajulaye OM. Toxicological effect of diazinon on African catfish (Clariasanguillaris). IOSR Journal of Environmental Science. Toxicology and Food Technology 2013;3(1): 64-71.
18. Saganuwan SA. Toxicity study of drugs and chemicals in animals: An overview. BJVM. 2016; ISSN 1311-1477.
19. Chen TH, Wu YT, Ding WH. UV-filter benzophenone-3 inhibits agonistic behavior in male Siamese fighting fish (Bettasplendens). J. Ecotoxicol. 2016;25:1–8.
20. Zarantoniello M, Randazzo B, Gioacchini G et al. Zebrafish (Daniorerio) physiological and behavioural responses to insect-based diets: a multidisciplinary approach. Sci Rep 2020;10:10648.
21. Kelley BP, Megan AS, Traska N. Exposure to the ultraviolet filter benzophenone-3 (BP3) interferes with social behaviour in male Siamese fighting fish. Animal Behaviour 2019;158:175-182.
22. Zebral YD, Anni ISA, Afonso SB, Abril SIM, Klein RD, Bianchini A. Effects of lifetime exposure to waterborne copper on the somatotropic axis of the viviparous fish *Poecilia vivipara*. Chemosphere. 2018;203:410–417.
23. Santamaria CG, Meyer N, Schumacher A et al. Dermal exposure to the UV flter benzophenone-3 during early pregnancy afectsfetal growth and sex ratio of the progeny in mice. Arch Toxicol 2020; https ://doi.org/10.1007/s00204-020-02776-5.
24. Dvergedal H, Odegård J, Overland M. et al. Selection for feed efficiency in Atlantic salmon using individual indicator traits based on stable isotope profiling. Genet SelEvol 2019; 51:13.
25. Mengistu S, Mulder HA, Benzie John AH, Komen, J. A systematic review of management factors affecting growth, feed conversion ratio and survival in Nile tilapia. International Conference and Exposition EAS. 2017:753-754.

26. Mazumder SK, Das SK, Bakar Y, Ghaffar MA. Effects of temperature and diet on length-weight relationship and condition factor of the juvenile Malabar blood snapper (Lutjanusmalabaricus Bloch & Schneider, 1801). J Zhejiang UnivSci B 2016;17(8):580-590.

Table 1: Cumulative mortality of Zebrafish during acute exposure to BP3 (n=20)

Concentration (mgL^{-1})	Number of mortality (%)			
	24 hours	48 hours	72 hours	96 hours
2	21.87	27.84	34.05	39.86
4	37.37	41.37	49.37	63.37
6	51.45	60.45	69.45	96.89
8	79.72	86.78	89.78	100
10	92.64	98.64	100	100

Table 2: LC_{50} Values with 95% confidence limits and regression co-efficient and χ^2 for Zebrafish exposed to UV filter BP3

Exposure period (hr)	**LC_{50} (mgL^{-1}) 95% fiducial limits (LCL-UCL)**	**Regression co-efficient (Y)**	**X2**	**R2**	Slope function (SF)
24	**4.468 (3.297- 6.054)**	**Y= 2.8989x + 3.1232**	0.312	0.871	**2.899**
48	**3.998 (2.858 - 5.594)**	Y=2.6035x + 3.4399	0.531	0.865	**2.603**
72	**3.25 (2.254 - 4.695)**	Y= 2.4213x + 3.7644	0.358	0.827	**2.421**
96	**2.549 (1.922 - 3.380)**	Y= 4.0906x + 3.3555	0.276	0.876	**4.091**

Significant at $p<0.05$; Control nil mortality; LC_{50}: lethal concentration that kills 50% of the exposed Zebrafish; LFL lower fiducial limits; UFL upper fiducial limits; x^2 : Chi-square value.

Table 3: Effect of environmentally relevant concentration (44 µg/L) BP3 on behavioural changes in *Danio rerio*

Nature of behaviour	**Control**	**BP3 (44 µg/L) 8 hours**	**BP3 (44 µg/L) 16 hours**	**BP3 (44 µg/L) 24 hours**
Hyper excitability	-	-	+	++
Opercular movement	-	+	+	+++
Fin movement	-	+	++	+++
Dispigmentation	-	-	+	++
Mucus secretion	-	-	+	++
Imbalanced swimming	-	-	+	++
Mortality	-	-	-	-

- = None (or) Normal; + = Mild effect; ++ = Moderate effect; +++ = High effect

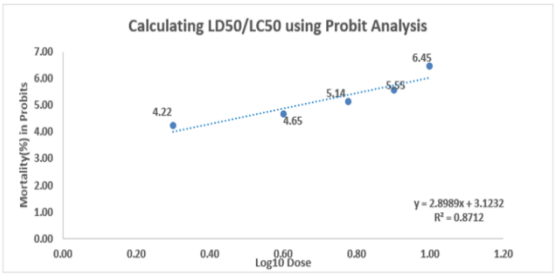


Figure 1. The graph shows regression lines between percent mortality (in Probit) of Zebrafish against log of various UV filter BP3 concentration for 24 hrs.

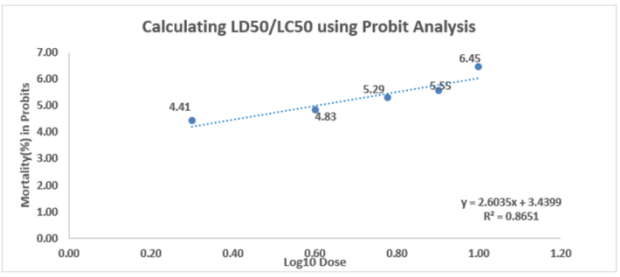


Figure 2. The graph shows regression lines between percent mortality (in Probit) of Zebrafish against log of various UV filter BP3 concentration for 48 hrs.

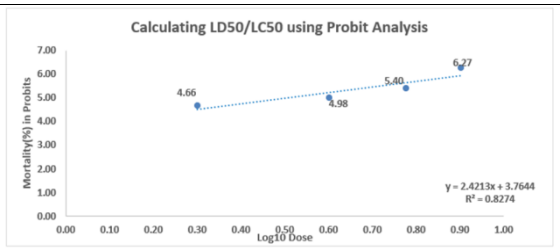


Figure 3. The graph shows regression lines between percent mortality (in Probit) of Zebrafish against log of various UV filter BP3 concentration for 72 hrs.

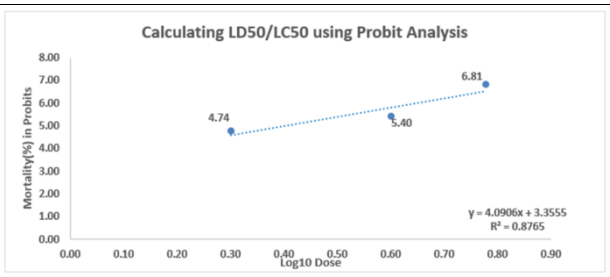
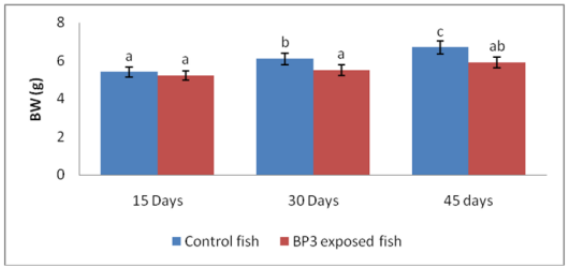
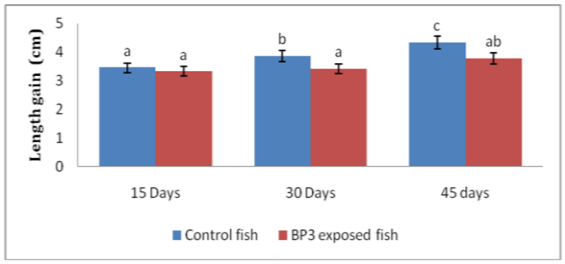


Figure 4. The graph shows regression lines between percent mortality (in Probit) of Zebrafish against log of various UV filter BP3 concentration for 96 hrs.





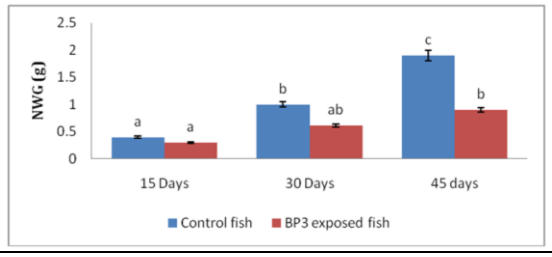


Figure 5. Effect of environmentally relevant concentration (44 µg/L) BP3 on growth performance in *Danio rerio*

A: body weight; B: Length gain; C; Net weight gain. Results are presented as mean ± SE. Bars with different letter indicates significant different (P<0.05) according to DMRT.

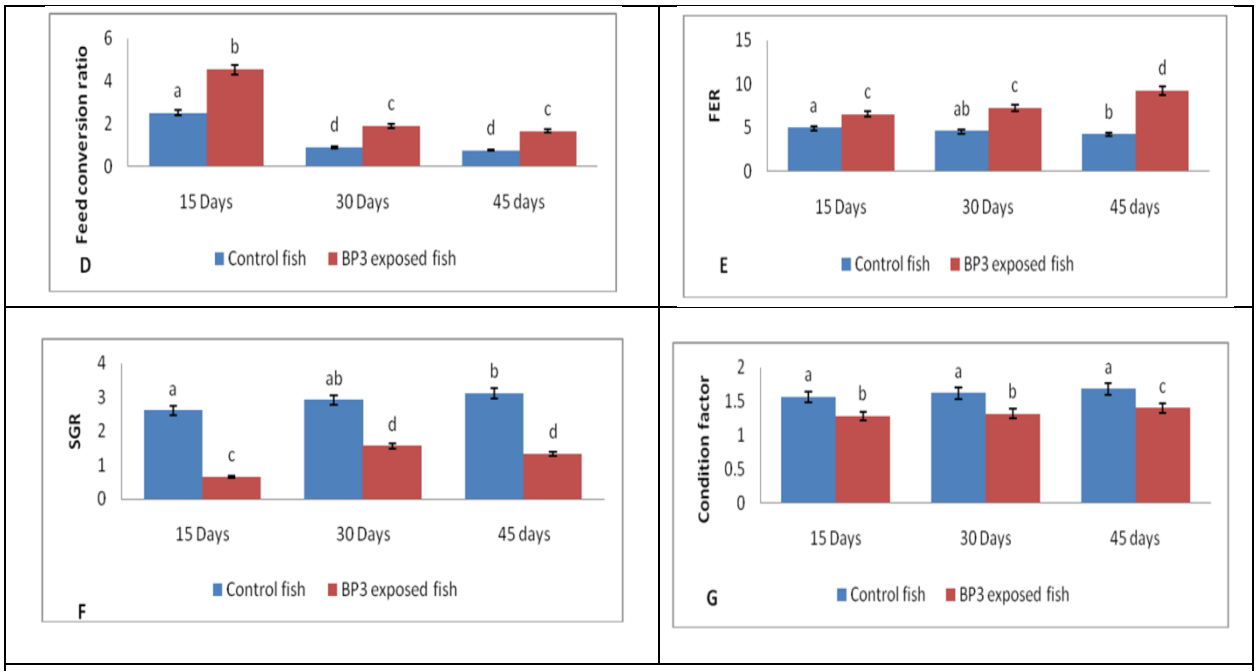


Figure 6: Effect of environmentally relevant concentration (44 µg/L) BP3 on growth performance in *Danio rerio*

D: Feed conversion ratio; E: Feed efficiency ratio; F; Specific growth rate; G: Condition factor (K).Results are presented as mean ± SE. Bars with different letter indicates significant different (P<0.05) according to DMRT.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Conflicts between Staff: Causes and Effects of School's Activities

Shandru Mariyadas[1]* and Saravanakumar AR[2]

[1]Ph.D Scholar, Department of Education, Alagappa University, Karaikudi, Tamil Nadu, India.
[2]Assistant Professor, Department of Education, Alagappa University, Karaikudi, Tamil Nadu, India.

Received: 01 Nov 2020 Revised: 20 Nov 2020 Accepted: 22 Dec 2020

***Address for Correspondence**
Shandru Mariyadas
Ph.D Scholar,
Department of Education,
Alagappa University,
Karaikudi, Tamil Nadu, India.

ABSTRACT

The conflict situations are a rationally frequent realism in every organization, including the school, and their recognition, consideration, and control represent a constant interest area for the psychologists and educational psychologist experts, mainly because of their impact on the individual and organizational performance. Even though it is a reality minimized or "hidden" by teachers and school managers, these conflicts affect the value of the learning environment and upward administration, as well as the teacher's performance. This paper presents how the professional conflicts are perceived and managed by teachers and principals, based on 10 I-AB schools from the total of 64 more numbers of staff included schools were being selected from research area by Random Sampling Method where principals, teachers, student's leader, and non-academic staff have selected as the respondents of primary data collection to fill up the questionnaire and face to face interview. The secondary data utilized to ensure the primary data as supportive documents for the study of conflict management theories. The study found that the causes for professional conflicts between teachers were identified as: different information and previous experience related to a certain issue, different perceptions for the same problem, different motivation, interests and personal objectives, unbalanced provision of tasks.

Keywords: School activities, Organizational ethnicity, Conflict solving strategies, learning environment, Communication, Educational psychologist.

INTRODUCTION

The common types of conflict usually arise between the school teachers on one hand and school authority on the other. Other forms of conflicts include intrapersonal conflict among teachers as well as administration regarding the causes of different norms and values, desires, and selfishness. If conflicts are not resolved in time, they have destructive impacts on teaching-learning and school development it may lead to violence. The modern conflict

theories outline that a conflict with low or medium strength and short duration could stimulate teachers' and creativity and their overall trained performance, while a high-intensity and long-term conflict are not favorable, neither to the organization nor to the involved people. The unnecessary association in conflict situations redirects the people's power and concentration away from their general objectives and often leads to emotional conflicts, in which case the trained performance of the respective conflict group tends to decrease drastically. Both short and long-term effects of the conflicts highlight the importance of studying and understanding this incident, and justify the effort of a school principal, for design, to handle it. Due to the causes of developing conflicts groups among students and teachers and its effects on school development. The common types of conflicts typically arise between the students on one hand and school authority on the other. Other forms of conflicts include intrapersonal conflict among teachers as well as non-academics and students regarding the causes of different norms and values, desires, and selfishness. If conflicts are not resolved in time, they have destructive impacts on teaching-learning, school development and it may lead to violence.

Statement of the Research Problem

The special effects of conflicts in school are measured insignificant by the greater part of the respondents. For them, the conflicts have a low intensity and short duration, and their effects are restricted in time. Some of the conflicts' effects mentioned by teachers are: the school climate deterioration, the communication between teachers may endure because of message distortion, isolation of some groups or individuals, the formation of some coalitions between teachers, all of this conducting to an unsociable atmosphere, nervousness and negative emotions, with a negative impact on their professional concert and school developments.

Objectives

The main objective of the study is to find the impact of developing conflict groups and their function-able activities through the destructive impacts in school development and it may lead to violence and identify the connection of school's effectiveness between staff satisfaction and principal's management.

- To identify the conflicts in school administration and reasons for the conflicts arises by review of documentaries
- To evaluate, the effects of staff and school developments by conflicts and its groups.
- To find out the impacts of conflicts between staff and give awareness for the suggestion of school development.

METHODOLOGY

This study presents how the professional conflicts are professed and managed by teachers and principals, based on 10 I-AB schools from the total of 64 more numbers of staff included schools were being selected from research area by Random Sampling Method where teachers and non-academic staff have selected as the respondents of primary data collection to fill up the Questionnaire and face to face interview. Moreover, secondary data would be utilized to ensure the primary data as supportive documents for the study of conflict management theories. The questionnaire had a multifarious composition, collecting personal opinions of the teachers concerning the following three aspects: the evaluation of their self-trust and self-esteem, the evaluation of some school cultural 02 elements that may manipulate the outcome of certain conflicts, and personal experiences regarding recent conflict phenomena in their schools.

RESULTS AND DISCUSSION

Note: Situation was created to choose the schools which consist more number of staff as being the number of variables and factors are higher in this study. Through which, conflicts based on individual and managerial drawbacks have been collected as well. Therefore, I AB schools have more staffs and students where the sampling

population has been higher than the other schools. The random sampling method has encouraged to ensuring the possibilities of getting equal chances by individuals.

Note: From the records, teachers, principal, vice-principal, and non-academic staff have been selected as samples. As being a higher number of teachers and students' leaders presented in the study location, random sampling has been carried out to select the sampling population

Note: Based on the ratio of 5:1, 180 teachers have been chosen as the sampling population. Along with the sample, random sampling would continue to select the number of samples too.

Causes of Conflicts by Teachers

The causes of conflicts between teachers have been categorized into three main groups: i. causes that are related to an individual, ii. causes that depend on school management, iii. causes deriving from the organizational culture. The 17 causes of conflict between teachers and their rate of incidence are presented in the table below. The person causes that can produce conflicts are circulated following a normal curve; the motives, interests and different objectives, perceptions concerning a problem or the accumulated experience may be important sources of conflict, to a large or very large extent, for over 20 percent of the interviewed people, and to a medium extent for about 30 percent. Nearly half of the teachers believe that the individual aspects only generate conflicts to a small extent, and even then, they are of small intensity and duration. Amongst the contextual factors, we can mention those of a managerial nature which are far less important, from the teacher's point of view, compared to the differences between the individual aspects. About 80 percent of the respondents have chosen either very low or low frequency of occurrence for this cause. A decade ago, the teachers were complaining about the technical void, now only 14 percent believe that there is a medium frequency for this cause, and only 5 percent of the teachers believe that it has a high or very high frequency of occurrence. Regarding the existing rules, only 26 percent of the questioned people believe that the lack of compliance with them has a medium, high, or very high frequency.

School Culture Components with Major Impact on Conflicts Evolution

At the organizational level, they have recognized three main causes that influence the time progress of every conflict: a. the complexity of the organization's environment; b. the differences between people, the fear of the new situations, and the normal resistance to change; c. the way of managing the divergent opinions as well as the resource limitations and the organizational constraints. The teachers were invited to classify their work environment through the behaviours which are accepted in their schools and there are encountered commonly. The predictable that, the most common behaviours in schools are the ones of collegiality, mutual support, and tolerance towards colleagues' different opinion, behaviours, and attitudes, conflict avoidance. There are, however, other behaviors found in schools that are considered harmful and which were mentioned by a limited number of respondents: bureaucracy, excessive control and obedient behaviour, inertia, verbal or behavioural aggression. To understand the teachers' familiar behavior in school, they were asked to select from a pre-defined list and to rank some important aspects of their school-life. In the diminishing order of the given importance, it was obtained the list: maintaining a pleasant work environment; the inspiration and improvement of the teachers; the correct and on-time completion of the allocated tasks; the opportunities for personal development.

Strategies used in School Conflicts Resolution

Inside any type of organization, there are functionalized several methods for preventive, discharging, or transforming a conflict, as parts of the "conflict resolution" or "conflict solving" process. According to Rubin, Kim and Pruitt, A classification of the strategies for conflict resolution is presented in the model of the bilateral concerns, two criteria: the concern regarding the personal results, respectively the concern regarding the counterpart's results. Based on this, the conflict resolution strategy could be: submission, if the concern towards own interests and results is low, while the other person's interests succeed; there are being introduced the ideas of self-sacrifice and self-interest; the strategy of submission is balancing to the direct strategy, of impressive; prevention, if the anxiety

towards the personal achievements and the other person's achievements is low; confrontation if the personal interests overshadow the concerns of the other; solving the problem if the interest in a satisfactory outcome is great. Andre Beaufre has offered a similar model; school is an organized system in which pupils in various cultural backgrounds pursue their studies at different levels and receiving instructions from the teacher. In involving instructional activities, teachers have to plan their activities by making use of their abilities and capabilities to give effective teaching to the learners. The attitude of novice teachers needs a better change through all other dimensions taken up in the study namely, internal locus of control and self-efficacy are reported to be satisfactorily good. But none of these dimensions involved in the academic performance of teachers, offered by Saravanakumar AR., and Paniadima A, who classifies the strategies into direct strategies, lateral, cooperative and conflictual, when the power of the partners and the cooperation skills are significantly unstable. Using an item with an open answer, there were highlighted the strategies most frequently used for solving the tensional situations among teachers. These are direct strategies, which involve imposing the solution without needing the approval of all the parties involved, this kind of strategy is used when there is a power imbalance; on the side of attrition strategies, which are used as a means to tire out the other party and forward its attention towards other minor problems; conflictual strategies, which involve tactics like threatening, blaming, warning, etc; problem-solving strategies; avoidance strategies; denial strategies. The first type, direct strategies, of imposing, are present in schools, but their frequency is perceived as low (very low - for 43% of the respondent teachers, low - for 18%, medium - for 24%).

These strategies involve a surrender of some partners, which means a give-up regarding some personal reward and reduced anxiety towards their interests. Only 15 percent of the respondents recognize the survival of imposing strategies as frequent or very frequent. Contrary to the initial potential, about a third of the questioned people estimate that the lateral strategies are used with a medium, high, or very high frequency, 22 percent of the teachers think that these types of strategies are rarely used, and the rest of 47 percent think that these are used with a really low frequency. The conflictual strategies still exist in schools, but to a very small or small extent (78% of the respondents). The rest of 22 percent think that these strategies are used to a high or very high extent. The cooperation strategies/problem solving are the most frequently used, and the results are regularly distributed between the choices of the answer "average frequency", "high frequency", and "very high frequency". The use of these strategies is frequent when both partners want to obtain the best result achieved by communal effort. Same as the accountability of teachers concerning the students' results (approximately 90%). The prevention strategies are frequent (32% of the respondents) or very frequent (24% of the respondents); 26 percent consider that these strategies arise with an average frequency, and the rest of 18 percent, rarely or very rarely. This type of strategy is used when no personal interests, but the school climate is perceived as important.

CONCLUSION

The foundation of conflict in schools are identical that can be found in any other organization, but the importance given to them by teachers is comparatively different. For example, only 20 percent of the teachers consider that bad communication in their school could create conflicts, while in some other type of organization, the perceived importance of communication as a conflict source could be significantly different. The conflict causes that produce the most divergent perceptions among the teachers are the individual ones, namely divergent objectives, motivations, or interests, different perceptions of the same issue, and different previous experiences and knowledge referring to a subject. School principles are still of relevance in that they offer a useful starting point in attempting to analyze the effectiveness of the plan of organization structure. Human relations writers verified that people go to work to satisfy the complexity of needs and not simply for financial reward and this is true if we recognize fundamental motivation. The principal should be the confrontational leader in school administration. According to the present study, about one-half of the respondents consider that the individual conflict causes can create conflicts among teachers quite often; while the other half think that the conflicts due to this cause are rare. The understanding of the conflict mechanism facilitates the identification of the most effective instruments for school conflict resolution.

A superior school climate will allow teachers to improve their overall professional performance and to deliver more value to their students.

REFERENCES

1. Beaufre, A. (1965). *Introduction to Strategy*. New York: Praeger.
2. Deep, S., & Sussman, L. (1966). Secretul oricarui succes: sa actionam inteligent. Bucuresti: Polimark.
3. Deutsh, M. (1973). *The Resolution of Conflict: Constructive and Destructive Processes*. New Haven: Yale University Press.
4. Gamble, T. K., & Gamble, M. (1993). *Communication Works*. New York: McGraw-Hill.
5. Prabakaran B., & Saravanakumar AR. (2020). Effectiveness of Interactive E-Content Module in Enhancing Students' Achievement in Mathematics. *International Journal of Control and Automation*, 13(2s), 84-94.
6. Prabakaran, B. & Saravanakumar AR. (2020). "An Interactive E-Content Module for Learning Mathematics -A Single Group Experiment". *International Journal of Advanced Science and Technology*, 29(5s), 1297-1313.
7. Radha, R., Mahalakshmi, K., Sathish Kumar, V & Saravanakumar, AR. (2020). E-Learning during Lockdown of Covid-19 Pandemic: A Global Perspective. *International Journal of Control and Automation*, 13(4), 1088-1099.
8. Ravichandran, T., & Saravanakumar, A.R. (2013). Enhancing Biological Sciences Laboratory Experimental Skills through Virtual Laboratory Techniques. *Indian Journal of Research*. 2(4), 70-72
9. Rubin, J.Z., Pruitt, D.G., & Kim, S.H. (1994). Social Conflict: Escalation, Stalemate and Settlement. New York: McGraw-Hill.
10. Saravanakumar AR (2008). Effectiveness of Motivational Strategies on Enhancing Academic Achievement, *Journal of Research and Reflections on Education*, 16(2).
11. Saravanakumar AR (2016). Role of ICT in transforming Sri Lankan Higher Education, *International Journal of Advanced Research Trends in Engineering and Technology*, 3(20).
12. Saravanakumar AR., & Padmini Devi KR. (2020). Indian Higher Education: Issues and Opportunities, *Journal of Critical Reviews*. 7(2), 542-545.
13. Saravanakumar AR, Paavizhi K., & Palanisamy P. (2019). Effectiveness of Video-Assisted Learning Module. *International Journal of Control and Automation*, 12(06), 268-275.
14. Saravanakumar AR. (2018). Role of ICT on Enhancing Quality of Education, *International Journal of Innovative Science and Research Technology*, 3(12), 717-719.
15. Saravanakumar, A. R. (2014). Present Scenario and Future Prospects of Higher Education in India. Proceeding of the Social Sciences Research *ICSSR*, Kota Kinabalu, Sabah, Malaysia. Organized by http://WorldConferences.net.
16. Saravanakumar, AR. (2014). Present Scenario and Future Prospects of Higher Education in India, *Proceeding of the Social Sciences Research ICSSR 2014* (e-ISBN 978-967-11768-7-0).
17. Saravanakumar, AR., & Paniadima, A. (2017). Cognitive Dissonance, Locus of Control, Selfef_cacy and Academic Performance of Novice Teachers, *Journal of Research and Reflection of Education,* 6.
18. Sivakumar, I., & Anitha, M. (2012). Education and Girl Children in Puducherry Region: Problems and Perspective. *International Journal of Social Science and Interdisciplinary Research*, 1, 175-184.
19. Sivakumar, I., & Usha V.T. (2013). Education and Women in India: In Theoretical Perspective, Development and Displacement: Social Justice Paradigm. (Ed) Gurusamy, S. APH: New Delhi; 141-148.
20. Sivakumar, I & Usha V.T. (2015). Gender Bias and Women's Education: Status of Illiterate Women in the Fishing Community, *Indian Journal of Women's Studies*, 8(8), 28-35.
21. Sivakumar, I & Usha V.T. (2012). Gender discrimination among the girl children in Puducherry: A marine fishing community study, *ZENITH International Journal of Multidisciplinary Research*, 2(11), 93-101.
22. Saravanakumar AR, Paavizhi K., & Balamurugesh KR. (2020). A Survey on Effectiveness of Video-Assisted Learning in Enhancing Knowledge Competencies for Teachers, *Test engineering and management*, 82, 5866–5872.

Table.1. Schools Information of Zonal Education – Batticaloa, Sri Lanka

Zonal Division	Types of School				Total
	I AB	IC	Type II	Type III	
Manmunai North	08	04	16	11	39
Eravur Pattu	01	03	03	02	09
Manmunai Pattu	01	03	04	08	16

(Zonal Education Office, Batticaloa, Sri Lanka - 2019)

Table. 2. Details of School for Research Study

Zonal Division, Batticaloa	Number of school for selection	
	IAB schools	Sample schools
Manmunai North	08	08
Eravur Pattu	01	01
Manmunai Pattu	01	01

Table.3. Selected internal Sampling Population

Name of the School	Principal and vice-principal	Teachers	Non-Academic staff
A	03	76	12
B	06	85	12
C	06	110	18
D	06	126	18
E	06	90	15
F	03	72	15
G	06	107	18
H	03	76	15
I	03	65	13
J	03	59	8

(School Record and Zonal Education Office, 2018-2019)

Table 4. Details of Sample

Name of the School	Total No. of Teachers	Ratio level	Sample of Teachers
A	76	5.1	15
B	85	5:1	17
C	110	5:1	24
D	126	5:1	26
E	90	5:1	18
F	72	5:1	14
G	107	5:1	21
H	76	5:1	15
I	65	5:1	13
J	59	5:1	12

Table 5. Causes of Conflict

SI. No	Causes of conflict	Frequency of occurrence (in %)				
		Very low	Low	Medium	High	Very High
A. Character cases						
1.	Divergent objectives, motivations, or interests	22	23	33	14	8
2.	Different perceptions of the same issue	20	22	33	23	2
3.	Different previous experiences and knowledge referring to a subject	17	23	33	23	4
B. Administrative cases						
4.	Inequitable task allocation	28	20	32	14	6
5.	Bad communication (lack of transparency, efficiency, clarity, addressability)	48	32	3	12	5
6.	Inequitable allocation of the school's resources	47	21	14	11	7
7.	Inequitable allocation of the administrative tasks	38	26	22	11	3
8.	Subjective performance appraisal	51	18	23	6	2
9.	Lack of clear, formal procedures	66	15	14	3	2
C. Other relative cases (depending on administration)						
10.	Lack of support for educational innovation	49	24	19	6	2
11.	Limitation of the access to personal/professional development	65	19	10	5	1
12.	Internal discussions that affect the image of other colleagues	48	24	19	7	2
13.	Limited promotion / career development options	49	19	16	7	9
14.	Integration of new people / Isolation of some people	59	20	15	6	-
15.	Breaking the internal rules and procedures	56	18	20	3	3
16.	Affective conflicts between teachers	52	26	13	6	3
17.	Dysfunctional relationship with the school management team	60	15	16	8	1

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Preliminary Phytochemical Screening and Acute Toxicity Studies of *Celtis philippensis* Blanco.

Sujith Thomas[1], Rohit Saraswat[1], R. Kothai[2] and B. Arul[2]*

[1]Department of Pharmacology, OPJS University, Churu, Rajasthan, India.
[2]Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University) Salem, Tamil Nadu, India.

Received: 24 Nov 2020 Revised: 06 Dec 2020 Accepted: 19 Dec 2020

***Address for Correspondence**
B. Arul
Department of Pharmacy Practice,
Vinayaka Mission's College of Pharmacy,
Vinayaka Mission's Research Foundation (Deemed to be University)
Salem, Tamil Nadu, India.
Email: arul1971@yahoo.com

ABSTRACT

Celtis philippensis is a large fugacious tree that belongs to the family of Ulmaceae. It is distributed in most parts of India at an altitude of 1400 m and in indestructible forests. The main aim of the present study is to investigate the phytochemical analysis of leaves of *Celtis philippensis* and to evaluate its acute toxicity study as per OECD guidelines. The phytochemical analysis was performed by following standard procedures. The phytochemical screening results of various extracts showed the presence of alkaloids, sterols, carbohydrates, glucosides, terpenoids and saponins, tannins, gums and mucilage and flavonoids. In the acute toxicity tests, single oral administration of 5, 50, 300 & 2000 mg/kg doses of various extracts of leaves of *Celtis philippensis* did not show any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence, it was concluded from the results that the possible oral toxic doses of *Celtis philippensis* is more than 2000mg/kg.

Keywords: *Celtis philippensis,* acute toxicity study, phytochemical compounds.

INTRODUCTION

Celtis philippensis is a large fugacious tree that belongs to the family of Ulmaceae commonly known as Vellai Tovarai in Tamil[1,2]. It is distributed in most parts of India at an altitude of 1400 m and in indestructible forests. Traditionally, the roots of *Celtis philippensis* has been used for diarrhoea and as astringent [3,4]. The sap leaves were used for parasites. Although limited pharmacological studies have been carried out with this plant, there is no experimental evidence on its phytochemical and toxicity studies. Hence, in the present study, we planned to evaluate

the phytochemical analysis of leaves of *Celtis philippensis* and to evaluate its acute toxicity study as per OECD guidelines.

MATERIALS AND METHODS

Plant Materials Collection and authentication

The leaves of *Celtis philippensis* Blanco was collected from Thirunelveli District, Tamilnadu in the month of October 2019. The collected plants (leaves) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu.

Extraction of the plant

About 500 g of dried leaves were coarsely powdered and subjected to maceration with different solvents of increasing order of polarity such as pet ether, chloroform, ethyl acetate, ethanol, and aqueous [5,6]. The extracts were dried under the rotary evaporator and the crude extract was obtained. The product of the crude extraction fraction was calculated and stored in an airtight container for further use for analysis[7].

Phytochemical screening of the crude drug

Qualitative phytochemical analysis of the Pet. ether, Chloroform, ethyl acetate, ethanol, and aqueous plant extracts was carried out to test the presence of phytochemicals such as alkaloid, flavonoids, terpenoids, sterols, tannins, glycosides, etc. The following tests were done for the preliminary phytochemical screening[7].

Test for flavonoids (Shinoda test)

2mL of the *Celtis philippensis* extracts were mixed in the methanol, to this a minor part of magnesium ribbon was added and 1 mL of concentrated hydrochloric acid added from the sides of the test tube. A magenta pink color designates the presence of flavonoids.

Test for saponins

To 5mL of the *Celtis philippensis* extracts, 5mL of distilled water was added, and mix well for the formation of froth which confirms the presence of saponins. Test for steroids: 1mL of plant extracts were taken in test tubes, to which 10mL of chloroform was added. After this 10mL of concentrated sulphuric acid was added along the sides of the test tubes. A color change from violet to blue/green confirms the presence of steroids in the samples.

Test for tannins

0.5mL of *Celtis philippensis* plant extracts were boiled in 10mL of water for 5-10 minutes and filtered. The filtrate was taken and 2mL Ferric chloride (0.1%) was added to the filtrate. The appearance of brownish-green or blue-black coloration formed confirms the presence of tannins.

Test for alkaloids

2 mL of the *Celtis philippensis* plant extracts were diluted to 10ml with acidified alcohol, boiled, and filtered. To 5mL of the filtrate 2mL of dilute ammonia was added. 5mL of chloroform was added and shaken gently to extract the alkaloid base. The chloroform layer was extracted with 10mL of concentrated acetic acid. Few drops of Wagner's solution were added to the chloroform solution and the presence of reddish-brown precipitate indicates the presence of alkaloids.

Test for cardiac glycosides

2mL of plant extracts were treated with 2 mL of glacial acetic acid containing a drop of $FeCl_3$ solution. This was treated with 1 mL of concentrated H_2SO_4. A brown ring obtained at the interface specifies the presence of de-oxy sugar characteristics of cardenolide.

Test for terpenoids

2 ml of *Celtis philippensis* extracts were treated with 2 mL of chloroform and concentrated H_2SO_4 was sensibly added to form a layer. A reddish-brown color creation at the interface confirms the presence of terpenoids.

Detection of Carbohydrate

Fehling's test

Plant extract (1mL) mixed with 1mL Fehling solutions A and B and was boiled on a water bath. The colour change was observed. A red precipitate indicated the presence of sugar.

Barfoed's test

To 1 mL of extract, 1 mL of Barfoed's reagent was added and heated on a boiling water bath for 2 minutes. The colour change was noted and recorded. A red precipitate indicated the presence of sugar.

Benedict's test

To 0.5 mL of extract, 0.5 mL of Benedict's reagent was added. The mixture is heated on a boiling water bath for 2 minutes and the result was observed. A red precipitate indicated the presence of sugar.

Detection of Proteins

The plant extracts were dissolved in 10 mL of distilled water and filtered through Whatman No.1 filter paper and the filtrate is exposed to many tests for proteins. Millon's test: To 2 ml of the plant filtrate, few drops of Millon's reagent are added. The result was detected and the form of white precipitate specified the presence of proteins.

Biuret test

To 2 mL of filtrate, a drop of 2% copper sulfate solution was added. To this, 1 mL of 95% ethanol was added, followed by an excess of potassium hydroxide solution (60%). The appearance of a pink colour in the ethanol layer designates the existence of proteins.

Total phenol content determination

The total phenol content was determined by Folin- Ciocalteu's assay using gallic acid as standard[8]. In this method, 0.5 ml of plant extracts were mixed with 1.5 ml Folin- Ciocalteu's reagent. After 5 minutes, 1.5 ml of 7% sodium carbonate solution was added. The final volume of the tubes was made up to 10 ml with distilled water and allowed to stand for 90 minutes at room temperature. The absorbance of the sample was measured against the blank at 750 nm using a Shimadzu 1601 UV spectrophotometer. All the readings were repeated three times for precision and values were expressed in mean ± standard deviation in terms of phenol content (Gallic acid equivalent, GAE) per g of dry weight.

Total flavonoid content determination

Total flavonoid content was determined by the Aluminium chloride method using quercetin as a standard[9]. 1 ml of the test sample and 4 ml of water were added to a 10 ml volumetric flask. To this 0.3 ml of 5 % Sodium nitrite and 0.3 ml of 10% Aluminium chloride were added after 5 minutes. The mixture was incubated for 6 min at room temperature, then 1 ml of 1 M Sodium hydroxide was added and the final volume was made up to 10 ml with distilled water. The absorbance of the sample was measured against the blank at 510 nm using a Shimadzu 1601 UV

spectrophotometer. All the readings were repeated three times for precision and values were expressed in mean ± standard deviation in terms of flavonoid content (Quercetin equivalent, QE) per g of dry weight.

Animals

Female Swiss albino mice weighing about 30-35g were used for the study. They were housed in polypropylene cages and fed with a standard chow diet and water ad libitum. The animals were exposed to an alternative cycle of 12 h of darkness and light each. Before each test, the animals have fasted for atleast 12h. The experimental protocols were subjected to the scrutinization of the institutional animal ethics committee and were cleared by the same.

Acute Oral Toxicity Study

The acute toxicity studies were performed as per OECD guidelines 423[10,11]. A total of 48 mice weighing between 30-35g were randomly divided into twelve groups of 3mice each. Animals were fasted prior to dosing (food but not water was withheld over-night). Following the period of fasting, the bodyweight of the animals was measured and the chloroform, ethyl acetate, ethanol, and aqueous extracts of leaves of *Celtis philippensis* was administered to each group at single doses of 5, 50,300, and 2000 mg/kg, respectively, by oral gavage. The control groups were treated with the same volume of distilled water. ceaselessly for cyanogenetic symptoms throughout the primary half-hour once dosing and discovered sporadically (with special attention given throughout the primary four hours) for consecutive twenty-four hours and then daily after that, for 14 days. Acute oral toxicity study of various extracts of *Celtis philippensis* leaves in mice was determined by observing the changes in skin and fur, eyes and mucous membranes, and behavioral pattern. Attention was given to observations of tremors, convulsions, salivation, lethargy, sleep, Diarrhoea, Respiratory, Circulatory, Autonomic, and Central nervous system, Somatomotor activity, changes in body weight, and mortality.

RESULTS AND DISCUSSION

Preliminary phytochemical analysis of leaves of *Celtis philippensis*

The present study was carried out to analyze the extractive value, percentage yield, and the presence of bioactive compounds in the various extracts of leaves of *Celtis philippensis*. The colors of the extracts were green color particularly ethyl acetate extract was greenish-brown. The percentage yield of these extracts was also measured, and it was the ethyl acetate extract 15.43% showed maximum yield in comparison with other solvent extracts. Chloroform and ethyl acetate extracts were sticky semisolid, ethanol, and aqueous extracts were powder in their consistency. The extractive values and percentage yield of leaves of *Celtis philippensis* were shown in Table.No.1. The qualitative phytochemical screening of Pet ether, chloroform, ethyl acetate, ethanol, and aqueous extracts of leaves of *Celtis philippensis* and its secondary metabolites were shown in Table No.2. The results showed the presence of phytochemical constituents, namely alkaloids, sterols, carbohydrates, glycosides, fixed oils and fats, phenolic compounds, proteins and aminoacids, terpenoids and saponins, tannins, Gums and mucilage and flavonoids. Among the four solvents used, ethyl acetate extracts yielded maximum bioactive compounds followed by ethanol extract, aqueous extract, and a minimum amount of compounds present in chloroform extract. Alkaloids, sterols and carbohydrates were present in all extracts in varying concentrations. Ethyl acetate and ethanol extracts were yielded similar bioactive compounds such as alkaloids, carbohydrates, sterols and flavonoids. Ethyl acetate extract yields the compounds are alkaloids, sterols, carbohydrates, flavonoids, tannins, phenols, protein, and amino acids. Ethanol and aqueous extracts showed the presence of all compounds of ethyl acetate extract. Glycosides, fixed oils and fats, phenolic compounds, proteins, and amino acids, terpenoids, saponins, tannins, gums, and mucilage and were absent in chloroform extracts. Further, ethyl acetate, ethanol, aqueous extracts were showed the absence of Glycosides, Fixed oils & Fats, Terpenoids, Saponins, Gums and mucilage.

Total phenol and flavonoid content

The main active compounds present in the extracts were found to be phenolic and flavonoids. In the present study, the total phenolic content of different extracts of leaves of *Celtis philippensis* Blanco was determined by the Folin–Ciocalteu reagent method and expressed as GAE/g of plant extracts. Ethanol extract exhibited the maximum amount of phenolic content among the extracts, i.e., (55.48±0.35) mg/g GAE followed by (41.37±0.21) mg/g GAE in the aqueous extract. Similarly, the total flavonoid content for all the extracts was measured with the aluminium chloride colorimetric assay using quercetin as standard. It showed 18.51±0.23 and 14.39±0.27 mg of quercetin equivalent /g for EECPB and AECPB respectively. Aluminium chloride forms acid-stable complexes with the C-4 keto groups and either the C-3 or C-5 hydroxide group of flavones and flavonols. Besides, it also forms liable complexes with ortho dihydroxide groups in A/B rings of flavonoids. The results are shown in Table No.3.

Acute oral toxicity study

To determine the safety of plant products and drugs for human use, toxicological evaluation is carried out in experimental animals to predict the toxicity and to provide for selecting 'safe' doses. In the acute toxicity tests, administration of various leaf extracts at different doses of 5,50,300& 2000mg/kg of *Celtis philippensi*s did not show any visual symptoms of toxicity or mortality in animals during the entire 14-days observation period. Hence the effective dose was found to be 200mg/kg.

CONCLUSION

In conclusion, it was observed that the plant *Celtis philippensis Blanco* contains several bioactive components and a high level of total phenolics and flavonoids content. Hence, the plant was considered as an enriched source of different phytocompounds. Acute toxicity studies also revealed the safe level of the plant extracts. This forms the basis for scientific evidence to conduct further studies and to investigate the lead compounds present in the plant, and to evaluate its various pharmacological activities.

ACKNOWLEDGEMENT

The authors are thankful to the authorities of Vinayaka Mission's Research Foundation (Deemed to be University), Salem for providing the facilities for carrying out this research.

REFERENCES

1. Sasidharan N. Biodiversity documentation for Kerala. Part 6: Flowering plants. Peechi: Kerala Forest Research Institute, Peechi; 2004. 436 p.
2. Sujith Thomas, Rohit Saraswat BA. Anti-Diabetic And Anti-Hyperlipidemic Evaluation Of Celtis philippensis Blanco On Albino Wistar Rats. Int J Pharm Res. 2020;12(4):2951–5.
3. Mary DA, Ganthi AS. Preliminary phytochemical and pharmacognostic studies of Celtis philippensis Blanco. Int J Res Pharm Chem. 2018;8(1):84–7.
4. Thomas S, Arul B, Saraswat R. Anti-diabetic in-vivo evaluation of Celtis philippensis Blanco by streptozotocin induced on albino wistar rats. J Nat Remedies. 2020;21(4 S1):26–31.
5. Arul B, Kothai R, Sureshkumar K, Christina AJM. Anti-Inflammatory Activity of Coldenia procumbens Linn. Pak J Pharm Sci [Internet]. 2005 Sep 14;18(3):17–20. Available from: http://www.pjps.pk/wp-content/uploads/pdfs/CD-PJPS-18-3-05/Paper-4.pdf
6. Arul B, Kothai R, Jacob P, Sangameswaran K, Sureshkumar K. Anti-inflammatory activity of Sapindus trifoliatus Linn. J Herb Pharmacother. 2004;4(4):43–50.
7. Harborne A. Phytochemical Methods A Guide to Modern Techniques of Plant Analysis. 3rd ed. Springer Netherlands; 1998. 282 p.

8. McDonald S, Prenzler PD, Antolovich M, Robards K. Phenolic content and antioxidant activity of olive extracts. Food Chem. 2001;73(1):73–84.
9. Olajire AA, Azeez L. Total antioxidant activity, phenolic, flavonoid and ascorbic acid contents of Nigerian vegetables. African J Food Sci Technol. 2011;2(2):22–9.
10. OECD. OECD guideline for testing of Animals. 2008. 2 p.
11. Manivannan E, Kothai R, Arul B. Acute and Subacute (28-Day) Toxicity Assessment of Ethanolic Extract of Seeds of Asteracantha longifolia Nees (Linn). Int J Pharm Res. 2020;12(4):2936–40.

Table 1: Colour, Extractive values and Percentage yield of various extracts of leaves of *Celtis philippensis*

Plant name	Part used	Method of extraction	Solvent	Colour of extract	Nature of extract	% yield of extract
Celtis philippensis	Leaves	Maceration	Chloroform	Green	Semisolid	5.56
			Ethyl acetate	Greenish brown	Greasy solid	15.43
			Ethanol	Green	powder	11.86
			Aqueous	Green	powder	12.45

Table 2: Preliminary phytochemical screening of the different extracts of leaves of *Celtis philippensis*

S.No	Constituents	Tests	Chloroform	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	Mayer's test	+	+	+	+
		Dragondraff's test	+	+	+	+
		Hager's test	+	+	+	+
		Wagner's test	+	+	+	+
2	Sterols	Burchard test	+	+	-	-
		Salkowski's	+	-	+	-
3	Carbohydrates	Molisch's test	+	+	+	+
		Fehling's test	-	+	+	+
		Benedict's test	+	+	+	+
		Bontrager's test	+	+	+	+
4	Glycosides	Legal test	-	-	-	-
		Kellerkiallani test	-	-	-	-
5	Fixed oils & Fats	Spot test	-	-	-	-
		Saponification test	-	-	-	-
6	Phenolic Compounds	Ferric chloride	-	+	+	+
7	Proteins & amino acids	Biuret test	-	+	+	+
		Ninhydrin test	-	+	+	+
		Millon's test	-	+	+	+
8	Terpenoids& Saponins	Foam test	-	-	-	-
		Haemolysis test	-	-	-	-
9	Tannins	Gelatin test	-	+	+	+
		$FeCl_3$ test	-	+	+	+
10	Gums & mucilage	Precipitation to 90%alcohol	-	-	-	-
11	Flavonoids	Shinoda test	-	+	+	+
		Lead acetate test	-	+	+	+
		Ferric chloride test	-	+	+	+
		Zinc HCL test	-	+	-	-

+Presence, -Absent

Table 3: Estimation of total phenolic and flavonoids from the various extracts of Leaves of *Celtis philippensis*.

S.No	Plant part used	Extracts	Total phenolic content(mg of gallic acid equivalent/ g dry material)	Total flavonoid content(mg of quercetin equivalent/ g dry material)
1	Leaves	Pet ether	11.43±0.27	2.30±0.20
2		Chloroform	20.53±0.25	5.36±0.19
3		Ethyl acetate	32.23±0.14	8.55±0.29
4		Ethanol	55.48±0.35	18.51±0.23
5		Aqueous	41.37±0.21	14.39±0.27

Values are expressed as mean ± SD, n=3

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Antimicrobial Activity of Two Indian Medicinal Plants against Pathogenic Bacteria

Aziz Mohammad Khan[1]*, Seema Bhadauria[2] and Rajesh Yadav[3]

[1]Department of Food and Biotechnology, Jayoti Vidyapeeth Women's University, Jaipur, Rajasthan, India.
[2]Department of Botany, University of Rajasthan, Jaipur, Rajasthan, India.
[3]Department of Zoolgy, JECRC University, Jaipur, Rajasthan, India.

Received: 01 Nov 2020 Revised: 21 Nov 2020 Accepted: 23 Dec 2020

***Address for Correspondence**
Aziz Mohammad Khan
Department of Food and Biotechnology,
Jayoti Vidyapeeth Women's University,
Jaipur, Rajasthan, India.
Email: khanazizmohammad@gmail.com

ABSTRACT

The present study was aimed to evaluate the antimicrobial activity of ethanol and ethyl acetate extract of indian medicinal plants *Allium sativum* and *Ocimum sanctum* against pathogenic bacteria. *Allium sativum* and *Ocimum sanctum* are daily used plants in India and native to Asia and Africa. The plant leaves were collected, shade dried, powdered and extracted in respective solvent. Antimicrobial activity of plant extract was conducted using agar disc diffusion method. The results of the present study indicate that both plants showed promising antimicrobial activity against pathogenic bacteria. It can be recommended that *Allium sativum* and *Ocimum sanctum* can be used as natural antimicrobial agent without any side effect.

Keywords: *Allium sativum*, *Ocimum santum,* Indian medicinal plants, Disc Diffusion, pathogenic, natural antimicrobial agent.

INTRODUCTION

Human beings have been utilizing plants for thousands of years for basic preventive folk medicine. Herbal medicines are being utilized in Ayurveda, Sidha, Unani and Homeopathy for their effective treatment (Dubey et al. 2011). Medicinal plants have been reported to have pharmacological properties. Since antiquity, many plants species reported to have pharmacological properties as they are known Pharmacological activity of plant is due to presence of some secondary metabolites like phenolic compounds, glycosides, saponins, flavonoids, steroids, tannins, alkaloids and terpenes (Lalitha *et al.* 2010; Khan and Bhadauria, 2017).

Microbial infection is common health problem all around the world. Development in science and technology has remarkable progress in the production of many synthetic drugs like antibiotics (Preethi*et al.* 2010). Antibiotics are produced from microbial origin. With the indiscriminate use of antibiotics for treatment of infection, antibiotic sensitivity may be developed in body. Antibiotic resistance has increased considerably in recent years. From the last few years, herbal medicines are being utilized as the solution of antibiotic resistance problem (Alagesaboopathi 2011). Medicinal plants have variety of pharmaceutical effects against pathogen and they can be used regularly without generating antibiotic resistance. Medicinal plants are being used for the treatment of many human disease as tradition medicine. Traditional use of medicinal plants has been used for anticancer, antiulcer, antioxidant and antidiabetic activity (Pankaj and Kaushik, 2011). Some medicinally plants are specifically used for anti-parasitic, antifungal, antimalarial and insecticide activity (Acharyya *et al.* 2011). The present study is aimed for antimicrobial activity of selected plant extract against some pathogenic bacteria.

MATERIALS AND METHODS

Plant material

In this study, two plants were selected for antimicrobial activity- *Allium sativum* and *Ocimum sanctum*.The plant leaves were collected from agriculture farms of Jaipur, Rajasthan and submitted to herbarium for Identification. The collected plant materials were washed thoroughly with running tap water to remove the surface contaminants and then dried under room temperature.After drying plant materials were finely powdered using mixer grinder and stored for further use.

Preparation of the plant extracts

The shade dried powder (50 gm) was used for the extracted in different solvents like Ethyl acetate and 80% Ethanolin Soxhlet extraction unit. All the extracts were evaporated by heating on water bath. Extracts of each plant materials were collected in sterile screw capped vials.

Antimicrobial activity of plant extract

The antimicrobial efficacy of plant extracts was evaluated against selected bacteria.The antimicrobial efficiency of plant extract of the *Allium sativum* and *Ocimum sanctum* was assessed against some pathogenic bacteria. For this, autoclaved Whatman filter paper discs were used and dipped in each plant extract separately for 24 hours for complete absorption of plant extraction**.**

Antimicrobial screening of plant extract

The antimicrobial activity of plant extract of the *Allium sativum* and *Ocimum sanctum* was evaluated using modified agar disc diffusion method as described by Gould and Bowie (1952) and Bauer and Kirby (1966). Antimicrobial susceptibility was tested on solid nutrient agar media plates. Prepared nutrient agar media was poured separately into Petri plates and allowed to solidify. Test cultures were inoculated onto agar media surface using sterile cotton swabs. Immediately after inoculation, plant extracts were placed on the surface of media plates separately. The plates were incubated at 37^0C for 24 hours. All the analysis of each test were made in triplicates for the calculation of standard error.

RESULTS AND DISCUSSION

In the present investigation, antibacterial activity of plant extracts of *Allium sativum* and *Ocimum sanctum* were evaluated against bacterial strains. The antimicrobial activity was determined using agar well diffusion method. Antibacterial activity of plant extracts was analyzed on the basis of zone of inhibition and their activity index. The results revealed that all the extracts are potent antimicrobials against all the microorganisms studied. From the two solvents extracts studied Ethyl acetate extract showed high degree of inhibition than Ethanol extract.

In Ethyl acetate extract of *Allium sativum* showed maximum inhibition zone with diameter of 19 ± 0.351 mm in *E. coli* and 17 ± 0.3 mm in *S.aureus*. The Ethanolic extract of garlic extract showed effective zone of inhibition for *E.coli* (17 ± 0.057mm) and *S.aureus* (15 ± 0.23 mm) respectively. *Ocimum sanctum* Ethyl acetate extract also exhibited promising zone of inhibition against *E. coli* (17 ± 0.057mm)and *S.aureus* (15 ± 0.23 mm). Similarly, Ethanol extract of *Ocimum sanctum* showed zone of inhibition of 14 ± 0.43 mm against *E.coli* and 11 ± 0.24 mm against *S. aureus*. In the present study, Antibacterial activity of different *Ocimum sanctum* and *Allium sativum* extracts against *E. coli* and *Staphylococcus aureus* were studied (Mittal*et al.* 2018).According to the results, of different *Ocimum sanctum* and *Allium sativum* extracts antibacterial activity against tested microbial pathogens (Rajesh *et al*. 2018). *Allium sativum* showed more antibacterial activity than *Ocimum sanctum*. Highest antibacterial activity was shown by *Allium sativum* ethyl acetate extracts against bot tested bacteria.

CONCLUSION

From the present study, it can be concluded that plant based herbal drugs can be developed which are similarly effective as synthetic drugs without any side effects.Commercial available drugs cannot be used regularly because body may develop antibiotic resistance against the drugs.The present study clearly indicates that *Allium sativum* and *Ocimum sanctum* contain potent antimicrobial properties and are easily available in India. From the present study, indian medicinal plants can be recommended as daily use antimicrobial agent against pathogenic micro-organisms with low cost and fewer side effects.

REFERENCES

1. Acharyya S, Rathore DS, Sundeep Kumar HK, Panda N, (2011): Screening of Anthocephaluscadamba(Roxb.) Miq. Root for Antimicrobial and Anthelmintic activities. Int.J. of Res. Pharma and Biomed Sci. 2 (1): 2229-3701.
2. Alagesaboopathi C, (2011). Antimicrobial Potential and Phytochemical Screening of *AndrographisAffinis*Nees an Endemic Medicinal Plant From India. Int. J. of Pharmaand Pharmaceutical Sci. 3 (2): 157- 159.
3. Bauer AW, Kirby WM, Sherris JC, Turck M, (1966) Antibiotic susceptibility testing by a standardized single disk method. American journal of clinical pathology, 45(4): 493.
4. Dubey R, Dubey K, Sridhar C, Jayaveera KN, **(**2011**).**Human Vaginal Pathogen Inhibition Studies on Aqueous, Methanolic and Saponins Extracts of Stem Barks of *ZiziphusMauritiana*. Int.J.Pharm. Sci. Res. 2(3): 659-663.

5. Gould JC,Bowie JH, (1952) The determination of bacterial sensitivity to antibiotics. Edinburgh medical journal, 59: pp-178-99
6. Khan AM, Bhadauria S,(2017).Isolation of some potential phytocompounds from *Adhatodavasica* through Gas Chromatography-Mass Spectroscopy analysis. Asian Journal ofPharmaceutical andClinicalResearch, 10(12): 328-332.
7. Lalitha P, Arathi KA, Shubashini K, Sripathi, Hemalatha S, Jayanthi P, (2010). Antimicrobial Activity and Phytochemical Screening of an Ornamental Foliage Plant, *Pothosaurea*(Linden ex Andre). An Int. J. of Chem. 1(2): 63-71.
8. Mittal R, Kumar R, Chahal HS, (2018). Antimicrobial activity of *Ocimum sanctum* leaves extracts and oil Journal of Drug Delivery & Therapeutics. 8(6):201-204
9. Pankaj G, Kaushik P, (2011). In *vitro* Evaluation of Antibacterial Activity of Various Crude Leaf Extracts of Indian Sacred Plant, *Ocimum sanctum* L. British Microbiology Research Journal; 1(3): 70-78.
10. Preethi R, Devanathan VV, Loganathan M, (2010). Antimicrobial and Antioxidant Efficacy of Some Medicinal Plants against Food Borne Pathogens. Adv. in Bio.Res. 4 (2): 122-125.
11. Rajesh T, Venkatanagaraju E, Goli D, Basha SJ, (2014). Evaluation of Antimicrobial Activity of Different Herbal Plant Extracts. International Journal of Pharmaceutical Sciences and Research. Vol. 5(4) 1460-1468.

Table 1: Antibacterial activity of different plant extracts of *Allium sativum* and *Ocimum sanctum*

S. No.	Plant extract	Microorganism	Zone of inhibition in mm		Zone of inhibition Chloramphenicol (10µg/ml)
			Ethyl acetate extract	Ethanol extract	
1	*Allium sativum*	*S. aureus*	17 ± 0.3	15 ± 0.23	21 ± 0.23
2	*Allium sativum*	*E.coli*	19 ± 0.351	17 ± 0.057	24 ± 0.18
3	*Ocimum sanctum*	*S.aureus*	13 ± 0.61	11 ± 0.24	21 ± 0.23
4	*Ocimum sanctum*	*E.coli*	15 ± 0.13	14 ± 0.43	24 ± 0.18

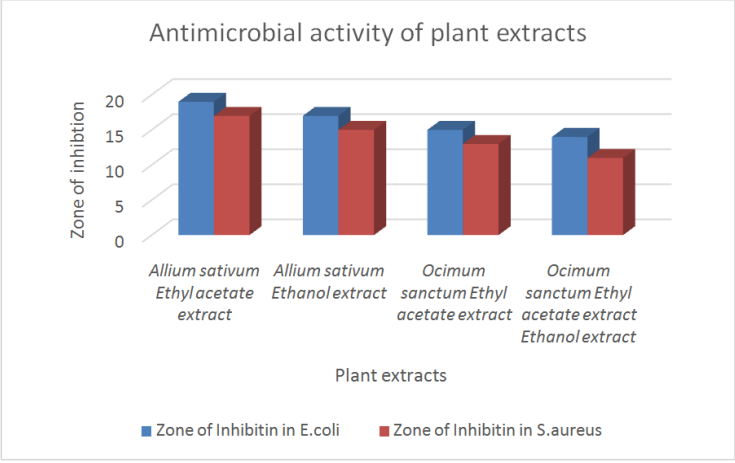


Figure 1: Antifungal activity of plant extracts

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Molecular Docking Studies of Three Mangostin Compounds from *Garcinia mangostana* Fruit with Apoptotic Proteins

Manimekalai I[1], Sivakumari K[2]*, Rajesh S[3] and Ashok K[3]

[1]Assistant Professor, Department of Zoology, Govt. Arts College for Men, Krishnagiri, Tamil Nadu, India.
[2]Associate Professor and Head, Department of Zoology, Presidency College, Chennai, Tamil Nadu, India.
[3]Research Scholar, Department of Zoology, Presidency College, Chennai, Tamil Nadu, India.

Received: 25 Nov 2020 Revised: 02 Dec 2020 Accepted: 24 Dec 2020

***Address for Correspondence**
Sivakumari K
Associate Professor and Head,
Department of Zoology,
Presidency College, Chennai,
Tamil Nadu, India.
Email : dr.sivakumari@rediffmail.com

ABSTRACT

In silico docking of Alpha, Beta and Gamma-mangostin compounds from *Garcinia mangostana* with apoptotic proteins with Argus Lab docking tool was done to find whether these compounds could be used as a drug against cancers. Alpha, Beta and Gamma-mangostins are the xanthones identified from *G. mangostana* fruit. Xanthones have many pharmacological properties like antioxidant, antimicrobial, antiulcer, anticancer, etc. From the results, docking score was highest in Alpha-mangostin; the score being -10.13 kcal/mol with Cyclin D, -10.00 with Caspase-3 and -9.90 kcal/mol with p53. Beta-mangostin gave a docking score of -9.49 kcal/mol with Cyclin D, -8.97 kcal/mol with p53 and -2.90 kcal/mol with Caspase-3. Likewise, Gamma-mangostin gave the docking score of -9.97 kcal/mol with Caspase-3, -9.90 kcal/mol with p53, and -8.16 kcal/mol with Cyclin D. When compared among these ligands, Beta-mangostin and Gamma-mangostin showed the least score for both Caspase-3 and Cyclin D. Hence, Alpha-mangostin seems to possess higher apoptotic activity than Beta-mangostin and Gamma-mangostin. Alpha-mangostin could be considered as a good therapeutic agent against cancers.

Keywords: Alpha-mangostin, Beta-mangostin, Gamma-mangostin, Caspase-3, Cyclin D, p53, ArgusLab.

INTRODUCTION

Computational biology and bioinformatics have the probable not just of speeding up the drug development, thus dipping the costs, but as well of shifting the way drugs are designed. Rational drug design helps to make easy and

speed up the drug designing progression, which involves range of methods to discover novel compounds [1-4]. Molecular docking is a well-established computational technique, which predicts the interaction energy between two molecules and this technique mainly incorporates algorithms like molecular dynamics, Monte Carlo stimulation and fragment-based search methods [5, 6]. Apoptosis is an evolutionarily preserved and tremendously coordinated form of cell death to facilitate the deletion of redundant, infected, injured or malformed cells during the normal life span in various biological systems which is an essential course of action in maintaining homeostasis in multi-cellular organisms. It is usually implicated in embryogenesis, metamorphosis, immune system and normal adult tissue remodelling as well as in a number of pathological disorders such as cancer, autoimmunity and degenerative diseases [7]. Commonly cancer cells themselves are more flat to undergo apoptosis and a comprehensive understanding of the molecular pathways that regulate apoptosis will assist in investigating novel cancer chemotherapeutic targets which in turn would offer new opportunities for the discovery and development of drugs [7-9]. In developing countries herbal medicine is the source of new discoveries for new drug leads towards various healthcare issues and synthesis of new formulations [10]. Vegetables and fruits contain numerous bioactive and potentially anti-carcinogenic substances including carotenes, dithiolthiones, flavonoids, indoles, isothiocyanates, phenols, folic acid and vitamins C and E [10-13]. Hence looking for an effective chemo-preventive agent has led to the identification of various naturally occurring compounds like xanthones from mangosteen (*Garcinia mangostana*) fruit which is known to possess number of pharmacologic properties such as antioxidant, antitumor, anti-allergic, anti-inflammatory, antibacterial, neuro-protective, antifungal, and antiviral activities [14-16]. A number of studies have exposed that xanthones obtained from mangosteen fruit have incredible biological activities. Alpha, Beta and Gamma-mangostins, garcinone E, 8-deoxygartanin and gartanin are the most studied xanthones [17]. Keeping the above facts in mind we decided to conduct *in silico* molecular docking studies of Alpha, Beta and Gamma-mangostin compounds present in *Garcinia mangostana* fruit with apoptotic proteins such as Caspase-3, Cyclin D and p53.

MATERIALS AND METHODS

Databases and Tools

Swiss-Prot

The proteins sequence of Caspase-3, Cyclin D and p53 were retrieved from Swiss-Prot database. The accession numbers are: P42574, P55273 and P04637.

Protein Data Bank (PDB)

The structure of Caspase-3, Cyclin D and p53 proteins were downloaded from PDBSum database and the PDB IDs are: 1CP3, 1BD8 and 1GZH.

RasMol

RasMol is a molecular graphics program intended for the visualization of proteins, nucleic acids and small molecules. The 3D structures of Caspase-3, Cyclin D and p53 visualized using RasMol Tool.

PubChem

The structure of the inhibitors Alpha, Beta and Gamma mangostin were downloaded from PubChem database; the ID being 5281650, 5495925 and 5464078, respectively.

ACD/ChemSketch

The structure of the ligands *viz*., Alpha, beta and Gamma-mangostins was drawn using ACD/ChemSketch.

Open Babel
The 'mol' formats of ligands are converted in to PDB format using Open Babel tool.

ArgusLab
The three ligands of Alpha, Beta and Gamma-mangostin were docked with apoptotic proteins Caspase-3, Cyclin D and p53 using ArgusLab docking tool.

PyMol Viewer
The docked complex was visualized by PyMol visualization tool and the docking scores were evaluated.

RESULTS AND DISCUSSION

The apoptotic proteins Caspase-3, Cyclin D and p53 sequence was downloaded from Swiss-Prot database IDs are P42574, P55273 and P04637. 3D structures of protein was downloaded from PDB Database IDs are 1CP3, 1BD8 and 1GZH and visualized by RasMol tool (Figure 1). The ligands Alpha-mangostin, Beta-mangostin and Gamma-mangostin were drawn using ACD ChemSketch and the 'mol' format of ligands are converted into PDB format using Open Babel tool (Figure 2, 3 and 4). The docking is a method which predicts the preferred orientation of one molecule which bound to the other molecule to form a stable complex; the docking being performed between the protein and the ligands using ArgusLab docking tool. The docked complex was visualized using PyMol software (Figure 5, 6 and 7). *In silico* molecular docking study revealed the interactions between ligand and protein and also calculated the minimum binding energy (kcal/mol) between the protein and ligand. The Alpha-mangostin showed the docking score of -10.00 kcal/mol with Caspase-3, -10.13 kcal/mol with Cyclin D and -9.90 kcal/mol with p53. Beta-mangostin showed docking score of -2.90 kcal/mol with Caspase-3, -9.49 kcal/mol with Cyclin D and -8.97 kcal/mol with p53. Likewise, Gamma-mangostin showed the docking score of -9.97 kcal/mol for Caspase-3, -8.16 kcal/mol for Cyclin D and -9.90 kcal/mol for p53 (Table 1). When compared among the ligands, Alpha-mangostin showed the best docking score for Caspase-3, Cyclin D and p53. Hence, Alpha-mangostin seems to possess higher apoptotic activity than Beta-mangostin and Gamma-mangostin. The interaction of Alpha-mangostin formed 2 hydrogen bonds with Caspase-3, 3 hydrogen bonds with Cyclin D and 3 hydrogen bonds with p53. Beta-mangostin formed 1 hydrogen bond with Caspase-3, 2 hydrogen bonds with Cyclin D and 4 hydrogen bonds with p53. Gamma-mangostin formed 1 hydrogen bond with Caspase-3, formed 1 hydrogen bond with Cyclin D and formed 3 hydrogen bonds with p53. The results revealed that bond formation was stronger in Alpha-mangostin compared than Beta-mangostin and Gamma-mangostin. Alpha-mangostin has more apoptotic potential than that of Beta-mangostin and Gamma-mangostin. From the above docking results, it is pragmatic that Alpha-mangostin docks well with Caspase-3, Cyclin D and p53, and good docking scores and strong hydrogen bond formation, and hence can be considered to be the best compound. The results of Lipinski rule suggest that the analyzed Alpha-mangostin compound as best therapeutic drug. Similar *in silico* docking studies of stearic acid against plasminogen and transferring proteins present in HepG-2 cell line to predict its anticancer property was carried out by Rajesh *et al*. (2016) [3]. Rutin compound against apoptotic proteins such as Tumor Necrosis Factor, Caspase-3, NF Kappa-B, P53, Collagenase, Nitric Oxide Synthase and Cytochrome C was carried out by Jayammena *et al*. (2018) [7]. Alginic acid and fucoidan compound present in *Sargassum wightii* against Caspase-3, Caspase-9 and β-Actin apoptotic proteins was carried out by Jayaprakash *et al*. (2018) [18].

Desulphosinigrin, Ethyl iso-allocholate, 9,12,15-Octadecatrienoic acid,2-[(trimethylsilyl)oxy]methyl]ethyl ester, [ZZZ]-, Pentadecanoic acid, 14-methyl-, methyl ester, 1-Monolinoleylglycerol trimethylsilyl ether, 16-Octadecenoic acid, methyl ester and Acetoxy-6a,11a-dihydroxy-16a,17a-propylmethylenedioxy pregna-1,4-diene-3,20-dione in propolis against Caspase-3, Caspase-9, β-Actin proteins was carried out by Flora Priyadarshini *et al*. (2018) [19]. Ascorbic acid, betalain and gallic acid from *Hylocereus undatus* against apoptotic proteins like Caspase-3, Caspase-9 and β-Actin was carried out by Karthika *et al*. (2018) [6]. Likewise propolis was docked with Caspase-3, Caspase-9,

Bcl-2, Bax and Bcl-xL by Rajini Selvaraj *et al*. (2019) [20]. Muricin J, Muricin K and Muricin L compound from *A. muricata* against caspase-3, caspase-9 and β-actin by Hemalatha *et al*. (2020b) [4]. Apoptosis is a familiar biological response exhibited by cells after suffer DNA damage and is a helpful indicator for screening compounds for succeeding development as probable anticancer agents [7, 21]. Apoptosis provides a number of clues with admiration to successful anticancer therapy, and many chemotherapeutic agents reportedly exert their antitumor effects by inducing apoptosis in cancer cells [22] The goal of ligand-protein docking is to predict the major binding model(s) of a ligand with a protein of known three dimensional structures [23]. Ligand binding is the key step in enzymatic reactions and, thus, for their inhibition. For that reason, thorough kind of communications between small molecules and proteins may outline the basis for a rational drug design approach [7]. Molecular docking, both structure-based and ligand-based, has become a powerful and inexpensive method for searching a novel lead compound. Molecular docking has been successful in discovering novel anticancer compounds against several protein targets, such as Caspase-3, Cyclin D and p53 as well. The docking result showed that there exists a binding interaction between each proteins and ligands, which was validated by the formation of hydrogen bond between the proteins and the ligands. Lipinski rule also suggests Alpha-Mangostin as the best therapeutic drug. The results clearly depicts that there is an interaction between the ligands and proteins. Hence, the *in silico* molecular docking studies suggests that Alpha-Mangostin can be utilized as a potential and green therapeutic agent to treat various diseases.

CONCLUSION

In conclusion, molecular docking binding interaction of Alpha-mangostin, Beta-mangostin and Gamma-mangostin compounds with apoptotic proteins results are helpful for manipulate and develop a novel drug that has better inhibitory activity against several types of cancers. When compared Alpha-mangostin showed better docking potential than the other two compounds. Hence, Alpha-mangostin from *G. mangostana* is one of the best anticancer agent to treat cancers. Further justification by wet lab studies for its accurate gathering as an anticancer drug waits.

ACKNOWLEDGEMENTS

The authors are thankful to Mrs. K. Shyamala Devi for the technical support.

REFERENCES

1. Christensen JG, Zou HY, Arango ME. Cytoreductive antitumour activity of PF-2341066, a novel inhibitor of anaplastic lymphoma kinase and c-met, in experimental models of anaplastic large-cell lymphoma. Mol Cancer Ther, 2007; 6: 3314-22.
2. Leite TB, Gomes D, Miteva MA, Chomilier J, Viloutreix BO. Frog: A free online drugs 3-D confirmation generator. Nucleic Acids Res, 2007; 35: 568-572.
3. Rajesh S, Sivakumari K, Ashok K. *In silico* docking of selected compound from *Cardiospermum halicacabum* Linn. leaf against human hepatocellular carcinoma (HepG-2) cell line. Int J Comp Bioinfor In Silico Mod, 2016; 5(2), 780-786.
4. Hemalatha G, Sivakumari K, Rajesh S, Shyamala Devi K. *In silico* molecular docking stuides of Muricin J, Muricin K and Muricin L compound from *A. muricata* against apoptotic proteins (caspase-3, caspase-9 and β-actin). Inn Int J Sci, 2020; 7(5): 1-4
5. Lengauer T, Rarey M. Computational methods for biomolecular docking. Curr Opin Stru Biol, 1996; 6: 402- 406.
6. Karthika S, Sivakumari K, Rajesh S, Ashok K, Shyamala Devi K. *In silico* molecular prediction of ascorbic acid, betalain and gallic acid from *Hylocereus undatus* against apoptotic proteins (caspase-3, caspase-9 and β -actin). J Pharm Sci Innov, 2018; 7(6): 215-220.

7. Jayameena P, Sivakumari K, Ashok K, Rajesh S. *In silico* molecular docking studies of Rutin compound against apoptotic proteins (Tumor Necrosis Factor, Caspase-3, NF-Kappa-B, P53, Collagenase, Nitric oxide synthase and Cytochrome C). J Cancer Res Treat, 2018; 6(2): 28-33.
8. Bailey K, Cook HW, McMaster CR. The phospholipid scramblase PLSCR1 increases UV induced apoptosis primarily through the augmentation of the intrinsic apoptotic pathway and independent of direct phosphorylation by protein kinase C δ. Biochim Biophys Acta, 2005;1733:199-209.
9. Alam JJ. Apoptosis: target for novel drugs. Trends Biotechnol, 2003; 21:479-83.
10. Hemalatha G, Sivakumari K, Rajesh S, Shyamala Devi K. Phytochemical profiling, anticancer and apoptotic activity of Graviola (*Annona muricata*) fruit extract against human hepatocellular carcinoma (HepG-2) cells. Int J Zool Appl Biosci, 2020a; 5(1): 32-47.
11. Abd Hadi N, Mohamad M, Rohin MAK, Yusof RM. Effects of Red pitaya fruit (*Hylocereus polyrhizus*) consumption on blood glucose level and lipid profile in type 2 diabetic subjects. Borneo Sci, 2016; 31, 113-129.
12. El Zawawy NA. Antioxidant, antitumor, antimicrobial studies and quantitative phytochemical estimation of ethanolic extracts of selected fruit peels. Int J Curr Microbiol Apl Sci, 2015; 4(5), 298-309.
13. Sancho LEGG, Yahia EM, García-Solís P, González-Aguilar GA. Inhibition of proliferation of breast cancer cells MCF7 and MDA-MB-231 by lipophilic extracts of papaya (*Carica papaya* L. var. Maradol) fruit. Food Nutr Sci, 2014; 5(21), 2097.
14. Li WQ, Xu JG. Profile of DNA damage protective effect and antioxidant activity of different solvent extracts from the pericarp of *Garcinia mangostana.* J Food Nutr Sci, 2015; 3(1-1): 1-6.
15. Manimekalai I, Sivakumari K, Ashok K, Rajesh S. Phytochemical profiling of mangosteen fruit, *Garcinia mangostana*. World J Pharm Sci, 2016a; 5(2): 221-252.
16. Manimekalai I, Sivakumari K, Ashok K, Rajesh S. Antioxiodant and anticancer potential of mangosteen fruit, *Garcinia mangostana* against hepatocellular carcinoma (HepG-2) cell line. World J Pharm Sci, 2016b; 5(2): 253-293.
17. Suksamrarn S, Komutiban O, Ratananukul P, Chimnoi N, Lartpornmatulee N, Suksamrarn A. Cytotoxic prenylated xanthones from the young fruit of Garcinia mangostana. Chem Pharm Bull, 2006; 54: 301-305.
18. Jayaprakash P, Sivakumari K, Ashok K, Rajesh S. In Silico Molecular Docking of Alginic acid and Fucoidan compound present in *S. wightii* against Apoptotic Proteins (Caspase-3, Caspase-9 and β-Actin). IJBPAS, 2018; 7(8): 1551-1565.
19. Flora Priyadarshini J, Sivakumari K, Ashok K, Jayaprakash P, Rajesh S. GC-MS analysis for identification of active compounds in propolis and molecular docking studies of selected compounds against apoptoic proteins (Caspase-3, Caspase-9 and β-Actin). J Biol Chem Res, 2018; 35(2): 349-358.
20. Rajini Selvaraj, Sivakumari K, Rajesh S, Ashok K. Molecular Docking interaction of Propolis with Caspase-3, Caspase-9, BAX, BCL-2 AND BCL-XL. IJRAR, 2019; 6(2): 33-38.
21. Yang HL, Chen CS, Chang WH, Lu FJ, Lai YC, Chen CC. Growth inhibition and induction of apoptosis in MCF-7 breast cancer cells by *Antrodia camphorata*. *Cancer Lett*, 2006; 231(2): 215-227.
22. Kamesaki H. Mechanisms involved in chemotherapy-induced apoptosis and their implications in cancer chemotherapy. Int J Hematol, 1998; 68(1): 29-43.
23. Mittal RR, McKinnon RA, Sorich MJ. Comparison data sets for benchmarking QSAR methodologies in lead optimization. *J Chem Inf Model*, 2009; 49(7): 1810-1820.

Table 1: Docking score of apoptotic proteins with three ligands

Name of the protein	Name of the ligand	Docking score (Kcal/Mol)	H-Bond
Caspase-3	Alpha-mangostin	-10.00	2
	Beta-mangostin	-2.90	1
	Gamma-mangostin	-9.97	1
Cyclin D	Alpha-mangostin	-10.13	3
	Beta-mangostin	-9.49	2
	Gamma-mangostin	-8.16	1

p53	Alpha-mangostin	-9.90	3
	Beta-mangostin	-8.97	4
	Gamma-mangostin	-9.90	3

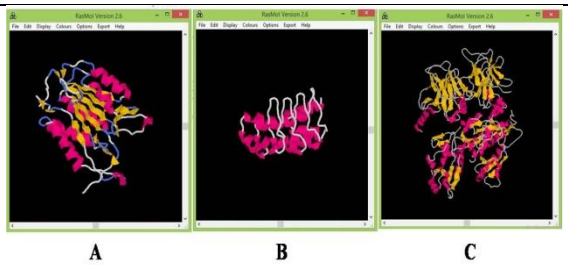


Figure 1: 3D Structure of Caspase-3 (A), Cyclin D (B) and p53 (C) visualized by RasMol

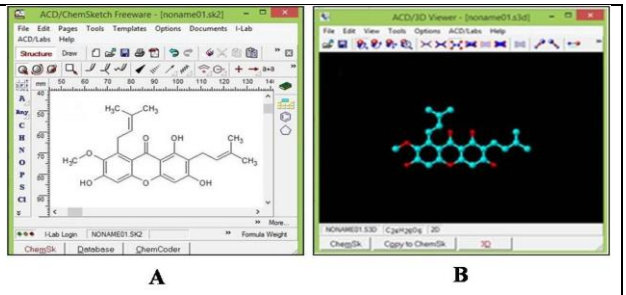


Figure 2: 2D (A) and 3D (B) structure of Alpha-Mangostin

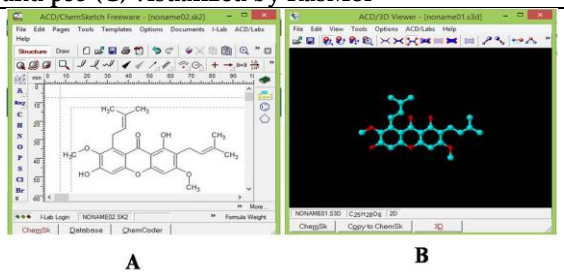


Figure 3: 2D (A) and 3D (B) structure of Beta-Mangostin

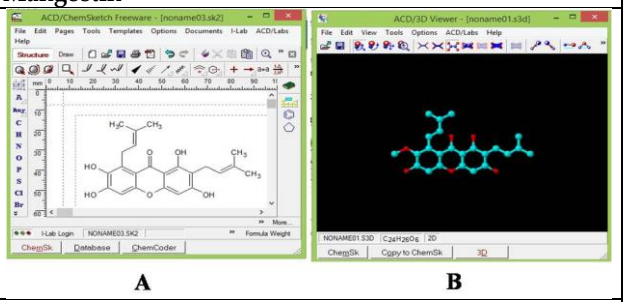


Figure 4: 2D (A) and 3D (B) structure of Gamma-Mangostin

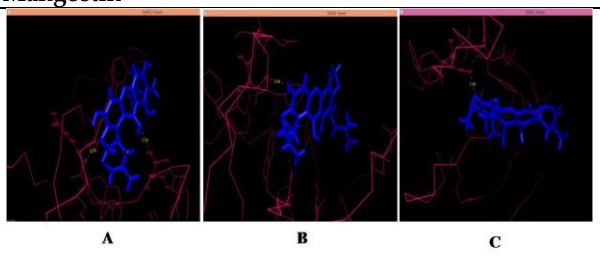


Figure 5: Docked complex of Caspase-3 with Alpha-Mangostin (A), Beta-Mangostin (B) and Gamma-mangostin (C)

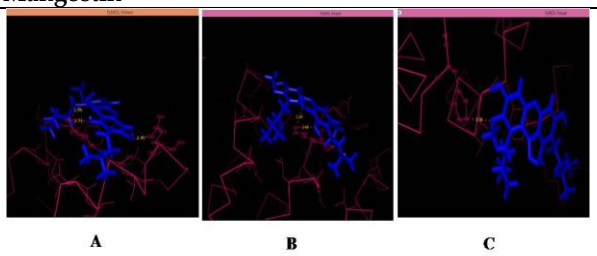


Figure 6: Docked complex of Cyclin D with Alpha-Mangostin (A), Beta-Mangostin (B) and Gamma-mangostin (C)

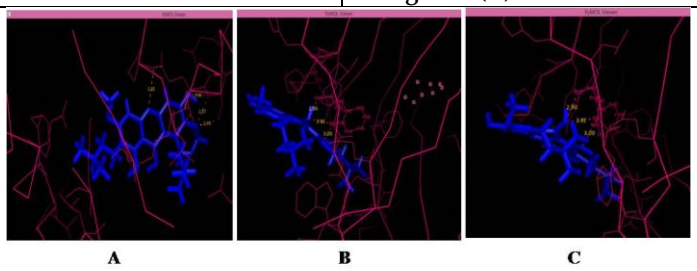


Figure 7: Docked complex of p53 with Alpha-Mangostin (A), Beta-Mangostin (B) and Gamma-mangostin (C)

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Subchronic Toxicity Studies of Ethanolic Extract of Seeds of *Cassia fistula* Linn

R. Kothai and B. Arul*

Department of Pharmacy Practice, Vinayaka Mission's College of Pharmacy, Vinayaka Mission's Research Foundation (Deemed to be University), Salem, Tamil Nadu, India.

Received: 09 Dec 2020 Revised: 16 Dec 2020 Accepted: 23 Dec 2020

***Address for Correspondence**
B. Arul
Department of Pharmacy Practice,
Vinayaka Mission's College of Pharmacy,
Vinayaka Mission's Research Foundation (Deemed to be University),
Salem, Tamil Nadu, India.
Email: arul1971@yahoo.com

ABSTRACT

Cassia fistula is a deciduous, medium-sized tree, commonly called a golden shower. This plant has been traditionally used for skin diseases and other various disorders. Also, used in ayurvedic and Unani systems of medicines. However, the toxicological profile of the plant was not yet scientifically validated. The present research aims to assess the toxicological profile of *Cassia fistula* Linn. in Wistar rats. The sub-chronic toxicity study was performed as per OECD guidelines. During sub-chronic studies, both male and female rats were treated with ethanolic extracts of seeds of *Cassia fistula* at the doses of 200,400, and 800mg/kg orally for 90 days. All the animals were observed weekly for changes in morphology, general behaviour property, food and water intake, body weight, and urine. At the end of the study, biochemical, hematological, macroscopy, and urine analysis were conducted. The results of the present study depicted that no signs of morbidity and mortality were observed. The studies also, revealed that the extract does not produce any signs of toxicity and adverse reactions related to general behavior, food and water intake, body weight, biochemical, hematological, and urine parameters. Thus it was concluded from the study, that the ethanolic extracts of seeds of *Cassia fistula* Linn were safe and non-toxic to rats even at higher doses and further chronic studies are needed to establish their therapeutic efficacy.

Keywords: *Cassia fistula* Linn, Sub-chronic toxicity, Bio-chemical parameters, Haematological parameters.

INTRODUCTION

Plants have been used for medicinal purposes long before the prehistoric period. For thousands of years, medicinal plants have been used to treat health disorders, add flavour and conserve food, and prevent disease epidemics.

Cassia fistula Linn is one such plant. It is a deciduous, medium-sized tree, commonly called a golden shower. It's one of the beautiful trees of tropical countries. It belongs to the family of Caesalpiniaceae and is called Kondrai or sarakondrai in Tamil [1]. It is widely grown in India, Ceylon, Malaya, and China. The bark is smooth and pale grey when young, rough and dark brown when old; branches spreading, slender. Leaves are long. Leaflets are ovate or ovate-oblong and bright green. Flowers long, slender and bright yellow. Seeds are broadly ovate. The plant has a wide spectrum of medicinal properties. In traditional medicine, it is used to treat skin disorders, leprosy, tuberculous glands, syphilis; cures burning sensation, laxative, antipyretic, liver problems, rheumatism. Itching and hyperglycemia [2]. The therapeutic potential of this plant was reported in Unani, Ayurvedha, and other traditional medical systems. It has been reported for its anti-microbial and antifungal [3-8], anti-plasmodial [9], anti-tumour [10], antifertility [11], anti-inflammatory [12], anti-oxidant [13-16], antiulcer [17], Hepatoprotective [18,19] and antiparasitic activities [20]. However, the adverse reaction and toxicological profile of this plant were not yet reported. The present research aims to assess the toxicological profile of ethanolic extracts of seeds of *Cassia fistula* Linn. in Wistar rats.

MATERIALS AND METHODS

Plant material

The seeds of the plant, *Cassia fistula* was collected from the foothill of Yercaud, Salem, and the tribal medical shops in October 2019. The collected plants (seeds) were identified and authenticated by the Botanical Survey of India, Tamilnadu, Agri University, Coimbatore, Tamilnadu. A voucher specimen (ACF-1) has been deposited in the museum. The plant parts were shade dried at room temperature for 10 days and coarsely powdered and passed through sieve No.60.

Preparation of extracts

About 500 g of dried seeds were coarsely powdered and subjected to continuous hot percolation with different solvents of increasing order of polarity such as pet ether, chloroform, acetone, ethanol, and aqueous. The extracts were dried under the rotary evaporator and then tested for various phytochemical constituents like alkaloids, flavonoids, glycosides, phenols, saponins, sterols, tannins, proteins, and carbohydrates [21].

Animals

Healthy adult wistar rats were used for the subchronic toxicity study. The animals were procured from CPCSEA listed suppliers of Sri venkateshwara Enterprises, Bangalore, India. Animals should be nulliparous and non-pregnant. The animals were kept in well-ventilated polypropylene cages at 12h light and 12 h dark schedule at 25°C and 55–65% humidity levels. The rats had been given a normal diet of pellets and free access to water. Each animal, at the commencement of the experiment, should be between 8 and 12 weeks old.

Preparation of animals

The animals were randomly selected, marked to permit individual identification, and kept in their cages for at least 5 days before dosing to allow for acclimatization to the laboratory conditions. Before each test, the animals were fasted for at least 12 hrs; the experimental protocols were subjected to the scrutinization of the Institutional Animals Ethical Committee (P.ceu/26/2019/IAEC/VMCP) and were cleared by the same. All experiments were performed during the morning according to CPCSEA guidelines for the care of laboratory animals and the ethical guideline for investigations of experimental pain in conscious animals. The standard orogastric cannula was used for oral drug administration in experimental animals.

Subchronic toxicity studies

Subchronic toxicity study was performed as per OECD (Organisation for Economic Co-operation and Development) – Guidelines 408[22]. A total of 48 rats weighing between 150-170g were randomly divided into 4 groups of 12 rats

each (6 male+6 female). Animals fasted before dosing (food but not water was withheld over-night). Following the period of fasting, the bodyweight of the animals was measured and the ethanolic extracts of seeds of *Cassia fistula* (ESCF) were administered to each group at single doses of 200,400 and 800 mg/kg, respectively, by oral gavage. The control groups were treated with the same volume of distilled water. Animals were observed individually after dosing at least once during the first 30 minutes, periodically during the first 24 h with special attention given during the first 4h, and daily thereafter for a total of 90 days. During the study, the body weights of all groups were measured once a week. Animals were also visually observed for mortality, changes in behavioural patterns, changes in physical appearance, and symptoms of illness. At the end of the study period, all rats fasted overnight (12–16h), and then anesthetized with urethane by intraperitoneal injection (1 mL/100 g body weight). Blood samples were collected for the measurement of hematological (EDTA-2K coated tubes) and biochemical (dry tubes) parameters. After euthanasia, the rats were sacrificed and subjected to an organ weight measurement, necropsy, and histopathological examination.

Body weight: Individual weights of animals were determined shortly before the test substance was administered and at least weekly thereafter. Weight changes were calculated and recorded. At the end of the test, surviving animals were again weighed.

Food and water intake: The food and water intake of each animal of both control and test groups were measured once per week throughout the study.

Urine analysis: The urine analysis was performed to investigate any abnormalities in the excretion pattern after exposure to the test drug for 90 days. The urine samples were collected from each animal early in the morning. The examination was carried by using a MULTISTIX reagent strip for urine analysis, which was procured from Ames miles Ltd.

Statistical analysis

The results were expressed as the mean ± SEM and analyzed statistically by one-way ANOVA followed by Dunnett's t-test by using SPSS version 16. $P < 0.05$ compared to control was considered to be statistically significant.

RESULTS AND DISCUSSION

The purpose of this research is to give scientific validation to the plants by collect and extract plant materials and then to screen them for potential phytochemical and Toxicological aspects. Several pharmacological studies have been reported with the leaves of this plant, there is no experimental evidence on its toxicity studies. Hence, the current research work focused on the toxic effects of ethanolic extracts of seeds of *Cassia fistula* Linn as per OECD Guidelines. Subchronic toxicity studies were performed to determine the long-term adverse effects of the drug when administered in a single dose orally for 90 days. It also indicates the safety of the drug in-vivo. In the present study, daily oral administration of extract for 90 days did not produce any obvious symptom of toxicity in rats of both sexes, including the highest dose tested at 800 mg/kg body weight. No deaths or obvious clinical signs were found in any group throughout the experimental period. Changes in body weight and general behaviour are some of the important parameters for the evaluation of the first sign of toxicity [23]. During the study, there were no significant changes observed in body weight, food, and water intake, urine and general behaviour pattern in all test groups animals were observed when compared to the control group. This indicates that food and water were well accepted by the rats, suggesting that the extracts did not in any way alter the metabolism of carbohydrate, protein, and fats in the rats. It also may denote that the nutritional status of the animals during the 90 days was not adversely affected by the extracts. This corroborates the traditional usage of the plant by the oral route. The organs isolated from various groups did not reveal any abnormalities on gross examination. The hematopoietic system is one of the important parameters for toxic substances and an index of physiological and pathological status; usually, blood profile gives

vital information on the response of the body to the injury or stress [24,25]. In the present study, no statistically significant differences were observed in the haematological, and biochemical parameters were shown in Table No.1-4. The macroscopic study of the organs of the animals treated with various doses of ESCF did not produce any changes in colour, size, and shape when compared with the control group rat organs. Hypertrophy of organs is one of the vital indications of toxicity of a chemical or biological substance. In this study, no hypertrophy of organs was observed amongst all the groups studied. Similarly, the histopathological studies with liver, stomach, spleen, kidney, heart, and lungs did not reveal any pathological changes in cell structure or any unfavorable effects and they were found to be normal as shown in fig no.1-6. Therefore, the results of the present study showed that the ESCF did not alter the liver, renal and other functions, and further support the non-toxic nature of ESCF.

CONCLUSION

Based on the findings of the present study, we may conclude that ethanolic extracts of seeds of *Cassia fistula* extract are not toxic in all the doses studied and did not produce any toxic signs or evident symptoms at sub-chronic oral toxicity. Cassia fistula extract did not cause any lethality or produce any remarkable histopathological signs or serum chemical alteration. These preliminary results suggest promising alternatives for exploring therapeutic and pharmaceutical interest in Cassia fistula Linn extract with a reduction of possible adverse effects. Further studies are required to determine the effects of seeds of Cassia fistula Linn extract on an animal fetus, on pregnant animals, and their reproductive capacity is needed to complete the safety profile of this herb.

REFERENCES

1. Kirtikar K.R. and Basu BD. Indian Medicinal Plants, International book distributors. 2006; 2:856-860.
2. Dutta A, De B. Seasonal variation in the content of sennosides and rhein in leaves and pods of *Cassia fistula*, Indian J. Pharmacol. Sci.1998; 60: 388-390.
3. Duraipandiyan, V. and S. Ignacimuthu. Antimicrobial and antifungal activity of *Cassia fistula* L. An ethnomedical plant. J. Ethnopharmacol.2007;112: 590-594.
4. Sony, P., Kalyani, M., Jeyakumari, D., Kannan, I., & Sukumar, R. G. In vitro antifungal activity of cassia fistula extracts against fluconazole resistant strains of Candida species from HIV patients. Journal de mycologiemedicale, 2018; 28(1): 93-200.
5. Irshad, M. D., Ahmad, A., Zafaryab, M. D., Ahmad, F., Manzoor, N., Singh, M., & Rizvi, M. M. A. Composition of Cassia fistula oil and its antifungal activity by disrupting ergosterol biosynthesis. Natural product communications.2013; 8(2): 261-264.
6. Bhalodia, N. R., Nariya, P. B., Acharya, R. N., & Shukla, V. J. In vitro antibacterial and antifungal activities of *Cassia fistula* Linn. Fruit pulp extracts. Ayu. 2012; 33(1): 123-129.
7. Irshad, M., Shreaz, S., Manzoor, N., Khan, L. A., & Rizvi, M. M. A. Anticandidal activity of *Cassia fistula* and its effect on ergosterol biosynthesis. Pharmaceutical Biology.2011;49(7): 727-733.
8. Jothy, S. L., Zakariah, Z., Chen, Y., & Sasidharan, S. In vitro, in situ and in vivo studies on the anticandidal activity of *Cassia fistula* seed extract. Molecules.2012; 17(6):6997-7009.
9. Grace, M. H., Lategan, C., Graziose, R., Smith, P. J., Raskin, I., & Lila, M. A. Antiplasmodial activity of the ethnobotanical plant *Cassia fistula*. Natural Product Communications.2012;7(10):1263-66.
10. Yadav, R., & Jain, G. C. Antifertility effect of aqueous extract of seeds of *Cassia fistula* in female rats. Advances in contraception.1999;15(4): 293-301.
11. Gupta, M., Mazumder, U. K., Rath, N., & Mukhopadhyay, D. K. Antitumor activity of methanolic extract of *Cassia fistula* L. seed against Ehrlich ascites carcinoma. Journal of Ethnopharmacology. 2000; 72(1-2): 151-156.
12. Ilavarasana, R., M. Mallika and S. Venkataraman, Anti-inflammatory and antioxidant activities of *Cassia fistula* Linn bark extracts. Afr. J. Trad. Compliment. Altern. Med.2005; 2: 70-85.

13. Siddhuraju, P., P.S. Mohan and K. Becker. Studies on the antioxidant activity of Indian Laburnum (*Cassia fistula* L.): A preliminary assessment of crude extracts from stem bark, leaves, flowers and fruit pulp. Food Chem. 2002; 79: 61-67.
14. Thabit, S., Handoussa, H., Roxo, M., El Sayed, N. S., De Azevedo, B. C., & Wink, M. Evaluation of antioxidant and neuroprotective activities of *Cassia fistula* (L.) using the Caenorhabditiselegans model. PeerJ, 2018; 6: e5159.
15. Abid, R., Mahmood, R., & Santosh Kumar, H. S. Hypolipidemic and antioxidant effects of ethanol extract of *Cassia fistula* fruit in hyperlipidemic mice. Pharmaceutical Biology.2016; 54(12): 2822-2829.
16. Luximon-Ramma, A., Bahorun, T., Soobrattee, M. A., & Aruoma, O. I. Antioxidant activities of phenolic, proanthocyanidin, and flavonoid components in extracts of *Cassia fistula*. Journal of Agricultural and Food Chemistry.2002; 50(18): 5042-5047.
17. Karthikeyan, S., & Gobianand, K. (2010). Antiulcer activity of ethanol leaf extract of *Cassia fistula*. Pharmaceutical biology.2010; 48(8): 869-877.
18. Bhakta, T., Mukherjee, P. K., Mukherjee, K., Banerjee, S., Mandal, S. C., Maity, T. K.,& Saha, B. P. Evaluation of hepatoprotective activity of *Cassia fistula* leaf extract. Journal of ethnopharmacology.1999; 66(3): 277-282.
19. Bhakta, T., Banerjee, S., Mandal, S. C., Maity, T. K., Saha, B. P., & Pal, M. Hepatoprotective activity of *Cassia fistula* leaf extract. Phytomedicine,2001; 8(3): 220-224.
20. Sartorelli, P., C.S. Carvalho, J.Q. Reimao, M.J.P. Ferreira and A.G. Tempone. Antiparasitic activity of biochanin A, an isolated isoflavone from fruits of *Cassia fistula* (Leguminosae). Parasitol. Res. 2009; 104: 311-314.
21. Arul B, Kothai R, Sureshkumar K, Christina AJM. Anti-Inflammatory Activity of Coldenia procumbens Linn. Pak J Pharm Sci [Internet]. 2005;18(3):17–20.
22. OECD.The OECD Guideline for Testing of Chemicals: 408 Subchronic Oral Toxicity-Rodent: 90-Day Study, OECD, Paris, France, 1998.
23. Sireeratawong S, Lertprasertsuke N, Srisawat U, Thuppia A, Ngamjariyawat A, Suwanlikhid N, Jaijoy K. Acute and sub-chronic toxicity study of the water extract from Tiliacora triandra (Colebr.) Diels in rats. Songklanakar in J Sci Technol 2008; 30: 729- 737.
24. Mukinda JT, Eagles FK. Acute and sub-chronic oral toxicity profile of the aqueous extract of Polygala fruticosa in female mice and rats. J Ethnopharmacol 2010; 128: 236-240.
25. Tan PV, Mezui C, Orock GE, Njikam N, Dimo T, Bitolog P. Teratogenic effects, acute and subchronic toxicity of the leaf aqueous extract of Ocimum suave Wild (Lamiaceae) in rats. J Ethnopharmacol 2008; 115: 232–237.

Table no.1. Effect of ethanolic extracts of seeds of *Cassia fistula* Linn on haematological parameters of male rats in subchronic toxicity studies

Group	Treatment	Dose mg/kg	Hb (g/dl)	RBC (million/mm³)	WBC (1000/mm³)	Differential count				
						Neutrophils %	Eosinophils%	Basophils %	Lymphocytes%	Monocytes %
I	Control	-	13.00±0.26	9.20±0.29	8.50±0.34	26.67±0.61	2.25±0.10	0.18±0.003	75.00±0.45	3.17±0.31
II	ESCF	200	13.50±0.22 ns	8.93±0.29 ns	9.17±0.48ns	25.67±0.33ns	2.67±0.09 ns	0.19±0.003 ns	76.67±0.33 ns	4.17±0.31 ns
III	ESCF	400	13.50±0.43 ns	9.17±0.34 ns	9.17±0.40ns	25.17±0.48 ns	2.05±0.08 ns	0.17±0.006 ns	74.50±0.22 ns	3.50±0.34 ns
IV	ESCF	800	14.17±0.48 ns	8.67±0.42 ns	9.33±0.49 ns	24.83±0.40 ns	2.45±0.08 ns	0.16±0.008 ns	76.50±0.22 ns	3.33±0.33 ns

Values were expressed as Mean ± SEM of 6 male rats in each group. The mean values observed in Hb, RBC, WBC and Differential cell count of extract treated groups were not significantly different from control group at the end of study (90 days).

Table no. 2. Effect of ethanolic extracts of seeds of *Cassia fistula* Linn on haematological parameters of female rats in subchronic toxicity studies

Group	Treatment	Dose mg/kg	Hb (g/dl)	RBC (million/mm^3)	WBC (1000/mm^3)	Differential count				
						Neutrophils %	Eosinophils%	Basophils%	Lymphocytes%	Monocytes %
I	Control	-	13.00±0.26	9.20±0.29	8.50±0.34	26.67±0.61	2.25±0.10	0.18±0.003	75.00±0.45	3.17±0.31
II	ESCF	200	13.50±0.22^{ns}	8.93±0.29^{ns}	9.17±0.48^{ns}	25.67±0.33^{ns}	2.67±0.09^{ns}	0.19±0.003^{ns}	76.67±0.33^{ns}	4.17±0.31^{ns}
III	ESCF	400	13.50±0.43^{ns}	9.17±0.34^{ns}	9.17±0.40^{ns}	25.17±0.48^{ns}	2.05±0.08^{ns}	0.17±0.006^{ns}	74.50±0.22^{ns}	3.50±0.34^{ns}
IV	ESCF	800	14.17±0.48^{ns}	8.67±0.42^{ns}	9.33±0.49^{ns}	24.83±0.40^{ns}	2.45±0.08^{ns}	0.16±0.008^{ns}	76.50±0.22^{ns}	3.33±0.33^{ns}

Values were expressed as Mean ± SEM of 6 female rats in each group.

The mean values observed in Hb, RBC, WBC and Differential cell count of extract treated groups were not significantly different from control group at the end of study (90 days).

Table no. 3. Effect of ethanolic extracts of seeds of *Cassia fistula* Linn on biochemical parameters of male rats in subchronic toxicity studies.

Group	Treatment	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Total bilirubin (mg/dl)
I	Control	-	191.83±1.28	80.33±0.67	230.50±0.56	7.23±0.03	121.33±0.42	0.50±0.004
II	ESCF	200	190.67±0.67^{ns}	80.00±0.58^{ns}	232.17±0.48^{ns}	6.81±0.05^{ns}	122.83±0.48^{ns}	0.43±0.021^{ns}
III	ESCF	400	188.00±0.37^{ns}	81.17±0.31^{ns}	233.83±0.48^{ns}	6.88±0.03^{ns}	120.17±0.48^{ns}	0.42±0.009^{ns}
IV	ESCF	800	191.33±0.49^{ns}	82.50±0.43^{ns}	230.83±0.48^{ns}	7.29±0.02^{ns}	120.00±0.58^{ns}	0.42±0.019^{ns}

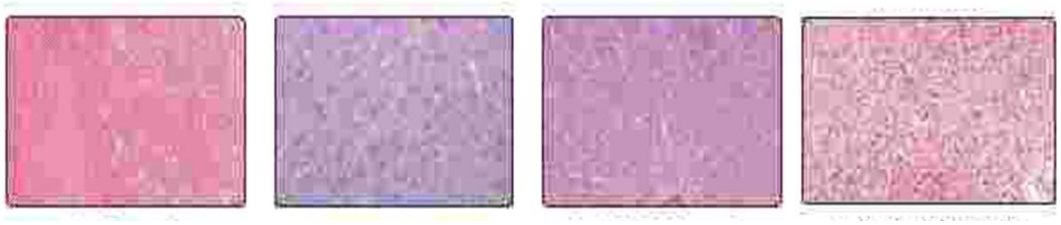
Values were expressed as Mean ± SEM of 6 male rats in each group.

The mean values observed in biochemical parameters of extract treated groups were not significantly different from control group at the end of study (90 days).

Table no. 4. Effect of ethanolic extracts of seeds of *Cassia fistula* Linn on biochemical parameters of female rats in subchronic toxicity studies

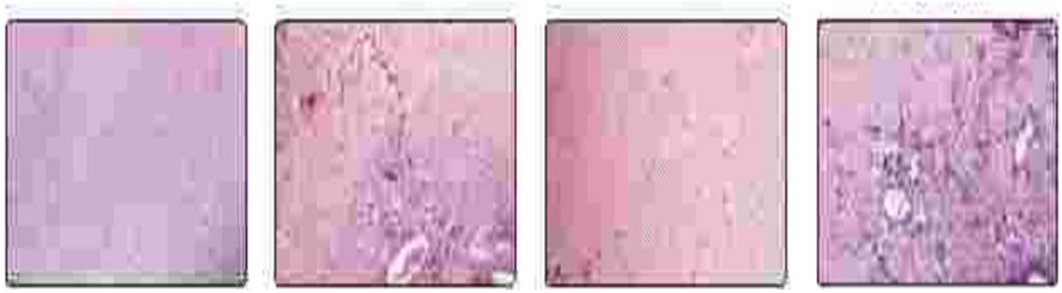
Group	Treatment	Dose (mg/kg)	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	Total Protein (g/dl)	Total cholesterol (mg/dl)	Total bilirubin (mg/dl)
I	Control	-	194.50±0.43	80.83±0.65	232.00±0.37	6.75±0.08	121.17±0.48	0.45±0.015
II	ESCF	200	191.00±0.52^{ns}	79.33±0.42^{ns}	231.17±0.70^{ns}	7.14±0.03^{ns}	122.83±0.60^{ns}	0.48±0.011^{ns}
III	ESCF	400	189.67±0.67^{ns}	81.50±0.56^{ns}	232.83±0.65^{ns}	7.10±0.05^{ns}	121.00±0.52^{ns}	0.45±0.010^{ns}
IV	ESCF	800	192.50±0.56^{ns}	81.33±0.49^{ns}	231.00±0.45^{ns}	7.23±0.04^{ns}	120.50±0.43^{ns}	0.44±0.011^{ns}

Values were expressed as Mean ± SEM of 6 male rats in each group. The mean values observed in biochemical parameters of extract treated groups were not significantly different from control group at the end of study (90 days).



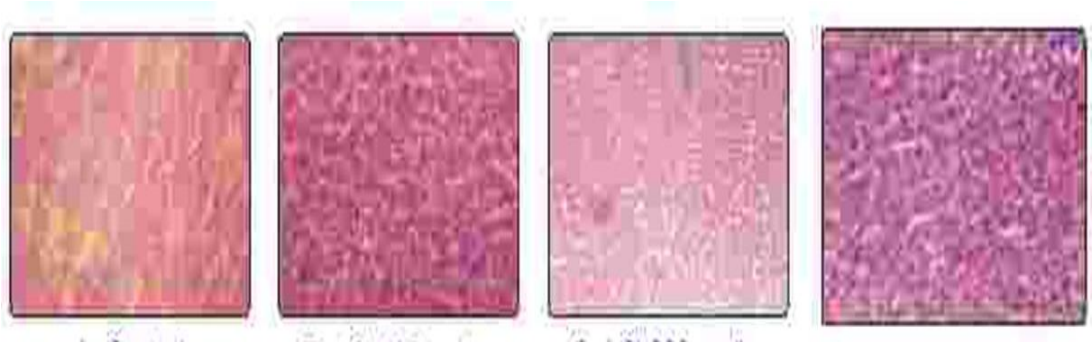
Control ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 1. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on liver in Subchronic toxicity.



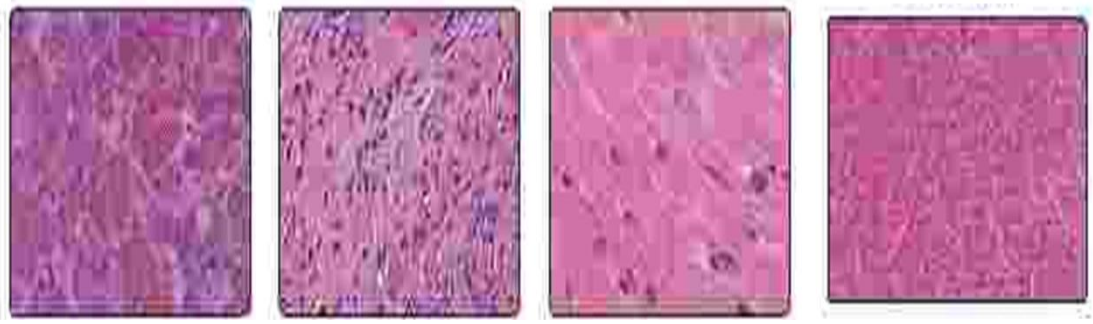
Control ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 2. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on stomach in Subchronic toxicity



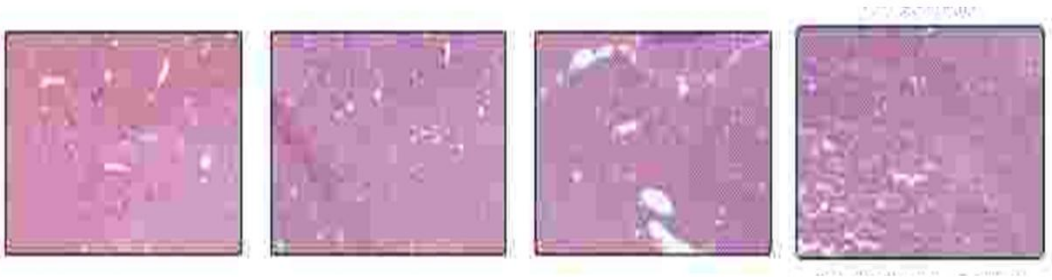
ontrol ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 3. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on kidney in Subchronic toxicity



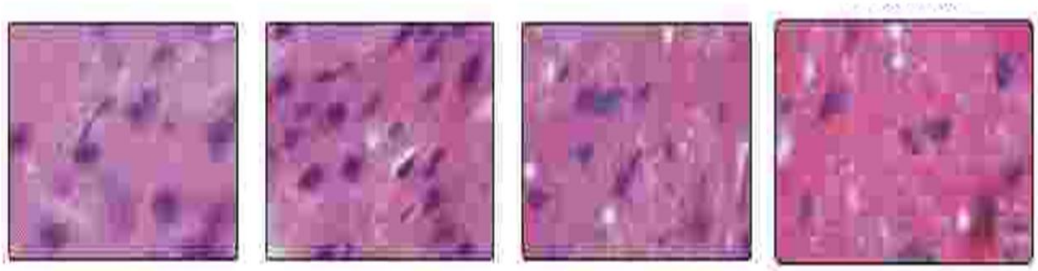
n
trol ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 4. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on heart in Subchronic toxicity



Control ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 5. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on lungs in Subchronic toxicity



Control ESCF 200mg/kg ESCF 400mg/kg ESCF 800mg/kg.

Fig. 6. Effects of ethanolic extracts of seeds of *Cassia fistula* Linn on spleen in subchronic toxicity.

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

REVIEW ARTICLE

Impact on Emerging Trends in Precision Agriculture and Agro Industry via Machine Learning Techniques: A Review

Mythili K[1]* and Rangaraj R[2]

[1]Associate Professor and Head, Department of Computer Technology, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, India.
[2]Professor and Head, PG and Research, Department of Computer Science, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, India.

Received: 03 Nov 2020 Revised: 24 Nov 2020 Accepted: 26 Dec 2020

***Address for Correspondence**
Mythili K
Associate Professor and Head,
Department of Computer Technology,
Hindusthan College of Arts and Science,
Coimbatore, Tamil Nadu, India.
Email: mythiliarul@gmail.com

ABSTRACT

India is the home for the largest malnourished population and also a higher proportion of the vegetarian population. The precision farming is expected to witness high growth rate in the forecast period. The key players of the market are concentrating on various strategies a smart agriculture market to sustain positive impact. Thence weather predicting approaches and existing approaches are discussed in detail and proposed design with an effective dimensionality reducing strategy as Self Organizing Map (SOM) incorporated with Latent Dirichlet Allocation (LDA).ie., a hybrid system of SOM and LDA is designed with multi-objective classifier DNN for enhancing the performance of season and crop prediction. After reducing the measurement, the dimensionality reduced information is utilized to forecast climate for a reasonable outcome. A reasonable season for an appropriate crop is arranged with the guide of Deep Neural Network (DNN) classification system. This research work depends on finding appropriate information model, which helps in accomplishing high precision and simplification for value forecast that enhances and analyses size of the global Agriculture on the basis of value and volume and to ensure good growth in agriculture sector.

INTRODUCTION

Agriculture is main source and supportive of economic India and acts as backbone. The lifespan of India begins from hands of farmer at necessitated food production. The 70% core work of India inclusive of rural and urban areas garners agriculture. India is second topmost producer of veggies. But the standstill challenges faced by agriculturists

are non-recognition and ignorance. The distress caused in industry of agriculture are as unpredictable weather condition, monsoon, floods and drought etc., Agriculture is standing next in the line to IT, banking, manufacturing, finance, healthcare by Machine learning making its entry. The platform features several applications in which the agriculture industry could further curtail its wings are being discussed in forthcoming sections.

A Study on Research Gap of Existing Algorithms

The agricultural production statistics in India have been a massive exercise. The structure of the exercise hides pockets of distress (and averages it over a larger area), and the system is not institutionally geared to broadcast distress in real-time to decision-making levels, as the compilation of yield results takes months after the harvest. Several techniques were proposed by various authors for crop yield prediction. Nowadays many experts are applying automated farming. In this scenario, a brief evaluation of some important contributions to the existing literatures is presented in table 1. The system proposed by Giannaros *et al*[1]exposes the assessment of numerical climate estimation model, in particular the climate research and estimating, with regards to the simulation of wind. Wind estimations were achieved from a system of surface synoptic climate stations were utilized for evaluating model execution. The assessment system concentrated on exploring the capacity of the model to duplicate replicating the wind asset.

Sellam, *et al* [2] explained various environmental parameters like Area under Cultivation (AUC), Annual Rainfall (AR) and Food Price Index (FPI) that influences the yield of crop and the relationship among these parameters was established. Using Regression Analysis (RA), Linear Regression (LR) the various environmental factors and their infliction on crop yield was analyzed. Hemageetha, *et al* [3] concentrates on the soil parameters like pH, Nitrogen, and moisture for crop yield prediction. Naive Bayes algorithm was used to classify the soil and 77% of accuracy was achieved. Appriori algorithm was used to associate the soil with the crops that could provide maximum yield. A comparison of accuracy achieved during classification using Naïve Bayes, J48 and JRIP is also presented. Sujatha, *et al* [4] described about the purpose of various classification techniques that could be utilized for crop yield prediction. A few of the data mining methods, such as the Naïve Bayes, J48, random forests, SVM, artificial neural networks were presented. A system using climate data and crop parameters used to predict crop growth has been proposed. Ankalaki, *et al* [5] presented a comparative study on DBSCAN and AGNES algorithm for clustering. Crop yield was forecasted using MLR (Multiple Linear Regression) and a formula was derived for each crops. but DBSCAN was more time consuming than the optimal and efficient number of clusters. Regression analysis performed for the forecasting that showed a highly dependency on the dataset. Proper data collection will make the model significant; otherwise it can lead to inaccurate results. Gayatri, *et al* [6] utilized IOT and web services to handle large amount of data. Sensors were used to collect the data and pass the data to data center. Agriculture field images were captured and GPS was used to accurately feed the data into repositories along with their location. Far and near nodes were communicated through cloud.

Fathima, *et al.* [7] utilized data mining techniques on real time data that help in knowledge discovery. They used k-means clustering algorithm to cluster the farmers based on the crop type and irrigation parameters. Appriori algorithm was used to determine, which two crops were selected as a frequent item set. They generally focus on the policies that government could frame by the cropping practices of farmers. Kaur, *et al.* [8] analyzed the different data mining techniques to find suitable data model that helps in achieving high accuracy for price prediction. Coimbatore market price of tomato data are collected and price was predicted using BP neural network and the result was simulated using MATLAB. Veenadhari, *et al.*[9] described the purpose of data mining methods in the area of agriculture. A few of the data mining methods, such as the k-means, ID3 algorithms, the k nearest neighbor, SVM, Artificial Neural Networks (ANN) were presented. Developed algorithms were user friendly and the accuracy of predictions were above 75% in all the crops. Raorane, *et al. [10]* discussed about the various data mining techniques for improving the crop production in agriculture. A few of Data mining methods, such as ANN, Decision Tree algorithm, Regression Tree, Bayesian network, SVM, k means were used for classification. J. Mariette, M. Olteanu, and N. Villa-Vialaneix [11] compared two extensions of the stochastic SOM for dis-similarity data, initially to

maintain a sparse representation of the prototypes at each step of the algorithm and in sequence of using a dimension reduction with feature space defined by the (K-PCA) dis-similarity.

C. Lennard, and G. Hegerl,[12] developed a supervised scheme: SOM for surface rainfall analysis associated with synoptic circulation for winter and summer time as mid-latitude based cyclones, whereas no circulations were related to spring and autumn rainfall. The paper evaluates the capability of SOMs to match the synoptic movers of observed rainfall record, which effectively downscales the large-scale data of synopses to an accurate resolute response of the surface. It helps in breaking down the change and its effects on circulation of atmospheric characteristics. A.Y. Abdulrahman, T.A. Rahman, et al, [13] updated the necessity for integrating crop-climate structures and also clarified that the integration can assist to overcome current difficulties like mismatch between farmer's requirement and obtainable predictions, risks and time related doubts, task in achieving institutional, financial and political provision, etc. it sustains the integration of crop weather representations for improvement of the predictions. A. Shastry, H.A. Sanjay, and E. Bhanusree,[14] proposed Regression Analysis (RA) to determine the environmental factors and their infliction on crop yield. RA was a multi-variate analysis approach for analyzing factors and groups them into response variables that helps to obtain a decision and enhanced by other factors like minimum support price, cost price index, whole-sale price index etc. J. Scheffel, K. Lindvall, and H.F. Yik, [15] proposed a time-spectral methodology based on Generalized Weighted Residual Method (GWRM) for numerical weather prediction. In this study, comparisons of accuracy and efficiency were carried out for both explicit and implicit time-stepping systems. It was found that the efficiency of GWRM shows better result compared to the existing methods in terms of accuracy. The GWRM has the additional advantage to produce analytical solutions in the form of Chebyshev series expansions.

Global Ml in Agriculture Market by applications

On the basis of application, the global market is segmented into irrigation management, weather tracking & forecasting, water quality management, crop scouting, field mapping, yield monitoring, and others. The yield monitoring segment is anticipated to grow at the highest rate during the forecast period owing to the growing need for efficient crop production with the availability of scarce resources. Some of global applications achieved by Machine Learning in Agriculture are forthcoming as

A Scalable Machine Learning System for Pre-Season Agriculture Yield Forecast

The neural network system concentrates on available inputs area unit treated on an individual basis. Static soil information in handled by fully-connected layers whereas dynamic meteorological information is handled by continual LSTM layers. This explicit design was trained with historical information for many soil properties, precipitation, minimum and most temperature against historical yield labels at county level. the most important lesson learnt from our experiments is that it's attainable get ascendable yield forecast as a result of the projected neural network model will notice and exploit redundant info each within the soil and within the weather information. The following figure demonstrates one of the systems proposed for yield forecast.

Machine learning approach for forecasting crop yield based on climatic parameters

The information mining techniques in predicting the crop yield supported the environmental condition input parameters. The developed webpage is user friendly and therefore the accuracy of predictions square measure higher than seventy-five percent all told the crops and districts designated within the study indicating higher accuracy of prediction. The user-friendly web content developed for predicting crop yield may be utilized by any user their alternative of crop by providing environmental condition knowledge of that place.

Crop Prediction on the Region Belts of India: A Naïve Bayes Map Reduce Precision Agricultural Model

The planned work introduces efficient degree economical crop recommendation system. Use of naïve mathematician makes the model terribly economical in terms of computation. The system is scalable because it may be wont to take a look at on totally different crops. From the yield graphs the simplest time of sowing, plant growth and gather of plant may be known. Conjointly the best and worst condition may also be incurred. The model focuses on all style of farms, and smaller farmers may also be benefitted.

Agricultural Production Output Prediction Using Supervised Machine Learning Techniques

Two supervised classification machine learning formula has-been enforced during this study. The choice Tree Learning-ID3 (Iterative Dichotomiser 3) and KNNR discover the patterns within the knowledge set containing average temperature and precipitation worth obtained and provides the prediction. be analyzed with more machine learning techniques to come up with crop predictions with higher exactness. Moreover, the analysis will result in profits and invention of advanced farming techniques which will improve our economy and can facilitate United States standout as a technologically advanced country.

Finds soil type and features crop mapping

Every time a crop is reaped from the land, the soil structure changes. It is hard to find the crop that would next suit the soil type. Some people in the agriculture industry maintain acres of land which makes it difficult to penetrate the potential problems in the other corner of their land piece. ML has a solution for all this by the building of digital maps for soil types and properties could make things easier. This aid in finding a solution quickly as it scans and gives an answer to what kind of crop could fit in the soil and reap most income.

Weather forecast to keep a check on crops health

A bad rain could soak the whole farm and earn zero penny while a no rain signal for too long could preferably yield the same. Therefore, weather inclusive of Rainfall and snow, Wind speed and direction, Humidity level, Amount and type of coverage of clouds in the sky, Temperature, Low-pressure areas, cyclones, tornadoes and depression, Sudden catastrophic changes like fog, frost, hail, thunderstorm and wind squalls plays a vital role in agriculture growth, development and yielding of crops. The findings brought about remarkable changes by sifting through database and studies to conclude things like the weather in the agriculture process.

Suggests pesticides and detects crop diseases

ML is informing the farmers how to manage pests and helps in forecasting the invasion of pests and the spread of microscopic diseases. Digital tools and data analysis in agriculture are being utilized to scientifically deal with harmful insects. Some companies have organized data science professionals to sensitize farmers on pesticide usage through user-facing platforms.

Automated irrigation system to minimize water use

The technological solution that is provided by ML for the facing water shortage issue is automated irrigation system. One kind of automated irrigation system functions based on small scale farms while the other uses weather predictions. It is capable of detecting the flood situation in areas through AI help.

Challenges of Agriculture

However, ML offers huge opportunities for application in agriculture, there still exists a lack of awareness with high tech machine learning solutions in farms across most of the region in a globe. Some of standstill challenges are Introduction of farming to external factors like weather conditions, soil situations and existence of pests, huge data requirement to train machines and to make accurate predictions setting of Market Trends, Agricultural Drones to Amplify the Growth of Market and predicting Geographic Overview Using of ML in agriculture is the worldwide.

The growth of the market is attributed to the high selection of trend setting innovations and item in agriculture part. Asia Pacific is estimated to meet high growth rate in the forecast period due to the rising demand from emerging nations, for instance, India and China. Also, rising adoption of the mechanical technology and IoT devices in agriculture is additionally evaluated to drive the ML in Agriculture market.

A Boom of Ml in Agriculture

According to a recent survey, the population will rise by 9.8 billion by 2050. Conversely, only 4% further land will come under farming by then. In this perspective, use of advance technological solutions to make cultivation more efficient, remains one of the greatest requirements. While, MLsees many direct use across sectors, i.e. ML-powered solutions will not only empower farmers to do better with less, it will also increase quality and assure faster go to market for crops. The report directed towards how ML can transform the agriculture landscape, the use of drone-made image processing techniques, exactitude farming landscape, the future of agriculture, challenges and overall Artificial Intelligence in Agriculture market position in forecast period. This is majorly owing to technological developments in the field of crop monitoring and artificial intelligence in agriculture.

The major players operating in the global smart agriculture market include

- IBM
- TOPCON Positioning systems
- Deere & Company
- AGCO Corporation
- SST Development Group
- AG Leader Technology

These players are implementing various strategies as strategic alliances and advanced product developments to obtain a robust market position. Global smart agriculture market is segmented into precision farming, livestock monitoring, fish farming, smart greenhouse, and others with qualitative and quantitative analysis on the global Agriculture via machine learning, computer vision, and predictive analytics. Machine learning held the largest market size owing to the growing adoption of this technology for various applications of agriculture such as precision farming, drone analytics, agriculture robots, and livestock monitoring. The successful ML algorithms bring huge success in Market Scope of Agriculture, Industry Dynamics, Drivers.

CONCLUSION AND FUTURE ENHANCEMENT

The weather predicting approaches and existing approaches are expensive and besides very unreliable for large datasets. On the way to overcome the drawbacks of the existing systems, an effective dimensionality reducing strategy: Self Organizing Map (SOM) is proposed along with Latent Dirichlet Allocation (LDA).ie., a hybrid system of SOM and LDA is designed with multi-objective classifier DNN for enhancing the performance of season and crop prediction. After reducing the measurement, the dimensionality reduced information is utilized to forecast climate for a reasonable outcome. A reasonable season for an appropriate crop is arranged with the guide of Deep Neural Network (DNN) classification system. This research work depends on finding appropriate information model, which helps in accomplishing high precision and simplification for value forecast. The research work focuses on the following:

- Analyzing the size of the global Agriculture on the basis of value and volume.
- Accurately computes vital factors of different segments of the global Agriculture market on yielding specific product.
- Showing the performance of different regions and countries in the global Agriculture market.
- Forecasting the market size and share of all segments, regions, and the global market.
- Crop yield and acreage forecasts for both cropping seasons available at district, state or all India level for single or multiple crops
- In-season tracking for near real-time monitoring of acreage, crop health and yield

- Premium calculator for efficient bidding and Pre-season yield and acreage forecast

Challenges exist, but one thing is sure: ML will significantly increase the efficiency of the farming industry. Undoubtedly, the need for quality ML solutions in agriculture will only grow with incorporation of appropriate research work.

REFERENCES

1. Giannaros, Theodore M., Dimitrios Melas, and Ioannis Ziomas, "Performance evaluation of the Weather Research and Forecasting (WRF) model for assessing wind resource in Greece", *Renewable Energy*, Vol.102, pp. 190-198, 2017.
2. Sellam,V, Poovammal, E., "Prediction of Crop Yield using Regression Analysis", *Indian Journal of Science and Technology*, Vol. 9, issue.38, pp.1- 5, 2016.
3. Hemageetha, N., "A survey on application of data mining techniques to analyze the soil for agricultural purpose", *3rd International Conference on Computing for Sustainable Global Development (INDIA-Com)*, pp.3112-3117, 2016.
4. Sujatha, R., Isakki, P., "A study on crop yield forecasting using classification techniques", *International Conference on Computing Technologies and Intelligent Data Engineering (ICCTIDE)*, pp.1-4, 2016.
5. Ankalaki, S., Chandra, N., Majumdar, J., "Applying Data Mining Approach and Regression Model to Forecast Annual Yield of Major Crops in Different District of Karnataka", *International Journal of Advanced Research in Computer and Communication Engineering*, Vol. 5, issue 2, pp.25-29, 2016.
6. Gayatri, M.K., Jayasakthi, J., Anandha Mala, G.S., "Providing Smart Agricultural Solutions to Farmers for better yielding using IoT", *IEEE Technological Innovation in ICT for Agriculture and Rural Development (TIAR)*, pp.40-43, 2015.
7. Fathima, G.N., Geetha, R., "Agriculture Crop Pattern Using Data Mining Techniques", *International Journal of Advanced Research in Computer Science and Engineering*, Vol. 4, issue 5, pp.781-786, 2014.
8. Kaur, M., Gulati, H., Kundra, H., "Data Mining in Agriculture on Crop Price Prediction: Techniques and Applications", *International Journal of Computer Applications*, Vol. 99, issue.12, pp.1-3, 2014.
9. Veenadhari, S., Misra, B., Singh, C.D., "Machine learning approach for forecasting crop yield based on climatic parameters", *International Conference on Computer Communication and Informatics*, pp.1-5, 2014.
10. Raorane, A.A., Kulkarni R.V., "Data Mining: An effective tool for yield estimation in the agricultural sector", *International Journal of Emerging Trends & Technology in Computer Science (IJETTCS)*, Vol. 1, issue 2, pp.75-79, 2012.
11. J. Mariette, M. Olteanu, and N. Villa-Vialaneix, "Efficient interpretable variants of online SOM for large dissimilarity data", Neurocomputing, Vol.225, pp.31-48, 2016
12. C. Lennard and G. Hegerl, "Relating changes in synoptic circulation to the surface rainfall response using self-organising maps", Climate Dynamics, Vol.44, No.3-4, pp.861-879, 2015.
13. A.Y. Abdulrahman, T.A. Rahman, I.M. Rafiqul, B.J. Olufeagba, T.A. Abdulrahman, J. Akanni, and S.A.Y. Amuda, "Investigation of the unified rain attenuation prediction method with data from tropical climates", IEEE Antennas and Wireless Propagation Letters, Vol.13, pp.1108-1111, 2014.
14. Shastry, H.A. Sanjay, and E. Bhanusree, "Prediction of Crop Yield Using Regression Techniques", International Journal of Soft Computing, Vol.12. No.2, pp.96-102, 2017. [16] J.
15. Scheffel, K. Lindvall, and H.F. Yik, "A Time-spectral Approach to Numerical Weather Prediction", arXiv preprint arXiv, pp.1704.00561, 2017.
16. Oliveira et al., "A Scalable Machine Learning System for Pre-Season Agriculture Yield Forecast", Published in 2018 IEEE 14th International Conference on e-Science (e-Science) 2018
17. Hardik Joshi et al., "Crop Yield Prediction Using Supervised Machine Learning Algorithm", IOSR Journal of Engineering (IOSR JEN), ISSN (e): 2250-3021, ISSN (p): 2278-8719, PP 35-42

18. Amarendra Goap et al., "An IoT based smart irrigation management system using Machine learning and open source technologies", Science Direct, Computers and Electronics in Agriculture, Volume 155, December 2018, Pages 41-49

Table 1- Brief Evaluation of Existing Literature

Existing Literature	Methodology Used	Evaluated result	Pitfalls
Giannaros, 2017	MM5 model is used for Numerical weather prediction model.	Evaluation of numerical weather prediction model, namely the Weather Research and Forecasting (WRF), with respect to the simulation of wind.	More complex while analyzing the input numerical data.
Sellam , 2016	RegressionAnalysis (RA), Linear Regression (LR) are Cited	Describes about various environmental factors that influence the crop yield and the relationship among these parameters is also established.	More complex to predict the optimized number of input parameter.
Hemageethaa , 2016	Naïve Bayes, Apriori algorithm are used for yield Prediction.	Focuses mainly on various soil parameters like pH, Nitrogen, moisture etc. and comparison accuracy is also presented.	Only 77% of precision is achieved.
Sujatha, 2016	Naïve Bayes, J48, random forests, support vector machines, artificial neural networks are implemented.	Climate data and Crop parameters are used for crop yield is predicted.	Other parameters like soil are not considered.
Ankalaki , 2016	DBSCAN, AGNES and MLR are used.	The comparative study between DBSCAN and AGNES is presented.	The formula is derived for each crop separately.
Gayatri , 2015	IOT and GPS Image capturing are used.	Far and near nodes are communicated through cloud.	Focuses mainly on image processing techniques.
Kushwaha , 2015	Hadoop Distributed File System (HDFS) is used.	The proposed prediction algorithm helps in building a decision support system for precision farming.	It only predicts the suitability of crop for the given soil parameters and not the yield.
Bendre , 2015	Map Reduce and Linear Regression algorithm are used for weather forecasting.	The effective model to improve the accuracy of rainfall forecasting is investigated.	The forecasting is done based on only a weather data.

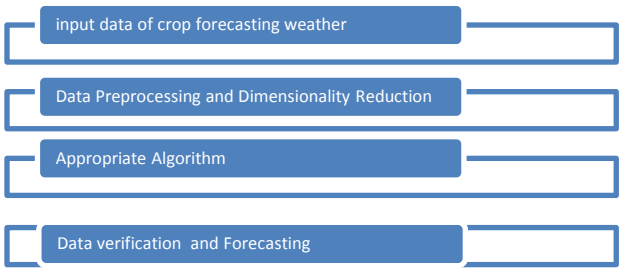


Figure 1: Generalized Block diagram of forecasting

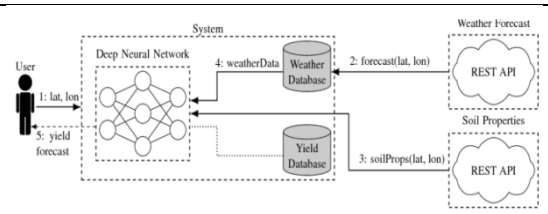


Figure 2. System Architecture for yield forecast [16]

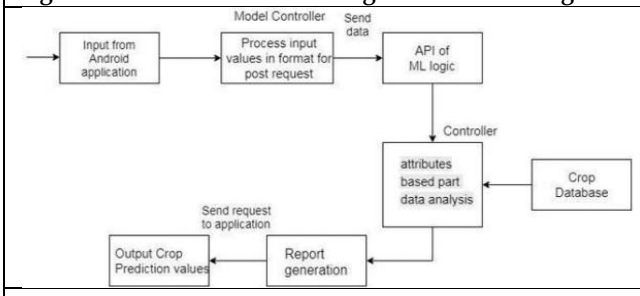


Figure 3. System flow for yield prediction [17]

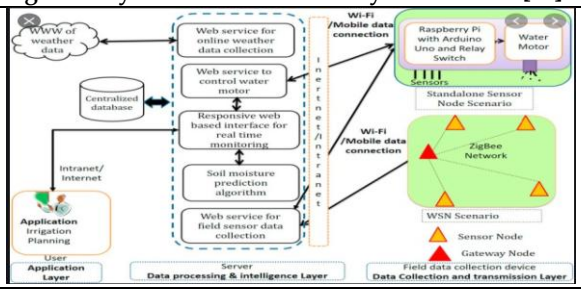


Figure 4. Smart Irrigation implemented by Machine learning [18]

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Studies on Heavy Metal Pollution Index of Groundwater in and around Tirunelveli Town, Near Thamirabharani River, Tamilnadu, India

S.S. Nawab Ali*, M. Mohamed Sihabudeen and J. Sirajudeen

PG and Research Department of Chemistry, Jamal Mohamed College (Autonomous), Affiliated to Bharathidasan University, Tiruchirappalli, Tamil Nadu, India.

Received: 16 Nov 2020 Revised: 19 Dec 2020 Accepted: 22 Jan 2021

***Address for Correspondence**
S.S. Nawab Ali
PG and Research Department of Chemistry,
Jamal Mohamed College (Autonomous),
Affiliated to Bharathidasan University,
Tiruchirappalli, Tamil Nadu, India.
Email: nawabchemist@gmail.com

ABSTRACT

The concentration of heavy metal present in the ground water in and around Tirunelveli town near thamirabharani river, tamilnadu was analysed with 10 ground water samples. The Heavy metal analyses of Cu, Fe, Zn, Pb, were performed for the water samples by using Atomic Absorption Spectrophotometer (AAS). The analytical data was compared with the guidelines given by WHO. The Calculated values HPI 146.13. The results reveal that the concentration of lead is higher than the permissible limit prescribed by the WHO. The result shows that the water quality of the study area is very poor and not suitable for drinking purpose. The heavy metal contamination may due to the discharge of waste, industrial and municipal wastewater, disposal of solid waste by land filling, and other anthropogenic influences in this region. It is known that heavy metal with higher concentration present in the ground water are highly toxic to human beings and living organisms.

Keywords: Heavy Metal Pollution Index, Human Health, Groundwater, Tirunelveli.

INTRODUCTION

Water is unarguably the most essential and precious. Life began in water and life is nurtured with water. Ninety seven percent of the world's water is found in oceans. Only 2.5% of the world's water is non-saline fresh water. There are organisms, such as anaerobes, which can survive without oxygen. But no organism can survive for any length of time without water. It is a universal solvent and as a solvent it provides the ionic balance and nutrients, which support all forms of life. In India the major source of water used to meet the domestic, agricultural and industrial needs is the ground water. The ground water is defined as water that is found underground in cracks and spaces in

soil, sand and rocks. This source has two distinct functions; firstly, it is a significant source of both urban and rural population's water supply and secondly it sustains many wetland ecosystems.

Groundwater is used for domestic, agriculture and industrial purpose in most parts of the world. The human activities like agriculture and domestic release large number of pollutants into the water bodies. In India ponds, rivers and ground water are used for the domestic and agriculture purposes [1]. The major sources of water are rainfall, surface water involving rivers, lakes and groundwater involving wells, bore wells etc. In recent years, the growth of industry, technology, population and water use has increased the stress upon both our land and water resources. Locally, the quality of ground water has been degraded. Municipal and industrial wastes, chemical fertilizers, herbicides and pesticides have entered the soil, infiltrated some aquifers and degraded the ground-water quality. Other pollution problems include sewer leakage, faulty septic-tank operation and landfill leachates. In some coastal areas, intensive pumping of fresh ground water has caused salt water to intrude into fresh-water aquifers. As the urbanization process continues, water pollution problems have become increasingly evident and have led to serious ecological and environmental problems. Industrial production without adequate regard for environmental impacts has increased water pollution has led to soil degradation and large scale global impacts such as acid rain, global warming and ozone depletion. All metabolic and physiological activities and life processes of aquatic organisms are generally influenced by water temperature [2].

The sources for ground water supply mostly depend upon the rainfall and the resulting percolation of the water into the earth. Another important factor is the quality of the soil. The heavy metals play a vital role in the normal functioning of human body. Imbalance of any of the heavy elements will disturb the normal function of human beings. Heavy metals are added to water system both from natural and man-made sources. Heavy metals in water refers to the heavy, dense, metallic elements that occur in trace levels, but are very toxic and tend to accumulate, hence are commonly referred to as trace metals. The major anthropogenic sources of heavy metals are industrial wastes from mining sites, manufacturing and metal finishing plants, domestic waste water and run off from roads. Many of these trace metals are highly toxic to humans, such as Hg, Pb, Cd, Ni, As, and Sn. Their presence in surface and underground water at above background concentrations is undesirable. Some heavy metals such as Hg, Pb, As, Cd, Fe, Co, Mn, Cr etc., have been identified as deleterious to aquatic ecosystem and human health.

Need for the Present Study

The main purpose of HPI (Heavy metal Pollution Index) is to turn complex water quality data in to information that is understandable and usable by the public. It gives the public a general idea of the possible problem with water in a particular region and evaluate the groundwater quality and its suitability for drinking, irrigation and domestic purpose in tirunelveli district, as the ground water is the only major source of water for drinking, irrigation and domestic purpose due to the lack of surface water in this region and also to link the quality of water in river thamirabharani through HPI and the distance from tirunelveli city. This shall be helpful for the efficient improvement in water quality management and policy making. To determine the spatial variation water quality by using HPI.

MATERIALS AND METHODS

Study Area

The study is carried out along the river bank of thamirabharani on a length of 125 kms starting from its origin at karayar of tirunelveli district to punnaikayal, the point where it drains into Bay of Bengal. There are 10 groundwater sampling locations have been selected, major towns, cities and also where different tributary connects of the river. The sites are chosen after conducting review literature, seeking connoisseur opinion and several site visits.

Collection of Groundwater Samples

Totally, 10 groundwater sampling locations were chosen during our reconnaissance investigation for the collection of water samples to do heavy metal pollution index. Outstanding care was given to distribution of spot throughout the study area during sampling point selection. The samples were collected in 1 liter polythene bottles. Prior to the collection, bottles were thoroughly washed with dil. H_2SO_4 and then with distilled water before filling the bottle with the sample. Each bottle was rinsed thoroughly to avoid any possible contamination in bottling and every other protective measure was taken [3].

Geology

The river thamirabharani is also known as the Porunai nathi (Tamil), which is one of the most significant perennial rivers among the 33 rivers of Tamilnadu. The river thamirabharani originates from the peak of Periyapothigai hill in the Western Ghats on Papanasam in the Ambasamudram taluk. It trespasses through Tirunelveli and Tuticorin districts before flowing into the Gulf of Mannar. Its total area of the catchment is 4500 km. It travelling up to 125 km (24 km in hilly ranges of Tirunelveli district and 40 km in Tuticorin district). It forms a delta in Punnaikayal village before falling into the Bay of Bengal, whose delta area is around 140.93 sq.km. It has about 50 large and small islands, the largest is with an area of 20 sq.km and the smallest is with an area of 0.1 sq.km. The river thamirabharani basin lies between 08° 8′ to 09° 23′ N latitude and 77° 09′ to 77° 54′ E longitude [4].

Sampling stations

1. Sivanthipuram (S1) 2.Mukkudal (S2) 3. Ambasamuthiram (S3) 4. Bazhavoor (S4) 5. Suthamalli vilakku (S5) 6. Kunnathoor (S6) 7. Senthimangalam (S7) 8. Kallidaikurichi (Bridge) (S8) 9. Seevalaperi (S9) 10. Tirunelveli town (S10).

REVIEW OF LITERATURE

Mohamed Sihabudeen et al, 2015

The heavy metal analysis values of all the ground water samples are within the permissible limit except Lead and Chromium. Hence it is recommended that suitable water quality management is essential to avoid further Lead and Chromium contamination in the ground water. Otherwise the ground water will be completely polluted and will become unfit to drink and for other purposes. Hence the ground water samples require treatment for Lead and Chromium before it is used.

J. Sirajudeen et al, 2015

The concentration of heavy metals like Zn, Pb, Ni, Fe and Cu have been determined by using Atomic Absorption Spectroscopy during pre-monsoon, monsoon and post monsoon. Based on the experimental data, the concentration of heavy metals during pre-monsoon is high compared to monsoon. This is due to evaporation during pre-monsoon. This study shows that most of the ground water samples have high content of Pb and Ni. On the basis of above discussion it may conclude that the underground drinking water at almost all sites in Edamalaipatti pudhur is highly contaminated. People dependents on this water are often prone to health hazards due to contaminated potable water. The high in the groundwater causes nausea, vomiting, diarrhea and if exceeding high in human system threatens life [5].

Heavy Metal Pollution Index

Heavy metal pollution index (HPI) is a technique of rating that provides the composite influence of individual heavy metal on the overall quality of water. The rating is a value between zero and one, reflecting the relative importance of individual quality considerations and inversely proportional to the recommended standard (Si) for each parameter

[6]. The calculation of HPI involves the following steps-First, the calculation of weight age of i^{th} parameter Second, the calculation of the quality rating for each of the heavy metal. The weightage of i^{th} parameter

$$Wi = k/Si \quad \text{.....(1)}$$

Where Wi is the unit weightage and Si the recommended standard for i^{th} parameter, (i=5), Where k is the constant of proportionality. Individual quality rating is given by the expression

$$Qi = 100\ Vi / Si \quad \text{..... (2)}$$

Where Qi is the sub index of i^{th} parameter, Vi is the monitored value of the i^{th} parameter in μg/l and Si is the standard or permissible limit for the i^{th} parameter.
The Heavy Metal Index (HPI) is then calculated as follows

$$HPI = \sum^{n}_{i=1} (Qi\ Wi) / \sum^{n}_{i=1} Wi \quad \text{..... (3)}$$

Where Qi is the sub index of i^{th} parameter. Wi is the unit weightage for i^{th} parameter, n is the number of parameters considered. The critical pollution index value is 75, above this value is not suitable for drinking purposes.

RESULTS AND DISCUSSIONS

The results regarding the mean values of the various metal concentration of ground water collected. The means result of the heavy metal analysis of all the samples are shown in Table 1 & Figures 1- 4 given below.

Copper (Cu)

Copper is an essential substance to human life, but chronic exposure to contaminant drinking water with copper can result in the development of anemia, liver and kidney damage[7]. Copper in large doses is dangerous to infants and people with certain metabolic disorders. On the other hand, lack of copper intake causes anemia, growth inhibition and blood circulation problems [8]. In this present study the concentration of copper content range in ground water from 0.026 ppm to 0.078 ppm. This study shows all samples have the level of copper than Permissible limit prescribed by WHO (2 ppm).

Iron (Fe)

Iron is biologically an important element which is essential to all organisms and present in hemoglobin system. High concentration causes slight toxicity, inky flavor, bitter and astringent taste. Iron contained water makes the teeth and nail black and weak, stickiness of hair and water. The shortage of iron causes a disease called anemia and prolonged consumption of drinking water with high concentration of iron may lead to liver disease called as haermosiderosis [9]. In our study the concentration of Fe ranges from is 0.019 ppm to 0.45 ppm. The Fe values in all ground water samples were found within the limit prescribed by WHO (1.0ppm)

Zinc (Zn)

Zinc is an essential trace element found in virtually all food and potable water in the form of salts or organic complexes. It plays an important role in protein synthesis. Zinc deficiency in human body may results in infantilism, impaired wound healing and several other diseases [10]. The values of zinc concentration obtained in this study, ranged from 0.02 ppm to 0.07 ppm. Zinc values for all samples were found within the permissible limit prescribed by WHO (3ppm).

Lead (Pb)

Lead is an undesirable trace metal less abundantly found in earth's crust. It is also found in soil, vegetation, animals and food. It is a serious cumulative body poison. Lead inhibits several key enzymes involved in the overall process of haemo-synthesis whereby metabolic intermediate accumulates [11]. The Pb values are found in the range of 0.02ppm to 0.07 ppm. In our study the high Pb values were observed for all water samples prescribed by WHO (0.01ppm). The Pb contamination of the ground water may be the result of entry from industrial effluents, household sewages, agricultural run-off containing phosphatic fertilizers, human and animal excreta. High Pb values may affect adverse changes in the arteries of human kidney and causes high blood pressure and kidney damage [12].

CONCLUSION

The ground water samples were collected from ten different places at tirunelveli town. The samples were subjected to analyse the concentrations of heavy metals like Cu, Fe, Zn and Pb by using Atomic Absorption Spectroscopy. In the present study, we found that Pb is present in relatively higher concentrations when compared to their permissible limits of WHO. Whereas Cu, Fe andZn concentration is below the permissible limit prescribed by WHO. The HPI is very useful tool in evaluating over all pollution of water bodies with respect to heavy metals. The HPI values of the present study indicate that the water samples ranged above 75, which shows that the water quality of the study area is very poor and not suitable for drinking purpose. Therefore the use of ordinary hand pump and bore well water should be discouraged. People dependents on this water are often prone to health hazards due to contaminated potable water. Therefore indigenous technologies should be adopted to make water fit for various purposes.

REFERENCES

1 Pramod N Kamble1, Viswas B Gaikwad , Shashikant R Kuchekarl, Der Chemica Sinica 2011, 2 (4):229-234.
2 Shah DG, Patel PS, Der Chemica Sinica, 2011, 2(5): 8-11.
3 Mohamed Sihabudeen M, 2020,Correlation Analysis of Heavy Metals in Ground Water Quality in and around Thamirabharani River at Tirunelveli District, Tamilnadu, India, Strad Research July,7(7): 0039-2049.
4 Rajesh .S, NVN. Nampoothiri, S.Vanitha, 2019, Water Quality Index of River Thamirabarani at Papanasam (upper & lower dam) region, Tamilnadu, India International Journal of Innovative Technology and Exploring Engineering (IJITEE) ISSN: 2278-3075, Volume-9 Issue-2.
5 J.Sirajudeen et al,2015, Studies on Heavy Metal Pollution Index of Groundwater of Edamalaipatti Pudhur Area near Korai River Tiruchirappalli District Tamil Nadu, India, IJSRD - International Journal for Scientific Research & Development| Vol. 3, Issue 08, | ISSN (online): 2321-0613.
6 Prasad B. and Kumari S., (2008), Heavy Metal Pollution Index of Ground Water of an Abandoned Open Cast Mine Filled with Fly Ash: a Case Study, Mine Water and the Environ., 27 (4).
7 Madsen, H., Poultsen L., and Grandjean., P., 1990), Risk of high copper content in drinking water. Ugeskr. Laegar. 152 (25).
8 Jennings, G.D., Sneed, R.E., Clair M.B, St. (1996) North Carolina cooperative Extension Service Publication no: AG – 473 -1. Electronic version 3.
9 Rajgopal., (1984), Groundwater quality assessment for public policy in India, 1st Annual report, Department of Geography, IOWA University, 10-11.
10 Khurshid S., Zaheeruddin and Shabeer M.U, (1997), Degradation of Water Quality due to Heavy Metal Pollution in Faridabad District, Hariyana, Poll. Res, 16(1), 41.
11 Verma N.K, Jain, O.P. and P.K. Shrivastava, 1995, Preliminary studies on heavy metals in ground water of Mandeep by Atomic Adsorption spectroscopy Proc, Acad, Environmental Biology, 4(1):123-126.
12 Abdul Jameel A. and Sirajudeen J. 2012, Advances research in Applied International Journal pelagia research library, 3 (1):424-429.

Table: 1: Mean value of metal ions concentrations of ground water samples collected in and around tirunelveli town

Stations	Heavy metals			
	Cu	Fe	Zn	Pb
S1	0.045	0.25	0.07	0.04
S2	0.037	0.28	0.05	0.05
S3	0.058	0.43	0.04	0.07
S4	0.026	0.025	0.02	0.03
S5	0.029	0.28	0.05	0.02
S6	0.028	0.28	0.05	0.05
S7	0.065	0.019	0.03	0.03
S8	0.054	0.19	0.05	0.05
S9	0.026	0.36	0.03	0.05
S10	0.078	0.45	0.02	0.06

Table 2: Calculation of HPI values for the heavy metal concentration of ground water in and around tirunelveli town

Heavy metals	Mean value in ppm(Vi)	Highest permitted value(WO) (Si)	Unit weightage (wi)	Wi×Qi
Cu	0.04	2.0	0.25	5.0
Fe	0.25	1.0	0.5	125.0
Zn	0.041	3.0	1.666	2.277
Pb	0.045	0.01	0.5	160.0

HPI =$\sum^{n}_{i=1}$ (Qi Wi) / $\sum^{n}_{i=1}$ Wi =146.13

Table 3: Status categories of HPI

HPI	Quality of water
0 – 25	Very good
26 – 50	Good
51 – 75	Poor
Above 75	Very poor (unsuitable for drinking)

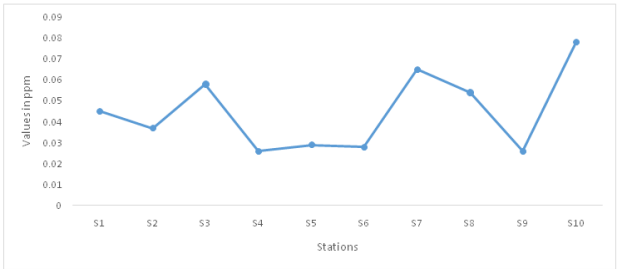


Chart. I. Variations of Copper (All the values are expressed in ppm)

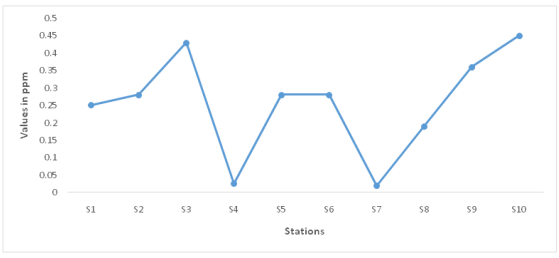


Chart. II. Variations of Iron

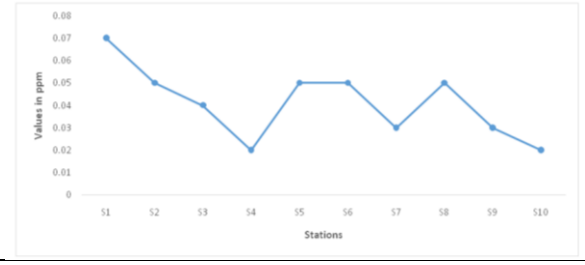


Chart. III. Variations of Zinc

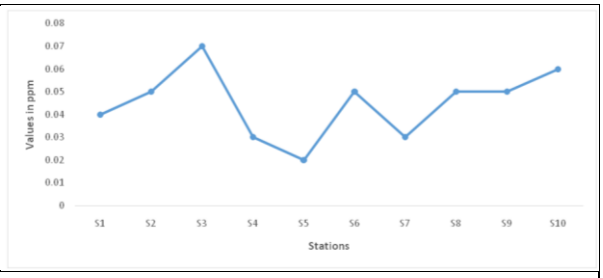


Chart. IV. Variations of Lead

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Severity of Risk Factors in Management of Cotton – As Perceived by Growers

Chitrasena Padhy[1]*, Pakalpati Satyanarayana Raju[2] and Rabindra Kumar Raj[3]

[1]Ph. D Student/Assistant Professor, Agricultural Extension, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha, India.
[2]Professor Emeritus, M.S. Swaminathan School of Agriculture, Centurion University of Technology and Management, Odisha, India.
[3]Professor and Head, Agricultural Extension and Communication, Institute of Agricultural Science, SOADU, Bhubaneswar, Odisha, India.

Received: 18 Nov 2020 Revised: 20 Dec 2020 Accepted: 22 Jan 2021

***Address for Correspondence**
Chitrasena Padhy
Ph. D Student / Assistant Professor,
Agricultural Extension, M.S. Swaminathan School of Agriculture,
Centurion University of Technology and Management,
Odisha, India.
Email: chitrasenapadhy@cutm.ac.in

ABSTRACT

Cotton is a crop with an uncertain growth habit having dynamic growth response to environmental changes and crop management practices. Adopting appropriate management practices helps in minimizing the deleterious effects of the production factors and improving cotton yield. The present study conducted on 240 cotton growers from four blocks in Gajapati and Rayagada districts of Odisha, India, revealed the risk factors and occur most frequently in cotton cultivation. These were: no control of Government on input dealers, unavailability of required manures and ignorance about alternate measures, severe weed menace, severe incidence of diseases and insect pests, lack of skill in topping, less reproductive branches, inability to provide life saving irrigation during dry spells, poor knowledge about dose and method of spraying, heavy dew during picking, non-availability of drying floors, no Government support for immediate purchase of the produce, risk of fire hazard during storage. Socio-economic attributes of growers such as education, caste, extension contact, occupation, and annual income, had significant influence in minimizing the frequency and severity of risk factors. The extension officials promoting cotton cultivation in the study area have to analyse all the risk factors and take possible approaches to minimise the frequency and severity of all these risk factors enabling better production and income generation of cotton farmers.

Keywords: Cotton, risk factors, frequency of risk, severity of risk, extension officials.

INTRODUCTION

India has the largest area under cotton production in the world (FAO, 2016). India accounts for about 17% of the world cotton production. Cotton plays a major role in the country's economy being one of the most important commercial fibre crops in India. Cotton is a crop with an uncertain growth habit having dynamic growth response to the environmental changes and crop management practices (Sikha *et al*., 2018). Cotton growers face various problems such as unexpected rain, incidence of insect pests and diseases, non-availability of quality seeds, and high costs of fertilizers, pesticides, and labour (Yadav and Goel, 2019). The farmers also face acute problems due to labour scarcity, poor storage facilities, low quality of inputs, and inadequate technical knowhow (Mohansundaram, 2015). If timely care is not taken to manage the various risk factors in cultivation of cotton, particularly crop management practices, there are chances of miserable situations (Gohil *et al*., 2016). The boll production of cotton is affected significantly by temperature, rainfall, wind and relative humidity. Boll retention is reduced by high temperature, especially more than 30° C. Strong winds may also reduce yield due to boll shedding (Cetin *et al*., 2010). This study was therefore made to analyse the risk factors involved in cotton crop management practices.

MATERIALS AND METHODS

The study was conducted in two tribal dominated districts of Gajapati and Rayagada in Odisha, India, where cotton is considered as the most remunerative crop for the sustainable livelihood of the resource-poor farmers. Rayagada and Kashinagar blocks in Gajapati district as well as Gunupur and Ramanaguda blocks in Rayagada district were randomly selected for the purpose of the study. Similarly, four gram panchayats in each block, i.e. Sanatundi, Kumelsingha, Karadasingi and Rayagad in Rayagada block, Budura, Khandaba, Alada and Goribandha in Kashinagar block, Gadhikhala, Sirijholi, Chalkamba and Jagannathpur in Gunupur block, and Buting, Nilamguda, Bhamini, and Golumunda in Ramanaguda block were randomly selected. Around 15% of the farmers cultivating cotton from all these panchayats were randomly selected. So, 67 farmers from Rayagada, 53 from Kashinagar, 62 from Gunupur and 58 from Ramanguda bock were randomly selected as the respondents for the study with the total sample size of 240. The data was collected personally through a semi-structured schedule pretested earlier. The data collected on the scale point of always, sometimes, and never on the severity of risk factors were analysed with the score value 3, 2, and 1, respectively. Statistical tools such as mean score, gap percentage, rank order, and multiple regression were employed to ascertain the results.

RESULTS AND DISCUSSION

Seed is the basic requirement for growing any crop. Farmers are always concerned about obtaining good quality seeds of chosen variety and their timely availability at reasonable price. The State Government Agricultural Department officials have to ensure that these requirements of the farmers are met. However, the study revealed that Government had no control over the seed dealers and the respondent farmers were exploited (Table-1). Moreover, quality seeds of chosen varieties at reasonable prices were not timely available. The respondents also emphasized the severity of risk factors that occurred most frequently which required appropriate action by the extension officials promoting cotton cultivation. Application of 8 to 10 tonnes of organic manure together with split doses of fertilizers is recommended for better growth and fruiting. The frequency and severity of risk factors as stated by the respondents on use of manures and fertilisers are given in Table-2. Unavailability of required manures, no skill competency in preparing quality manure, ignorance about alternate sources of manure, no idea about bio-fertiliser use, unavailability of required quantity of fertilizers along with its quality, no competency in calculating recommended dose of fertiliser, and above all exploitation by traders and dealers on sale price of fertililiser. Improving the knowledge and skill competency of cotton farmers through training and demonstration on preparing

quality manures along with proper monitoring of supply of quality fertiliser with fair price are essential to minimize the frequency and severity of risk factors on the use of manures and fertilisers. Cotton crop requires well pulverised soil for which summer ploughings as well as deep ploughing upto 15-20 cm and 2-3 cross harrowings with laddering are essential. It is advisable to prepare clean and friable seed beds for better root growth and crop stand. If this is not possible and late monsoon rains delayed sowing, the frequency and severity of risk factors in land preparation as observed in Table-3 need feasible solutions by the extension officials.

Cotton crop needs to be weed-free at least for six weeks after sowing. Therefore, timely and skillful weeding, hoeing, gap filling, earthing-up, and drainage are essential cultural practices. The data analysed and presented in Table-4 revealed that severe weed menace, inadequate skill in topping at one meter height for more reproductive branches, inability to provide life saving irrigation during dry spell, inability to make weed-free up to 30-40 days of sowing, and excess soil moisture causing inconvenience while top dressing with urea were the severe risk factors occurring regularly. Cotton crop is usually affected by a number of insect pests and diseases. The economic loss caused by insect pests and diseases is of serious concern. The data analysed on insect pest and disease management (Table-5) revealed severe incidence of insect pests and diseases, exploitation by the local dealers and suppliers of chemicals, ignorance of bio-pesticides and bio-control agents, no control of the Government on dealers and suppliers, limited supply of chemicals by the Government, incompetency in diagnosis of diseases and insect pests, poor knowledge of dose and method of spraying, as well as spraying sometimes being restricted by rain, were risk factors that occurred severely and frequently and caused economic loss to the respondent farmers. Harvesting of cotton is done manually by hand picking. Normally, farmers have to go for 2-5 pickings to complete harvest. Picking of cotton bolls is tedious work and is ten times costlier than irrigation as well as twice of weeding. Unavailability of skilled labourers is another constraint in picking. The frequency and severity of risk factors as stated by the respondents (Table-6) were labour scarcity for timely harvesting, smoke and rat menace causing discoloration and damage of lint in storage, fire hazard in storage, no Government support for immediate sale, non-availability of drying floors, low yield due to less reproductive branches, and heavy dew during picking causing inconvenience while harvesting, and improper post-harvest management.

Comparative analysis of the risk factors (Table-7) revealed that the respondents of both Gajapati and Rayagada districts were of similar opinion as significant differential gap percentages were not observed on the various aspects of risk factors studied. The respondents had more risk factors on harvest and post-harvest, insect pest and disease management, cultural practices, as well as manure and fertiliser use when compared to seed availability and land preparation. However, the mean score value indicated that the respondents had severity of risk factors on all aspects of management. Socio-economic attributes often solve the problems faced by farmers in their activities. Multiple regression analysis was therefore made to screen out the pertinent variable minimising the effect of risk factors in cotton cultivation. It revealed (Table-7) that the best fitted regression equation could explain 50.6% of the total variance in minimising the risk factors. Among the selected 15 variables, education, caste, extension contact, occupation, and annual income had significant influence in minimising the frequency and severity of risk factors in cotton cultivation.

CONCLUSION

Cotton is one of the most important commercial crops generating substantial income, particularly for the resource-poor farmers towards sustainable livelihood. But the frequency and severity of risk factors caused severe economic losses. The study revealed that the respondents faced risks in all the aspects of cotton cultivation covered in the study. The respondent farmers had more of risks in harvest and post-harvest, insect pest and disease management, cultural practices, and manure and fertiliser use compared to seed availability and land preparation. No control of Govt. on dealers and input suppliers, unavailability of required manures, ignorance of alternate manures, severe weed menace, inadequate skill in topping, inability to provide life saving irrigation during dry spell, severe

incidence of insect pests and diseases, poor knowledge about dose and method of spraying, heavy dew during picking, non-availability of drying floors, less reproductive branches, no Govt. support on immediate sale of the produce, and fire hazard during storage were the severe risk factors in cotton cultivation that occur frequently. Socio-economic attributes such as education, caste, extension contact, occupation, and annual income had significant influence in minimising the frequency and severity of the risk factors. The findings suggested that the extension officials promoting cotton cultivation have to analyse all the risk factors and take appropriate approaches to minimise the frequency and severity of risk, thus enabling improved production and income generation of the cotton farmers.

REFERENCES

1. FAO, 2016: Organisation for economic co-operation and development, Food and Agriculture Organisation, OECD-FAO Agricultural outlook, OECD Agriculture Statistics (database), http://dx.doi.org/10.1787/agr-outl-data-en.
2. Sikha, A., Maharana, P., Singh, K.K., Dimri, A.P. and Niwas, R. 2018: Cotton Crop in changing climate, Current Science, 115(5): 948-954.
3. Yadav, R.D. and Goel, R. 2019: Problems of Cotton farmers in Haryana, International Journal of Current Microbiology and Applied Sciences, 8(3):2139-2142.
4. Mohansundaram, P. 2015: Cultivation of Cotton: A study on factors and problems, International Journal of Arts, Humanities and Management Studies, 1(3):1-6
5. Gohil, G.R., Nakum, P.B., Kalsariya, B.N and Patel, R.I. 2016: knowledge level of cotton growers about crisis management for cotton cultivation, International Journal of Agriculture Sciences, 8(25): 1503-1505.
6. Cetin, O and Basbag, S. 2010: Effects of climatic factors on cotton production in semi-arid regions-A review, Research on crops, 11(3): 785-791.

Table 1: Frequency and severity of risk factors on seed availability

Sl. No.	Factor	Mean Score		Pooled Mean Score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Chosen variety not available	2.44	2.51	2.48	3
2	Unavailability of quality seeds	2.42	2.50	2.46	4
3	Seeds not available timely	2.08	2.06	2.07	7
4	Not available at reasonable price	2.48	2.42	2.45	5
5	Supply of spurious and F_2 seeds	2.08	2.03	2.05	8
6	Exploitation by dealers and other supply agencies	2.82	2.86	2.84	2
7	Seeds not available in easy access	2.28	2.16	2.22	6
8	Supply of admixture seeds by the supplier	2.11	1.99	2.05	8
9	No control of Govt. on seed dealers	2.86	2.93	2.89	1

Maximum obtainable score: 3

Table 2: Frequency and severity of risk factors on manures and fertilizers

Sl. No.	Factors	Mean Score		Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Unavailability of required manures	2.60	2.69	2.65	2
2	No skill competence in preparing quality manure	2.65	2.59	2.62	4
3	Ignorance about alternate manures	2.63	2.46	2.54	6
4	Fertilisers not available in time	2.29	2.22	2.25	11
5	Chosen fertiliser not available	2.44	2.42	2.43	10
6	Quality of fertiliser not ensured	2.36	2.58	2.47	7
7	Required quantity of fertiliser not available	2.51	2.43	2.47	7
8	Exploitation on sale price by the traders and dealers	2.77	2.73	2.75	1
9	Ignorance about integrated nutrient management	2.44	2.48	2.46	9
10	No idea about bio-fertiliser use	2.60	2.59	2.60	5
11	No competency in calculating recommended dose of fertiliser	2.66	2.59	2.63	3

Maximum obtainable score: 3

Table 3: Frequency and severity of risk factors on land preparation

Sl. No.	Factors	Mean Score		Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Summer ploughing not possible	2.31	2.25	2.28	7
2	Unable to make deep ploughing of 15-20 cm	2.33	2.32	2.33	3
3	Difficulty in line sowing	2.18	2.26	2.22	9
4	Well pulverised soil not possible	2.30	2.13	2.22	9
5	Not possible to prepare clean and friable seed bed	2.54	2.46	2.50	1
6	Ignorant about use of ploughing implements	2.35	2.24	2.30	6
7	Ploughing implements available not feasible	2.43	2.21	2.32	5
8	Bullocks not pulling available implements	2.40	2.25	2.33	3
9	Poor germination of seeds	2.18	2.28	2.23	7
10	Monsoon rain delayed sowing	2.48	2.44	2.46	2

Maximum obtainable score: 3

Table 4: Frequency and severity of risk factors on cultural practices

Sl. No.	Factors	Mean Score		Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Severe weed menace	2.92	2.96	2.94	1
2	Drainage development difficult	2.36	2.34	2.35	8
3	Difficulty in gap filling due to wet soil	2.43	2.36	2.39	6
4	Excess soil moisture restricting urea top dressing	2.50	2.32	2.41	5
5	More vegetative growth due to excess moisture	2.44	2.34	2.39	6
6	Timely hoeing not possible due to rain	2.43	2.27	2.35	8
7	Not possible to make weed free up to 30-40 days	2.67	2.53	2.60	4
8	Inadequate skill in topping	2.92	2.90	2.91	2
9	Inability to provide life saving irrigation during dry spell	2.75	2.78	2.76	3

Maximum obtainable score: 3

Table 5: Frequency and severity of risk factors on pests and diseases management

Sl. No.	Factors	Mean Score		Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Severe incidence of insect pests and diseases	2.83	2.93	2.88	1
2	Spraying sometimes not possible due to rain	2.56	2.39	2.48	8
3	Poor knowledge about management practices	2.37	2.43	2.40	9
4	Incompetency in diagnosis of insect pests and diseases	2.51	2.47	2.49	7
5	Poor knowledge of dose and method of spraying	2.52	2.48	2.50	6
6	Recommended chemicals not available in time	2.34	2.35	2.35	10
7	Exploitation by local dealers and suppliers	2.94	2.78	2.86	2
8	No control of Govt. on dealers and suppliers	2.64	2.72	2.68	4
9	No idea about integrated management methods	2.45	2.25	2.35	10
10	Limited supply of chemicals by the Govt.	2.62	2.55	2.58	5
11	Ignorance of bio-pesticides and bio-control agents	2.77	2.78	2.78	3

Maximum obtainable score: 3

Table 6: Frequency and severity of risk factors in harvest and post-harvest

Sl. No.	Factors	Mean Score		Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)		
1	Heavy dew drops during picking	2.51	2.53	2.52	7
2	Non-availability of drying floor	2.60	2.71	2.65	5
3	Moisture stress causing boll dropping	2.27	2.36	2.31	9
4	Low yield due to less reproductive branches	2.58	2.61	2.60	6
5	Difficulty in separating trash and other contaminants	2.37	2.31	2.34	8
6	No Govt. support in immediate sale	2.68	2.66	2.67	4
7	Smoke and rat menace causing discolouration and damage in storage	2.75	2.84	2.80	2
8	Fire hazard in storage	2.65	2.70	2.68	3
9	Labour scarcity for timely harvesting	2.83	2.90	2.87	1

Maximum obtainable score: 3

Table 7: Comparative analysis of risk factors

Sl. No.	Factors	Mean Score		Diff (%)	Pooled mean score (n=240)	Rank
		Gajapati District (n=120)	Rayagada District (n=120)			
1	Seed availability	2.40	2.38	0.83	2.39	5
2	Manures and fertilisers	2.54	2.53	0.39	2.53	4
3	Land preparation	2.35	2.28	3.01	2.32	6
4	Cultural practices	2.60	2.53	2.72	2.57	3
5	Pest and diseases management	2.60	2.56	1.55	2.58	2
6	Harvesting and post harvesting	2.58	2.62	1.53	2.60	1

Maximum obtainable score: 3

Table 8: Influence of socio-economic attributes in minimising risk factors

Sl. No.	Attribute	Unstandardized Coefficients		Standardized Coefficients	't' value	Probability
		B	Standard Error	Beta		
X_1	Age	0.790	1.222	0.046	0.646	0.519
X_2	Education	1.040	0.649	0.112	3.602	0.011*
X_3	Holding size	-0.676	1.275	-0.050	0.531	0.596
X_4	Farming experience	-0.425	0.791	-0.041	0.537	0.592

X_5	Caste	-1.788	1.683	-0.074	4.062	0.028**
X_6	Social participation	-0.154	0.588	-0.023	0.261	0.794
X_7	Cosmopoliteness	0.007	0.535	-0.001	0.013	0.990
X_8	Extension contact	-0.194	0.524	-0.148	3.745	0.002**
X_9	Source of information	0.136	0.423	0.028	0.322	0.748
X_{10}	House type	-0.235	1.356	-0.012	0.173	0.863
X_{11}	Occupation	-1.007	1.308	-0.056	0.770	0.032*
X_{12}	Annual income	-1.100	1.522	-0.069	0.723	0.004**
X_{13}	Social aptitude	-0.124	0.514	-0.021	0.240	0.810
X_{14}	Economic aptitude	0.176	0.445	0.035	0.395	0.693
X_{15}	Scientific orientation	0.093	0.352	0.025	0.265	0.792

R^2= 0.506, Adj. R^2 = 0.318

* = Significant at 0.05 level

** = Significant at 0.01 level

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Prediction of Liver Disease with Feature Selection and Classification Techniques

Jeyalakshmi K* and Rangaraj R[2]

[1]Associate Professor, Department of Computer Science, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, India.
[2]Professor and Head, PG and Research, Department of Computer Science, Hindusthan College of Arts and Science, Coimbatore, Tamil Nadu, India.

Received: 16 Nov 2020 Revised: 01 Dec 2020 Accepted: 28 Dec 2020

***Address for Correspondence**
Jeyalakshmi K
Associate Professor,
Department of Computer Science,
Hindusthan College of Arts and Science,
Coimbatore, Tamil Nadu, India.
Email: jeyas1201@gmail.com

ABSTRACT

Data Mining is one of the predominant revealing segments of predetermined request and distinctive evidence. It includes quite good number of data mining strategies to scrutinize valuable data. In the current health care industry, the most vital aspect is to offer medical facilities that are readily accessible for every last one. In the recent times, one of the prominent reasons for loss of life is caused by health issues related to liver. Though huge volume of data is being gathered by the health care industry, there is major setback in the proper mining and best possible usage of the collected data. For well-being of the mankind, prompt declaration of the liver disorder is indispensable. It has to be well-thought-out by planning suitable observant arrangements for the prompt analysis of liver problems. Thence this paper sort out general intelligent software model, analysis among Machine learning techniques and its algorithms that attaining optimised result in existing related works, .

Keywords: liver disorder, data mining, classification, Naïve bayes, SVM

INTRODUCTION

Generally for majority of the organisations, data and information turn out to be the chief resources and the level to which the collected data is operated determines the growth of any organisation. Also the data serves as the key input for the process of strategic decision making that advances the business. In the current century, maintaining a customer database assists in business management since with the intention of providing better services, customer

preference and behaviour has to be decided [1]. The role played by data mining in the health care industry is much higher. As per the record produced by WHO, the second principal fatality cause is cancer and for about 9.6 million deaths in 2018 was due to cancer. Low consumption of fruits and vegetables, high usage of tobacco and alcohol, lack of physical activity are few of the primary reasons for the cause of cancer. Also approximately 7,82,000 deaths were caused by liver cancer all over the world, in the year 2018. As per the survey by Times of India, one in every five person in India is affected by some sort of liver disorders [2]. One of the major organs in the human body that plays a primary role in maintaining the metabolism and carrying out various essential functions is the liver. The primary functions associated with immunity, digestion, metabolism and nutrients storage are all accomplished by liver, without which the body tissues would rupture. There exists a number of elements that accelerates the chances for liver diseases. According to the professionals, it is anticipated that India would turn out to be the "World Capital for Liver Cancer" by the year 2025 [2]. Several types of Liver disorders such as Bile Duct, Cirrhosis, Liver Cancer, Acute Hepatitis and Chronic Hepatitis exists which certainly need the attention of the medical experts [3]. Datamining and its associated Machine Learning (ML) is viewed as the technology that comes into view which has produced a drastic transformation in the information domain. It refers to the approach of evaluating the data from various viewpoints and précising it into constructive information using several analytical tools and techniques [4]. Thus it is a technique for discovering patterns or correlations between lots of fields in large relational databases. Hence data mining comprises of strategic functional elements which convert data onto data warehouse, deal with data present in a multidimensional database, enables data access by information analysts or professionals, assess data by applying application techniques and tools, and meaningfully deliver data to make available beneficial information [5].

Intelligent Systems Models

In the healthcare industry, the developed software have a fundamental function of offering effective services to the medical experts which in turn eventually results in providing superior treatment to the patients. A proficient healthcare software could contribute efficiently in various undertakings such as predicting the diseases grounded on the historical data of a different patient, image processing of medical images, a data warehouse for administrating the entire establishment and so on. In this regard various development models for the software are explained as follows [6]

Waterfall Model – Suitable for small and demanding projects and would offer good results in case the projects are complicated and larger. The lifecycle includes the following five stages:

- Requirement analysis
- Designing
- Implementation & Testing
- Integration & System testing
- Operations & Testing

Agile Model – founded on the principle of clustering together the approaches connected to the development of software that embraces alike principles. Small increments, minimum long-term planning and speedy release of high-quality software are the features of this model. The software development lifecycle includes:

- Planning
- Small Iterations
- Delivering software well in advance
- Obtaining frequent feedback from the clients

Incremental Model – A range of standalone modules are developed. Since the output of the preceding iteration acts as the input of the following, the former iteration has a vital role. Following are the phases in each of the iteration:

- Requirement gathering
- Designing
- Coding
- Testing

An Overview of Methodologies and Algorithms

In the timely realising of illnesses, chance factor investigation, Decision making, Treatment and medication, Data mining plays a crucial part in the therapeutic field. Information retrieval is a fundamental practice in determining produced information highlights, propensities and structures in vast datasets, and communicates approved identifications stimulated by the reason or condition of specific situations. Information mining is related to the domain of bioinformatics in various applications like disease visualization [7], disease conclusion [8],quality finding [9], protein recognition [10], protein work derivation [11], protein sub-cell position gauge [12],function theme identification [13], protein and quality participation arrange recovery [14], infection prescription improvement, information laxative [15]. Prediction and description are the two major objectives of Data mining in which prediction is achieved by utilising the variables existing in the database for predicting the unknown values that are desirable and description aims at unearthing the patterns that defines the data. The most widely employed data mining technique is Classification in which a set of pre-classified instances are used to develop a model that could classify the population of records in bulk. Based on the data type, classification type and constraint different classification algorithms are offered. Few of the popular classification models are as depicted below:

- **Naive Bayes Classifier:** works on the principle of Bayes theorem in which posterior probability is calculated based on the probability of likelihood of the event, predictor prior probability, class prior probability
- **J-48:** works by creating a decision tree, performing data analysis and in case a matching data with the training data is found, categorizes them. Termination of a branch is made for an ambiguous situation and thus classification is carried out.
- **SMO:** Sequential minimal optimization is employed for Support Vector Machine (SVM) and when utilised for binary classification, best results are attained.
- **Random Forest Algorithm:** a supervised classification algorithm that could manage classification still when the data is missing and also has the ability of operating for the categorical values too.
- **IBk:** instance-based classification is a supervised learning algorithm that uses neighbor nodes for which the values are resolved by the value of k.
- **Zero R:** the simplest classification approach that depends on the target and disregards all the predictors. Fundamentally, this classifier predicts the majority class.
- **Multilayer perceptron:** is a feed forward artificial neural network model that charts sets of input data onto a set of appropriate outputs. MLP is a variation for the standard linear perception and can differentiate data that are not linearly separable [17, 18].
- **Margin Curve:** defined as the difference between the probability predicted for the actual class and the highest probability predicted for the other classes
- **VFI algorithm:** each feature participates in the classification in this technique and each feature offers a vote for one of the classes out of the n available classes. It is an incredibly faster algorithm and in certain instances, it outperforms other classifiers too. In sequence to Classification, Feature selection is proceeded. Irrelevant attributes and redundant data may present in the dataset taken. Thus to optimize the accuracy data must be included in a feature selection technique. In general, the Correlation-based Feature Selection Subset Evaluator was used as Feature evaluator and Greedy Stepwise used as search method are obtained a good result.

Related Works

L. Anand et al [18] developed an Liver Disease Classification using Deep Learning Algorithm. The developed work do analysis on the Indian Liver Patient (ILPD) Dataset inclusive of 416 liver patient records and 167 non liver patient narratives including 441 male patient records and142 female patient records collected from Andhra Pradesh. Based on the LFT test outcomes, 11 attributes were taken in to consideration. As a result of work, the researcher used extracted features using M-PSO and ANN for classifying the features. This results in achieved calculation as 96.25% of the estimating purpose of Precision and is 96.16% of the F1 measure. Jagdeep Singh et al [6], designed a Software-based Prediction of Liver Disease with Feature Selection and Classification Techniques. Their effort considered 583

instances with eleven attributes from the (ILPD) Indian Liver Patient Dataset from the UCI Repository. They employed a database with 416 patient records among which 167 are non-liver disease instances. Objective of this work is to predict and analyse the liver diseases efficiently applying feature selection technique and classification algorithms effectively. Kefelegn [19] performed Prediction and Analysis of Liver Disorder Diseases by using Data Mining Technique: Survey. The research work, from the UCI data repository clumpses the dataset. Support Vector Machine (SVM) and Naive Bayes (NB) are the data mining approaches utilised inpatient classification to sort out liver disease occupied persons. C4.5 Decision Tree algorithm is thought out for performance evaluation of liver disease classification using UCI dataset. This work performs NB, SVM comparisons to achieve performance analysis. The general work flow used are shown below. Fig 1 exhibits Work flow of this approach.

Jatav and Sharma [20] proposed an algorithm in medical diagnosis for classification based data mining approach. Its objective attains classification of the patients with liver disease possibility by using data mining and machine learning approaches. Algorithms such as NB, KNN, SVM, Neural network, Fully Convoultional Network (FCN) are analysed and it is revealed that SVM outclasses other approaches. Serveral steps of the this research work are as depicted below in Fig 2. Vijayarani et al. [21] proposed an algorithm for Liver Disease Prediction using SVM and NB. For classifying the patients with liver disorders and others, Naïve Bayes and Support Vector Machine (SVM) classification techniques are used in this research work and from the UCI repository, the dataset is taken. In terms of execution time, it is shown that SVM achieves better results when compared to Naïve Bayes classifier. The general architecture of the research work is as follows. Nagaraj and Sridhar [22] Neuro SVM: A Graphical User Interface for Identification of Liver Patients. In this work, following the data collection from the UCI repository, the process of dual feature selection is performed. Depending on the functions of various algorithms, the features are chosen utilising boruta package and classification is carried out applying SVM, NB, Random forest and Bagging. Also the implementation is done in R platform. The workflow of this research is shown in the below figure. The general and common dataset utilised in above all existing works are depicted in following table 1 and its authenticated reference values are depicted in table 2 correspondingly.

CONCLUSION

The crucial objectives of this survey study are to make available diverse classification algorithms popular in the field of data driven prediction of liver disease. The general methods and algorithms described help expert to predict the liver disease at an early stage. Thence it is attained by performing a comparative study of various papers. Quite a good number of data mining methodologies have been proposed and formulated for the implementation in health care industry and in particular for diagnosing liver diseases since any liver disorder may result in liver cirrohis. The precision of predicting the liver infections by means of clinical data mining, normally clings upon the determination of the components. The success lies on determining the measures of clinically significant results. Machine learning techniques and algorithms play a key role in this aspect in appropriately classifying the liver disorder patients by which the results are then effectively analysed for further processing. From this evaluated study, systemised architecture, its workflow, attained output is known on several existing papers discussed.

REFERENCES

1. Tapas Ranjan Baitharu et al., "Analysis of Data Mining Techniques For Healthcare Decision Support System Using Liver Disorder Dataset", International Conference on Computational Modeling and Security (CMS 2016), Procedia Computer Science 85, 2016 862 – 870
2. Hajera Subhani et al., "A Study of Liver Disease Classification Using Data Mining and Machine Learning Algorithms", Springer Nature Switzerland AG 2020, pp. 630–640.
3. Larose D T, Discovering knowledge in data: an introduction to data mining, John Wiley, New York, 2005.

4. Goldschmidt P S, Compliance monitoring for anomaly detection, Patent no. US 6983266 B1, issue date January 3, 2006, Available at: www.freepatentsonline.com/ 6983266.html
5. Bace R, Intrusion Detection, Macmillan Technical Publishing, 2000.
6. Jagdeep Singh et al., "Software-based Prediction of Liver Disease with Feature Selection and Classification Techniques", International Conference on Computational Intelligence and Data Science (ICCIDS 2019), Science Direct, Procedia Computer Science 167 (2020) 1970–1980
7. M.-J. Huang, M.-Y. Chen, and S.-C. Lee, "Integrating data mining with case-based reasoning for chronic diseases prognosis and diagnosis," Expert systems with applications, vol. 32, pp. 856-867, 2007
8. J. Soni, U. Ansari, D. Sharma, and S. Soni, "Predictive data mining for medical diagnosis: An overview of heart disease prediction," International Journal of Computer Applications, vol. 17, pp. 43-48, 2011
9. B. Stapley, L. A. Kelley, and M. J. Sternberg, "Predicting the sub-cellular location of proteins from text using support vector machines," in Biocomputing 2002, ed: World Scientific, 2001, pp. 374-385
10. J. Ji, A. Zhang, C. Liu, X. Quan, and Z. Liu, "Survey: Functional module detection from protein-protein interaction networks," IEEE Transactions on Knowledge and Data Engineering, vol. 26, pp. 261-277, 2014
11. M. Deng, K. Zhang, S. Mehta, T. Chen, and F. Sun, "Prediction of protein function using protein–protein interaction data," Journal of computational biology, vol. 10, pp. 947-960, 2003
12. B. Stapley, L. A. Kelley, and M. J. Sternberg, "Predicting the sub-cellular location of proteins from text using support vector machines," in Biocomputing 2002, ed: World Scientific, 2001, pp. 374-385
13. J. D. Watson, R. A. Laskowski, and J. M. Thornton, "Predicting protein function from sequence and structural data," Current opinion in structural biology, vol. 15, pp. 275-284, 2005.
14. J. Xu and Y. Li, "Discovering disease-genes by topological features in human protein–protein interaction network," Bioinformatics, vol. 22, pp. 2800-2805, 2006
15. M. G. Tsipouras, T. P. Exarchos, D. I. Fotiadis, A. P. Kotsia, K. V. Vakalis, K. K. Naka, et al., "Automated diagnosis of coronary artery disease based on data mining and fuzzy modeling," IEEE Transactions on Information Technology in Biomedicine, vol. 12, pp. 447-458, 2008.
16. Sta´nczyk, U. Establishing relevance of characteristic features for authorship attribution with ANN. In: Decker, H., Lhotska, L., Link,S., Basl, J., Tjoa, A., editors. Database and Expert Systems applications; vol. 8056 of Lecture Notes in Computer Science. Springer Berlin Heidelberg; 2013, p. 1 - 8.
17. Sta´nczyk, U.. Rough set and artificial neural network approach to computational stylistics. In: Ramanna, S., Jain, L.C., Howlett, R.J., editors. Emerging Paradigms in Machine Learning; vol. 13 of Smart Innovation, Systems and Technologies. Springer Berlin Heidelberg;2013, p. 441-470
18. L. Anand et al., "Liver Disease Classification using Deep Learning Algorithm", International Journal of Innovative Technology and Exploring Engineering (IJITEE) ISSN: 2278-3075, Volume-8 Issue-12, October, 2019
19. Kefelegn S (2018) Prediction and analysis of liver disorder diseases by using data mining technique: survey. Int J Pure Appl Math 118(9):765–770
20. Jatav S, Sharma V (2018) An algorithm for predictive data mining approach in medical diagnosis. Int J ComputSciInfTechnol 10(1)
21. Vijayarani S, Dhayanand S (2015) Liver disease prediction using SVM and Naïve Bayes algorithms. Int J Sci Eng Technol Res 4(4)
22. Nagaraj K, Sridhar A (2014) Neuro SVM: a graphical user interface for identification of liver patients. Int J Comput Sci Inf Technol 5(6):8280–8284

Table 1. Data set – Liver patients of India

No	Attributes
1	Age
2	Gender
3	TB Total Bilirubin
4	DB Direct Bilirubin
5	Alkphos Alkaline Phosphotase
6	SgptAlamine Aminotransferase
7	Sgot Aspartate Aminotransferase
8	TP Total Proteins
9	ALB Albumin
10	A/G Ratio Albumin and Globulin Ratio
11	Selector field used to split the data into two sets

Table 2. Liver Disease Patients' Dataset attributes

Attributes	Description
Age	A numeric value having range [4-90] In the year
Gender	having two nominal value "male" or "female"
TB (Total Bilirubin)	A numeric value having range [0.4-75]
DB (Direct Bilirubin)	Numeric value having range [0.1-19.7]
Alkphos (Alkaline Phospotase)	A numeric value having range [63-2110]
Sgpt (Alamine Aminotransferase)	Numeric value having range [10-2000]
Sgot (Aspartate aminotransferase)	A numeric value having range [10-4929]
TP (Total Proteins)	A numeric value having range [2.7-9.6]
ALB (Albumin)	Numeric value having range [0.9-5.5]
Albumin and Globulin Ratio (A/G Ratio)	A numeric value having range [0.3 2.8]
Class	having the class value "1" represents Liver Disease present and "2" represent Liver
(Selector)	Disease not present

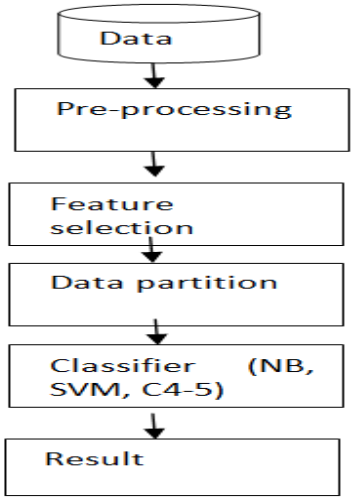


Fig.1 Workflow of methodology developed by Kefelegn et al

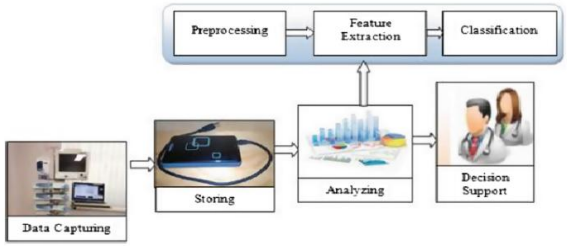


Fig 2. System architecture of the approach

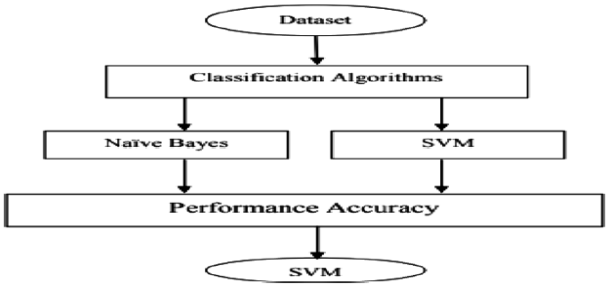


Fig 3. workflow of approach by Vijayarani et al.

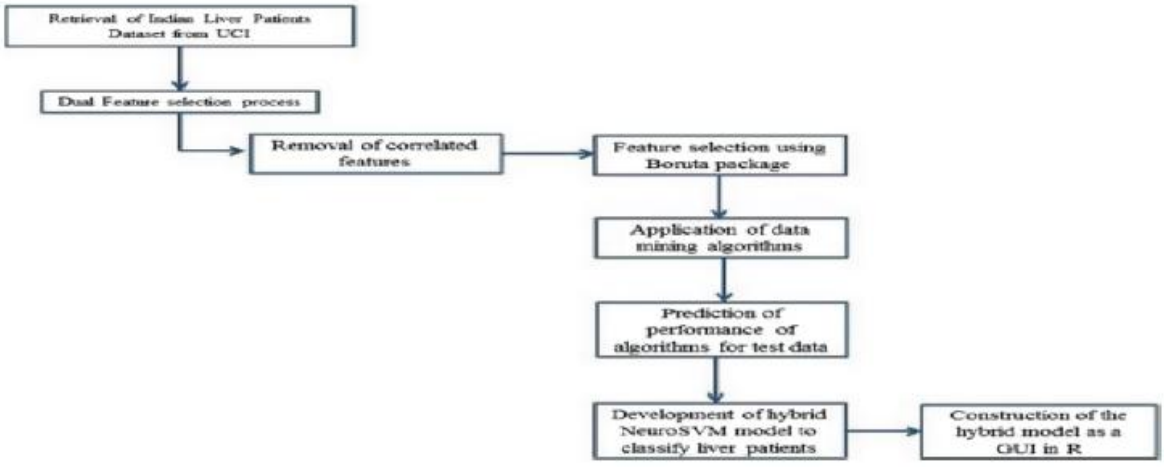


Fig 4. System architecture of the Neuro SVM approach

Indian Journal of Natural Sciences www.tnsroindia.org.in ©IJONS

Vol.11 / Issue 64 / February / 2021 *International Bimonthly* *ISSN: 0976 – 0997*

RESEARCH ARTICLE

Species Composition and Diversity of Macro and Meio Benthos in Thangassery Fishing Harbour Area of Kerala, South West Coast of India

Ambily.K.Sukumaran[1]*and R.Sunil Kumar[2]

[1]Ph.D.Zoology, Post Graduate and Research Department of Zoology, Catholicate College, Pathanamthitta, Affiliation: Mahatma Gandhi University, Kottayam, Kerala, India.
[2]Associate Professor, Post Graduate and Research Department of Zoology, Catholicate College, Pathanamthitta, Affiliation: Mahatma Gandhi University, Kottayam, Kerala, India.

Received: 23 Nov 2020 Revised: 25 Dec 2020 Accepted: 27 Jan 2021

***Address for Correspondence**
Ambily.K.Sukumaran
Ph.D.Zoology,
Post Graduate and Research Department of Zoology,
Catholicate College, Pathanamthitta,
Affiliation: Mahatma Gandhi University,
Kottayam, Kerala, India.
Email: ambilyasheraby@gmail.com

ABSTRACT

The objective of the study is to examine the species composition and diversity of macro and meio benthos of Thankasserry fishing harbour along with environmental parameters. Sampling is carried out for one year, October 2015 to September 2016. Percentage composition of macro benthos indicates polychaetes are the dominant group (68.94%) followed by gastropods (21.49%), bivalves (9.4%) and other organisms (0.17%). Percentage composition of meio benthos indicates nematodes are the dominant group (56.22%) followed by foraminiferans (41.70%) and other organisms (2.08%). Macro and meio benthic composition and abundance and its correlation with hydrological and sedimentological parameters are analyzed. It reveals there is no direct impact of ecological factors on benthic organisms. On the other hand, classical diversity indicates that the harbour is polluted and the macro and meio benthic community is under stress due to natural and/ or anthropogenic factors. Overall the ecosystem dynamics and stability of the fishing harbour area appeared to be determined by the large scale anthropogenic impact factors.

Keywords: Harbour, Macro benthos, Meio benthos, Species composition, Diversity, South west coast of India.

INTRODUCTION

Benthic organisms play a significant role in energy pathway and nutrient cycling and also an essential link in aquatic food chain (Snelgrove, 1998, Shafiqul Islam et al., 2013). Benthic communities have been extensively used in assessing the health of marine environment and biodiversity (Millward & Grant, 2000; Dovgal et al. 2008; Ingole et al. 2009). Macro benthos are an integral and important element of all ecosystems (Asadujjamanet al., 2012). Survival, distribution and abundance of macro benthos depend on the characteristics of their environment such as water and sediment characteristics and ability to construct permanent burrows in the substratum (Perkins,1974; Dahanayakar and Wijeyaratne,2006). Polychaetes, bivalves, molluscs and crustaceans are the most established macro benthic organisms and are regarded as environmental indicators (Ingole et al., 2006). Meiofauna are phyletically more diverse and the most abundant metazoan known to science. Meiofauna may be more sensitive to sediment pollution than macrofauna. They have certain inherent advantages over macrofauna in the determination of the effects of biological pollutants at the community level. They also have higher species richness than macrofauna (Heip et al., 1985). Disturbances may affect in growth rate, recruitment and mortality of organisms (Tablado et. al., 1994; Johnston and Keough, 2002).

Harbours, as a major interface between coastal cities and the sea, are often under heavy pressure from human activities and increasingly suffer from environmental risks linked to poor water and sediment quality (Estacio et al. 1997). Harbours are enclosed areas considered as the most altered coastal habitats characterized by depleted oxygen in the water column, high concentration of pollutants in water as well as sediment, low hydro dynamism and low biodiversity (Darbra et al., 2005; Guerra-Garcia and Garcia-Gomez, 2004; Danulat et al., 2002; Rivero et al., 2005). Although, harbours are the lifeline of a country's economy as the bulk of the trade takes place through them. The fisheries sector plays a vital role in Indian economic development, provides many employment opportunities and nutritional security to the nation. The west coast of India is recognized as a biodiversity hotspot and contributes to >70% of the countries marine capture fishery (Ingole, 2009). Objective of the present study is to examine the macro and meio benthic species composition and diversity in Thangassery fishing harbour along with environmental factors.

MATERIALS AND METHODS

Study area

Thangassery (8°52'35"N;76°34'E) (Fig. 1) is situated in Kollam district, Kerala along the south west coast of India. Sample collection for the present study is carried out for one year from October 2015 to September 2016. Ten sampling stations were fixed in harbour and the average value taken for analysis for all factors

Hydrological analysis

Water samples for hydrological analysis are collected at subsurface level (0.5m depth) along the water column from study station. Temperature, dissolved oxygen, chloride and salinity, pH, conductivity, nitrate, phosphate and sulphate are measured (APHA, 1995; Grasshoff et al.,*1983)*.

Sedimentological analysis

Approximately 150 grams of wet sediment grab sample collected from each station for the studies of the sediment characteristics. pH, organic carbon and organic matter (Walkley and Black, 1934), nitrate, phosphate, sulphate (Grasshoff et al., 1983) are measured. The soil texture was estimated by combined sieving and pipette analysis as described by McIntyre, (1969).

Macro benthic sample collection

Duplicate macro benthic samples are collected by using Van Veengrab (0.1m²) from each stations. The sediment washed through a 0.5 mm mesh sieve, the retained organisms on the sieves are considered as macro benthos. These macro benthos were preserved in 5% formalin. The separated macrofauna were counted and identification was carried out to species level for major groups' polychaetes, molluscs and gastropods. Some specimens could not be identified due to damage or unresolved taxonomic problems, were considered as 'others' group of organisms. The identified organisms are sorted, counted and identified upto species level with a stereo microscope (40X magnification, Leica digital stereo microscope) and the counting are made astotal individual per 0.1m² (ind./0.1m²). Numerous taxonomy references used for identification, the most often used are Fauvel (1953) and Day (1967).

Meio benthic sample collection

Meio benthic samples are collected by using 10 cm graduated PVC tube corer with an inner diameter of 2 cm was used to sub sample the meiofauna from sediment sample collected by Van veen grab. The tube corer is inserted into the undisturbed sediment sample upto a depth of 5 cm, duplicate core sample was taken. Sediment containing meiofauna was stained with Rose Bengal biological stain (Pfannkuche and Thiel, 1988) and preserved in 5 % formalin. Later the samples were separated by sieving through two sieves 0.5mm and 0.063 mm mesh size to separate the meiofauna (Giere, 2009). Washing was carried out till the clear sediment with meiofaunal organisms remain on the 0.063mm sieve. The separated meiofauna were counted and identification was carried out to possible lower level. The major groups were identified as nematodes, foraminifera and harpacticoidea. Nematodes are identified upto genus level whereas foraminiferans and harpacticoids are species level. Some meiobenthic organisms were present only occasionally and some organisms could not be identified due to damage or unresolved taxonomic problems, were considered as 'others' group of organisms. Numerous taxonomic references used for identification, the most often used is Giere (2009). Organisms are enumerated and expressed in 10cm² area.

Data Analysis

Species composition and diversity indices were calculated using PRIMER Version 6 for windows (Clarke & Warwick, 2001; Clarke & Gorley, 2006). For quantitative and qualitative analysis of diversity, various classical diversity indices are used such as Shannon diversity index (H') (Shannon, 1949) Margalef richness index (d) (Margalef, 1958), Pielou's evenness index (J') (Pielou, 1975), Simpson dominance index (λ), Simpson diversity index (1-λ) (Simpson, 1949).Correlations between hydrological and sedimentological parameters with macro and meio benthos are calculated by SPSS Version1.6 and also percentage composition is calculated from the data.

RESULTS

Hydrological factors

Water temperature has the highest value in pre monsoon (29.9^{0}C) followed by monsoon (28.8^{0}C) and lowest value is in post monsoon (27.3^{0}C)]. Atmospheric temperature shows the similar variation as water temperature and it has the highest value in pre monsoon (31.9^{0}C) followed by monsoon (31.2^{0}C)] and lowest value is in post monsoon (30.3^{0}C)]. Ph shows the highest value in monsoon season (7.92) followed by post monsoon season (7.86) and lowest is in pre monsoon (7.76). Conductivity shows the similar variation as pH. It has shown the highest value in monsoon season (43 mS/cm) followed by post monsoon season (41.9mS/cm) and lowest is in pre monsoon (40.1mS/cm). Chloride has The Highest Value in Monsoon (18.85mg/l) followed by pre monsoon (18.65mg/l) and lowest value is in post monsoon (17.70mg/l). Salinity has the highest value in Monsoon (34.04‰) followed by pre monsoon (33.44‰) and lowest value is in post monsoon (31.97‰). Dissolved Oxygen has the highest value in pre Monsoon (5.75mg/l)] followed by monsoon (4.95mg/l) and lowest value is in post monsoon (4.82mg/l). Highest value of nitrate is observed in pre monsoon (1.76mg/l) followed by post monsoon (0.855mg/l) and lowest value was observed in monsoon (0.620mg/l). Highest value of phosphate is observed in monsoon (0.048mg/l) followed by pre monsoon (0.038mg/l) and lowest value was observed in post monsoon (0.025mg/l). Highest value of sulphate is observed in monsoon

(97.583mg/l) followed by post monsoon (80.5mg/l) and lowest value was observed in pre monsoon (79.698mg/l) , (Table. 1).

Sedimentological factors

Sediment pH has the highest value in monsoon (7.86) followed by pre monsoon (7.58) and lowest value is in post monsoon (7.43).Organic carbon has the highest value in post monsoon (2.67%) followed by pre monsoon (2.65%) and lowest value is in monsoon (2.51%). Organic matter has the highest value in post monsoon (4.59%) followed by pre monsoon (4.57%) and lowest value is in monsoon (4.33%). Highest value of nitrate is observed in pre monsoon (0.71 mg/l) followed by monsoon (0.66mg/l) and lowest value was observed in post monsoon (0.63 mg/l). Highest value of phosphate is observed in post monsoon (0.650mg/l) followed by pre monsoon (0.630mg/l) and lowest value was observed in monsoon (0.530mg/l). Highest value of sulphate is observed in pre monsoon (71.55mg/l) followed by monsoon (70.91mg/l) and lowest value was observed in post monsoon (67.27 mg/l). Highest value of sand in post monsoon (67.48%) followed by monsoon (65.91%) and lowest value is in pre monsoon (57.30%). Highest value of silt in pre monsoon (11.72%) followed by monsoon (9.58%) and lowest value is in post monsoon (8.80%). Highest value of clay in pre monsoon (30.97%) followed by monsoon (24.49%) and lowest value is in post monsoon (23.71%),(Table.2).

Species and percentage composition of macro benthos

Polychaetes, gastropods and bivalves are the major macro benthic organisms reported from the study area. There are total 58 species of macro benthic organisms reported from the study period. Among them 34 species of polychaetes, 14 species of gastropods and 7species of bivalves are present. Others which include crustaceans and fishes contribute only 3 species. Species from polychaete group, *Platynereis dumerili* of the family *Nereididae* is the dominant polychaete worm contributing 3.14% of total organisms. Followed by another polychaete worm *Ampharete acutifrons* of the family Alucitoidea which are contributing 2.97% of total organisms. *Epitonium scalare* of the family Epitonidae is the dominant gastropod species contributing 2.01% of total organisms. Followed by another gastropod species *Oliva nebulosa* of the family *Olividae* which are contributing 2.01% of total organisms. *Anadara rhombea* (1.56%) and *Anadara granosa* (1.54%) of the family *Arcidae* which are the dominant species from bivalve group. *Penaeus indicus (0.13%), Charybdis cruciata* (0.02%) and *Leiognathus splendens (0.02%)* are the least contributing species.

Species and percentage composition of meio benthos

Foraminiferans and nematodes are the major meio benthic organisms reported from the study area. There are total 41 species of meiobenthic organisms reported from the study period. Among them 25 species of foraminiferans, 14 species of nematodes and 2 species of ostrocodes are present. Species from foraminiferans group, *Bolivina abbreviate* of the family Bolivinitidaeis dominant contributing 4.35% of total organisms followed by another foraminifera *Lagena apiculata* of the family Nodosariidae which are contributing 3.71% of total organisms. *Desmodora*sp. of the family Des modoridae is the dominant nematode species contributing 7.73% of total organisms. Followed by another nematoda species *Viscosia* sp. of the family Oncholaimidae which are contributing 7.71% of total organisms. *Parastenocypris canaliculata* (0.81%) and *Strandesia elongate* (1.27%) of the family Cyprididae are the least species from ostrocodes group.

Diversity indices of macro benthos

Classical biodiversity indices are used to compare spacio-temporal variations in diversity, which plays a significant role in the quantitative analysis of biodiversity in the ecosystem. In the current study, seasonal diversity indices of the macro benthos analysed. The diversity indices show only slight variations among seasons. Margalef's richness index (d), weights number of species in the community rather than individuals. "d" value shows all three seasons show slight variations, but premon soon season have a higher value of 7.689 than postmon soon 7.466 followed by monsoon 7.445. Equitability is often expressed as Pielou's evenness index (J') and the values lie between one and zero. The index refers to how close numbers of each species are in an ecosystem. In this study, Pielous evenness

index was observed high in the monsoon season (0.9874) followed by pre-monsoon (0.9834) and postmon soon (0.9805). Generally, the Shannon diversity index (H' ^{log2}) varies between 0.0 – 5.0 (Turkmen & Kazanci, 2010). More than 5 are uncommon (Magurran, 2004). In the present study, the Shannon diversity index shows the high value in pre monsoon (5.736) followed by post monsoon (5.719) and monsoon (5.708). Simpson diversity index ($1 - \lambda$)is an equitability or evenness index and the value of $1 - \lambda$ is always <1. In this study, three seasons shows similar values and all are <1. Pre monsoon season (0.98107) followed by monsoon (0.98074) and post-monsoon (0.98077).

Diversity indices of meio benthos

Classical biodiversity indices are used to compare spacio-temporal variations in diversity, which plays a significant role in the quantitative analysis of biodiversity in the ecosystem. In the current study, seasonal diversity indices of the meio benthos were analyzed. The diversity indices show only slight variations among seasons. Margalef's richness index (d), weights number of species in the community rather than individuals. "d" value shows all three seasons show slight variations, but monsoon season have a higher value of 5.6795than pre monsoon 5.2299 followed by post monsoon 4.9921. Equitability is often expressed as Pielou's evenness index (J') and the values lie between one and zero. The index refers to how close numbers of each species are in an ecosystem. In this study, Pielous evenness index was observed high in the post monsoon season (0.9433) followed by pre-monsoon (0.9378) and monsoon (0.9).Generally, the Shannon diversity index (H' ^{log2}) varies between 0.0 – 4.0 (Turkmen & Kazanci, 2010). More than 4 are uncommon (Magurran, 2004). In the present study, the Shannon diversity index shows the high value in pre monsoon (4.7708) followed by post monsoon (4.7585) and monsoon (4.6887).Simpson diversity index ($1 - \lambda$)is an equitability or evenness index and the value of $1 - \lambda$ is always <1. In this study, three seasons shows similar values and all are <1. Post monsoon season (0.9568) followed by pre monsoon (0.9564) and monsoon (0.9505).

Correlation Analysis

Correlation of macro benthos with hydrological parameters

Salinity showed significant correlation with chloride (r = 0.988**) at 0.01 level. Atmospheric temperature showed significant correlation with water temperature (r = 0.669*) at 0.05 level. Gastropod showed significant negative correlation with sulphate (r = -0.645*) at 0.05 level. (Table. 7)

Correlation of macro benthos with sedimentological parameters

Organic matter showed significant correlation with organic carbon (r = 1.000**) at 0.01 level. Sand showed significant negative correlation with sulphate (r = -.586*) at 0.05 level. Silt showed significant negative correlation with sand (r = -.675*) at 0.05 level. Clay showed significant negative correlation with sand (r = -.967**) at 0.01 level. Polychaete showed significant negative correlation with sulphate (r = -0.836**) and silt (r = -0.644*) () at 0.01 and 0.05 level respectively.

Correlation of meio benthos with hydrological parameters

Salinity showed significant correlation with chloride (r = 1.000**) at 0.01 level. pH showed significant correlation with chloride (r = 0.895*) and salinity (r = 0.895*) at 0.05 level. Nitrate showed significant correlation with atmospheric temperature (r = 0.871*) at 0.05 level. Foraminiferans showed significant negative correlation with chloride (r = -0.875*), salinity (r = -0.875*) and pH (r = -0.813*) at 0.05 level.

Correlation of meio benthos with sedimentological parameters

Organic matter showed significant correlation with organic carbon (r = 1.000**) at 0.01 level. Clay showed significant negative correlation with sand (r = -.966**) at 0.01 level.

DISCUSSION

Temperature is a factor of prime importance in the physical environment of organisms. This has a universal influence controlling the activities and distribution of animals and plants. Significance of water temperature is massive as it controls different a biotic characteristics of an aquatic eco system (Singh and Mathur, 2005; Ramachandra and Solanki, 2007). Steele (1983) reported that temperature has considered as the most critical factor seminal to the ecological boundaries of marine species allocation. In present study pre monsoon showed the highest value and post monsoon showed the lowest value of water and atmospheric temperature in both stations. Pre monsoon maximum may due to summer sea son in the Indian peninsula. Pre monsoon maxima were observed in many studies (Sahu et al., 2012). During post monsoon months or the at monsoon was prominent in Kerala this may due to the lowest value. Temperature did not show significant correlation with any factors in study station. Hydrogen ion concentration (pH) is a necessary chemical factor for marine life. pH can determine the survival rate and reproductive success of marine organisms. Various biological activities cause variations in pH (Gupta et al.,1996). In the present study, the range of variation in pH was found to be very narrow among seasons. Monsoon sea son showed highest values of pH in study area. This may due to discharge of effluents long with fresh water flow. pH did not show significant correlation with any factors study area. Conductivity is a measure of the capacity of water to conduct an electric current. It is proportional to the concentrations of total dissolved solids. In the present study monsoon sea son showed highest values of conductivity. This may be due to intense wave action in monsoon season. Study based on seawater quality in Gujarat coast (Bhadja et al.,2012) observed the high values of conductivity in monsoon season as the intense wave action of Arabian Sea. Rough tidal action may also vary conductivity values (Vaghela et al.,2010).

Conductivity did not show significant correlation with any factors. The constituent that occurs in sea water in the greatest abundance is Chloride. Chloride ion accounts for 55. 04 percent of the dissolved solids in any sample of ocean water. The present study observed highest value in monsoon season may be due to the river run off from terrestrial area. Chloride did not show significant correlation with any factors in study area. Salinity is among the most imperative environmental factor and wields various effects on the vitality of marine organisms. The decreasing or increasing trend of salinity aids the deteriorating effect on the growth rate of organisms. The present study observed that the salinity highest value in monsoon and lowest in post monsoon. This may due to the fresh water run of fin monsoon season. Fluctuations in salinity might occur during a tidal cycle or by heavy rains specially in tropics (Ingole & Parulekhar, 1998) .Salinity show significant correlation with chloride. The dissolved oxygen plays a major role in survival capacity of an organism. Dissolved oxygen is important for respiration of organisms. Oxygen levels are highest in surface waters, particularly coastal waters due to constant mospheric interaction and turbulence. Dissolved oxygen can be the critical source which offers information about the biological, biochemical and in organic chemical reactions taking place in aquatic environment. Highest dissolved oxygen was observed in pre monsoon and lowest was observed in post monsoon in study area. It may be attributed to photothetic activity by phytoplankton during summer periods (Sahu et al., 2012). Pre monsoon maxima of dissolved oxygen were reported in many studies (Rajagopalan et al., 1992). Oxygen did not show significant correlation with any factors in study station.

In the present study, sediment pH showed slight variations among seasons. The highest pH was observed in the monsoon season and the lowest in post monsoon season. In monsoon season the river runoff was very high, it affects the water quality and sediment pH. Ingole et al., (1998) reported a normal pH value 7-7.6 from the sediments of the west coast of India. Present study agrees with this finding. In benthic communities, it is generally expected that increases concentrations of total organic carbon in the sediment will result in increases abundance and productivity, with decreased species richness and diversity in changes to the benthic community's structure and possibly to functions. Post monsoon season showed the highest value of organic carbon and matter whereas monsoon season had the lowest. In post monsoon season north east monsoon was prominent in Kerala. This may lead to a high nutrient load to the sea. A positive relationship between the abundance of benthic fauna and concentration of

organic carbon in sediments had been documented by many workers (Damodaran, 1973; Parulekar et al., 1975; and Anbuchezhian et al., 2009). Texture and reworking also influence the organic carbon variaions (Thamban et al., 1997). The surface sediments of the west coast contained a greater quantity of total organic matter (Jacob et al., 2008). Organic matter may influence benthos through the availability of food supply and the consumption of organic matter bound sediment and subsequent generation of fecal pellets will alter the mechanical composition of sediments. Different workers studied the organic matter of sediments from various coastal areas along the west coast of India and substantiated the general fact that finer sediments retained more organic matter than coarse one.

In the present study, both water and sediment nutrient values showed slight variations among seasons. Analyzing the seasonal values nitrate was high in monsoon season whereas post monsoon and pre monsoon seasons were very low. The increased nitrate level may be due to fresh water inflow and terrestrial runoff in monsoon season (Santhanam et al.,2003).In the present study analyzing the season wise average of phosphate values was high in post monsoon season whereas pre monsoon season was very low. North east monsoon was prominent in Kerala at post monsoon season this may lead to a higher value. But in water post monsoon season showed the lowest value of phosphate. In the present study analyzing the season wise average sulphate values was high in post monsoon season whereas monsoon season was very low. North east monsoon was prominent in Kerala at post monsoon season this may lead to a higher value. River flow is considered to be a major source of organic load in harbours (Webber and Kelly, 2003). In the present study, the textural analysis indicates all seasons showed dominating the sand particles. This indicates the study area becomes sandy in nature. Along the southwest coast of India sediment was dominated by sand (Jacob et al., 2008). Analysis of water and soil revealed that there is no ecological master factor which may affect the composition of macro and meio benthic organisms.

The detailed understanding of the bottom fauna is necessary to obtain the comprehensive picture of the fishery potential of that area (Damodaran, 1973). Since the study area is a fishery area is a fishery harbour, evaluating the community structure of benthic fauna and its qualitative and quantitative analysis have immense significance in assessing the fishery as well as ecological aspects of the area. As the polychaetes often dominate in terms of composition and abundance, these are the important component in macro benthic community (Sivadas et al., 2009). Among macro benthos polychaete worms are dominant in the study area. Dominance of polychaete worms was as expected for harbour area and compares well with other harbour studies (Raman, 1995; Belan, 2003; Guerra-García & García-Gómez, 2004; Rivero et al. 2005). Within meio benthos, nematodes are dominant group in the study area. Among meio benthos nematodes are an excellent taxon as ecological indicators of benthic environment (Schratzberger et al. 2000). They have a ubiquitous distribution, with high density, high diversity and short generation time (Heip et al. 1985). According to Khan et al. (2004), the Margalef richness index will be higher (2.5-3.5) in healthy environment. In the present study the richness index was above 7 for macro benthos and 5 for meio benthos, this indicates the unhealthy environment of the harbour. Meiofauna have higher species richness than macrofauna (Heip et al., 1988). Generally, the Shannon diversity index varies between 0.0 – 4.0 (Turkmen & Kazanci, 2010). In the present study diversity index shows higher than that of normal range in macro (5) as well as meio benthos (4). This analysis clearly indicates that the harbour is polluted and the macro and meio benthic community is under stress due to natural and/ or anthropogenic factors. Anthropogenic disturbances may affect in growth rate, recruitment and mortality of organisms (Tablado et al., 1994; Johnston and Keough, 2002). Anthropogenic activities had gross influence in the harbours where the co-contamination of several toxicants such as petroleum products, heavy metals and pesticides show cumulative effects (Millward & Grant 2000). Simpson diversity index ($1 - \lambda$) is always <1. Present study observed both the macro and meio benthos are <1 and they are evenly distributed among seasons.

The present study revealed the fact that the ecosystem dynamics operating in the fishery harbour area appeared to be determined not only by the interactions among the benthic communities but also due to the large scale anthropogenic impact factors. Apart from this, it seems to be no direct impact of ecological factors both water and

edaphic on the bioceoenosis of benthic fauna and no ecological master factor which influences the bottom dwelling benthic organisms.

ACKNOWLEDGEMENTS

The authors are grateful to the Principal, Catholicate College, Pathanamthitta for the facility provided. First author thanks to Mr. M. S. Sileesh, EcoStatAssissance, Ernakulam for helping the statistical data analysis.

REFERENCES

1. Anbuchezhian, R.M., Rameshkumar, G. and Ravichandran, S., (2009). Macrobenthic Composition and Diversity in the Coastal Belt of Thondi, South east coast of India. *Global Journal of Environmental Research*, 3(2):68-75.
2. APHA.,(1995). *Standard methods for the examination of water and waste water.* In. Michel, J.T.,E.C. Arnold, R.D. Hoakand R and (Eds). American Public Health Association (APHA) Pub. WashingtonD.C.,19th Edn.pp:874. .
3. Asadujjaman, M., Hossain, M.B., Shamsuddin, M., Amin, M.A. and Azam, A.K.M., (2012). Occurrence and abundance of macro benthos of Hatiya and Nijhum Dweep Islands, Bangladesh. *Middle-EastJ.Sci.Res.*,11:184-188pp.
4. Badhja, P. and Kundu ,R., (2012). Status of seawater quality at few important industrial coasts of Gujarat, India, off Arabian Sea. *Indian Journal of Marine Science*.
5. Clarke K.R. and Warwick R.M., (2001) Change in marine communities: an approach to statistical analysis and interpretation, 2nd edition. Plymouth: Primer-E.
6. Clarke K.R. and Gorley R.N. (2006) PRIMER v6: user manual/tutorial. Plymouth: PRIMER-E, 192 pp.
7. Dahanayakar, D.D.G.L. and Wijeyaratne, M.J.S., (2006). Diversity of macro benthic community in the Negombo estuary, Srilanka, with special reference to environmental conditions. *Srilankan J. Aquat. Sci.*,11:43-61.
8. Damodaran, R., (1973). Studies on the benthos of the mudbanks of Kerala coast. *Bulletin of Department of marine Sciences,* University of Cochin, 6:1-126.
9. Danulat, E., Muniz, P., García-Alonso, J. and Yannicelli B.,(2002).First assessment of the highly contaminated harbour of Montevideo, Uruguay. *Marine Pollution Bulletin*, 44, 554– 565.
10. Darbra, R. M., Ronza, A., Stojanovic, T. A., Wooldridge, C. and Casal, J.,(2005). A procedure for identifying significant environmental aspects in sea ports. *Marine Pollution Bulletin* 50: 866-874.
11. Day, J.H., (1967). A mono graph on the polychaeta of southern Africa. Parts 1 and 2. *British Museum (Nat.Hist.). London* .pp;87 8.
12. Dovgal I., Chatterjee T., Ingole B. & Nanajkar M.,(2008)**.** First report of *Limnoricusponticus* Dovgal & Lozowskiy (Ciliophora: Suctorea) as epibionts on Pycnophyes (Kinorhyncha) from the Indian Ocean with key to species of the genus *Limnoricus. Cahiers de Biologie Marine*, **49**: 381-385.
13. Estacio F. J., García-Adiego E. M., Fa D. A., García-Gómez J. C., Daza J. L., Hortas F. & Gómez-Ariza J. L.,(1997). Ecological analysis in a polluted area of Algeciras Bay (Southern Spain): external "versus" internal outfalls and environmental implications. *Marine Pollution Bulletin*, 34: 780-793.
14. Fauvel. P.,(1953). The fauna of India including Pakistan, Ceylon, Burma and Malaya. *Annelida: Polychaeta*, Allahabad, 507pp.
15. Giere, O., 1993. Meiobenthology : The Microscopic Fauna in Aquatic Sediments. Springer, Berlin.
16. Grasshoff, K., Ehrharrot, M. and Kremling, K.,(1983). *Methods for seawater analysis (second revised edition)* Verlag. Chemic. Weinheim, Germany pp: 419.
17. Guerra-Garcia, J. and Garcia- Gomez,J.,(2004). Polychaete assemblages and sediment pollution in a harbour with two opposing entrances. *Helgoland Marine Research*,58(3),183-191
18. Gupta, R.K., Sharma, M., Gorai, A.C. and Pandey, P.N., (1996). Impact of Coal Mining Effluents on the physico-chemical characteristics of Raja Tank, Jaria (Dhanbad). *J. Fresh water Biol.,* 8(2):63-73pp.

19. Heip C., Vincx M. & Vranken G.,(1985).The ecology of marine nematodes. Oceanogra. *Marine Biology-Annual Review*, 23: 399-489.
20. Ingole,B.S. and Parulekar, A.H. (1998). Role of salinity in structuring the intertidal meiofauna of a tropical estuarine beach: Field evidence. *Indian Journal of Marine Sciences*,27:356-361pp.
21. Ingole, B., Sivadas, S., Goltekar, R., Clemente, S., Nanajkar, M., Sawant, R., D'Silva, C., Sarkar, A. and Ansari, Z., (2006). Ecotoxicological effect of grounded MV River Princess on the intertidal benthic organisms of Goa, *Environment International,* 32, 284-289.
22. Ingole, B., Sivadas, S., Nanajkar, M., Sautya ,S. & Nag, A .(2009). A comparative study of macro benthic community from harbours along the central west coast of India. *Environmental Monitoring and Assessment,* 154(1-4),135-146.
23. Jacob, J., Chandramohankumar, N., Jayaraj, K.A., Raveendran, T.V., Balachandran, K.K., Joseph, T., Nair, M., Achuthankutty, C.T., Nair, K.K.C., George, R., and Zeena P.R., (2008). Biogeochemistry of the Surficial Sediments of the Western and Eastern Continental Shelves of India. *Journal of Coastal Research,* 24(5): 1240-1248.
24. Johnston, E.L. and Keough, M.J., (2002). Direct and indirect effects of repeated pollution events on marine hard-substrate assemblages. *Ecol. Appl*., 12,1212-1228pp.
25. Khan A., Murugesan S.M. and Lyla P.S.,(2004).A new indicator macro invertebrate of pollution and utility of graphical tools and diversity indices in pollution monitoring studies. Current Science 87, 1508–1510.
26. Margalef R., (1958) Information theory in ecology. General Systems 3, 36–71.
27. McIntyre, A.D., (1969). Ecology of marine meiobenthos. *Biological Review of the Cambridge Philosophical Society*, 44:245-290.
28. Millward R. N. & Grant A.,(2000).Pollution-induced tolerance to copper of nematode communities in the severely contaminated Restronguet creek and adjacent estuaries, Cornwall, United Kingdom. *Environmental Toxicology and Chemistry*, 19(2): 454-461.
29. Pfannkuche, O. and Thiel, H., (1988). Sample processing. In: Introduction to the study of meiofauna. Smithsonian Institution press, London, 488 pp.
30. Parulekar, A.H., Rajamanickam, G.V. and Dwivedi, S.N., (1975). Benthic studies in Goa estuaries. Biomass and faunal composition in the Zuari estuary. *Indian J.Mar.Sci.,* 4:202-205.
31. Peres,J.M.,(1982). General features of oceanic assemblages in pelagical and benthal. *In: Kinne, O. (Ed.) Marine Ecology., 591.John Wiley and Sons Ltd. NewYork*, 119–186.
32. Perkins,E.J.,(1974).Thebiologyofestuarineandcoastalwaters,*AcademicPress,London,*pp:678
33. Pielou, E.C., (1975). A general book on ecological diversity, Entropy based diversity measures (Models for distribution of species). *Ecological diversity,* Newyork: Wiley.
34. Rajagopalan, M.S., Thomas Mathew, P.A., Naomi, K.J., Kaladhran, P., Balachandran,V.K. and Geetha Antony, (1992). Productivity of the Arabian Sea along the south west coast of India. *Bull. Cent. Mar. Fish. Res. Inst.*,45:9-37pp.
35. Raman, A.V.(1995). Pollution effects in Visakhapatnam harbour, India: an overview of 23 years of investigation and monitoring. *Helgol and Meeresunters,* 49,633-645.
36. Ramachandra, T.V. and Solanki, M.,(2007). Ecological Assessement of Lentic Water Bodies of Bangalore. *Envis Technical Report: 25* ,Indian Institute of Science, Bangalore
37. Rivero, S.M., Elías, R. and Vallariona, E.A., (2005). First survey of macro infauna in the Mardel Plata Harbour (Argentina), and the use of polychaetes as pollution indicators. *Revistade Biologia Marinay Oceanografía,* 40 (2), 101-108.
38. Sahu, G., Satpathy, K.K., Mohanty, A.K. and Sarkar, S.K., (2012). Variations in community structure of phytoplankton in relation to physico-chemical properties of coastal waters, south east coast of India. *Indian J. Geo Mar. Sci.,* 41(3):223-241pp.
39. Santhanam, P. and Perumal, P., (2003). Diversity of zooplankton in Parangipettai coastal waters, south east coast of India. *J. Mar. Biol. Assoc. India*, 45: 144- 151pp.

40. Schratzberger M., Gee J. M., Rees H. L., Boyd S. E. and Wall C. M.,(2000). The structure and taxonomic composition of sublittoral meiofauna assemblages as an indicator of the status of marine environments. *Journal of the Marine Biological Association of the United Kingdom*, 80(6): 969-980.
41. Shafiqul Islam, M., Nurul Azim Sikder, M., Al Imran, M., Belal Hossain, M., Debbrota Mallick and Monjur Morshed, M., (2013). Intertidal Macrobenthic Fauna of the Karnafuli Estuary: Relations with Environmental Variables. *World Applied Sciences Journal*, 21(9):1366-1373pp.
42. Shannon C.E. and Weaver W., (1949).The mathematical theory of communication. Urbana-Champaign, IL: University of Illinois Press.
43. Simpson, E.H., (1949). Measurement of Diversity. *Nature*, 163:688pp.
44. Singh, R. P. and Mathur, P.,(2005). Investigations of variations in physico-chemical characteristic of a fresh water reservoir of Ajmercity, Rajasthan. *Ind.J.Env.Sci.*,9:57–61pp.
45. Sivadas S.K., Ingole B., Ganesan P., Sautya S., Nanajkar M.,(2012). Role of environment heterogenecity in structuring the macrobenthic community in a tropical sandy beach, west coast of India. *Oceanogr*., Vol. 68;295-305pp.
46. Sivadas, S., Ingole, B. and Nanajkar, M., (2009). Benthic poly chaetes as good indicators of anthropogenic impact. *Indian Journal of Marine sciences.* Vol 39(2),pp.201-211.
47. Snelgrove, P.V.R., (1998). The biodiversity of macrofaunal organisms in marine sediments. *Biodiversity and Conservation*, 7, 1123-1132.
48. Steele, D.H.,(1983). Marine ecology and zoogeography. In: South, G.R.(ed.). *Biogeography and Ecology of the Island of Newfoundl and. Dr. W. Junk Publishers, The Hague* ,421-465pp.
49. Tablado, A., Lopez Gappa, J.J. and Magaldi, N.H., (1994). Growth of the pulmonate limpet *Siphonarialesson* (Blainville) in a rocky intertidal area affected by sewage pollution. *Journal of Experimental Marine Biology and Ecology,* 175:211–226pp.
50. Thamban, M., Purnachandra Rao, V. and Raju, S.V., (1997).Controls on organic carbon distribution in sediments from the eastern Arabian Sea Margin. *Geo-Marine Letters*, 17:220-227.
51. Turkmen G. and Kazanci N.,(2010) Application of various biodiversity indices to benthic macro invertebrate assemblages in streams of a national park in Turkey. Review of Hydrology 3, 111–125.
52. Vaghela, A., Bhadja, P., Ramoliya, J.,Patel, N. and Kundu, R., (2010). Seasonal variations in the water quality, diversity and population ecology of intertidal macrofauna at an industrially influenced coast. *Water Science and Technology*,61(6):1505-1514pp.
53. Walkley, A. and Black I. A., (1934). Method for determining soil organic matter and a proposed modification of the chromic acid titration method. *Soil. Sci*., 37: 29-37pp.
54. Webber, D.F. & Kelly, K.W.,(2003).Characterization of sources of organic pollution to Kingston Harbour, the extent of their influence and some rehabilitation recommendations. *Bulletin of Marine Science,* 73(2), 257-271.

Table 1. Hydrological factors of study area

Hydrological factors	Post monsoon	Pre monsoon	Monsoon
Water temperature (^{0}C)	27.3	29.9	28.8
Atmospheric temperature (^{0}C)	30.3	31.9	31.2
pH	7.86	7.76	7.92
Conductivity	41.9	40.1	43.0
Chloride (mg/l)	17.70	18.65	18.85
Salinity (‰)	31.97	33.44	34.04
Oxygen (mg/l)	4.82	5.75	4.95
Nitrate (mg/l)	0.85	1.76	0.62
Phosphate (mg/l)	0.025	0.038	0.048
Sulphate (mg/l)	80.50	79.69	97.58

Table 2. Sedimentological factors of study area

Sedimentological factors	Post monsoon	Pre monsoon	Monsoon
pH	7.43	7.58	7.86
Organic carbon (%)	2.67	2.65	2.51
Organic matter (%)	4.59	4.57	4.33
Nitrate (mg/l)	0.63	0.71	0.66
Phosphate (mg/l)	0.650	0.630	0.530
Sulphate (mg/l)	67.27	71.55	70.91
Sand (%)	67.48	57.30	65.91
Silt (%)	8.80	11.72	9.58
Clay (%)	23.71	30.97	24.49

Table 3. Species and Percentage composition of macrobenthos

Sl No	Species	% of composition
1	*Ampharete acutifrons*	2.97
2	*Armandia longicaudata*	2.46
3	*Armandia intermedia*	1.88
4	*Capitella capitata*	1.93
5	*Cirratulus africanus*	2.30
6	*Cossura coasta*	2.10
7	*Dorvillea gardineri*	1.93
8	*Euchone rosea*	1.99
9	*Eunice indica*	2.01
10	*Eulalia macroceros*	2.37
11	*Glycera alba*	1.78
12	*Glycinde capensis*	2.31
13	*Goniada emerita*	1.93
14	*Goniadella gracilis*	2.27
15	*Lanassa capensis*	2.30
16	*Leanira hystricis*	2.31
17	*Leocrates claparedii*	2.25
18	*Lumbrineris brevicirra*	2.27
19	*Maldane sarsi*	1.88
20	*Nephtys sphaercocirrata*	1.82
21	*Nereis capensis*	1.78
22	*Noto cirrus australis*	2.01
23	*Notomastus aberans*	2.05

24	*Perinereis cultrifera*	2.65
25	*Platynereis dumerili*	3.14
26	*Phyllodoce malmgreni*	1.93
27	*Pisionidens indica*	2.01
28	*Pistaherpini*	2.05
29	*Pista quadrilobata*	1.95
30	*Saccocirruspapillocercus*	1.97
31	*Scolelepis squamata*	1.71
32	*Sternapsis scutata*	1.43
33	*Sternapsis affinis*	1.24
34	*Trichobranchusglacialis*	1.16
35	*Bullia vittata*	1.71
36	*Cerithium coralium*	1.71
37	*Chicoreus virgineus*	1.35
38	*Epitonium scalare*	2.01
39	*Natica vitellus*	1.18
40	*Naticarius onca*	1.63
41	*Nassarius variegatus*	1.58
42	*Oliva nebulosa*	2.01
43	*Pirenella cingulata*	1.52
44	*Trochus maculatus*	1.45
45	*Turritella attenuata*	1.13
46	*Turritella cingulifera*	1.39
47	*Umbonium vestiarium*	0.79
48	*Cellana tramoserica*	0.88
49	*Anadara granosa*	1.54
50	*Anadara rhombea*	1.56
51	*Donax scortum*	0.96
52	*Meretrix meretrix*	1.41
53	*Modiolus metcalfei*	1.43
54	*Paphia undulata*	1.41
55	*Perna viridis*	1.07
56	*Penaeus indicus*	0.13
57	*Charybdis cruciata*	0.02
58	*Leiognathus splendens*	0.02

Table 4. Species and Percentage composition of meio benthos

Sl No	Species	% of composition
1	*Ammonia beccarii*	3.65
2	*Ammonia tepida*	1.16
3	*Bolivina abbreviata*	4.35
4	*Cyclammina cancellata*	1.68
5	*Diffusilina humilis*	2.32
6	*Discorbis patelli formis*	0.34
7	*Eliphidium crispum*	2.55
8	*Globigerina bulloides*	0.17
9	*Globigerinoides sacculifer Ra*	2.14
10	*Lagenaa piculata*	3.71
11	*Lagena marginata*	1.01
12	*Lagena semistriata*	0.75
13	*Loxostominaperrectum*	0.3
14	*Neoconorbinacrustata*	0.17
15	*Operculina cumingii*	3.15
16	*Planorbulinellalarvata*	1.21
17	*Quinqueloculina sulcata*	0.17
18	*Rosalina bertheloti*	1.04
19	*Rosalina bradyi*	1.62
20	*Rosalina globularis*	3.07
21	*Spirillinalimbata*	2.61
22	*Spiroloculina bidentata*	1.21
23	*Spiroloculina depressa*	0.98
24	*Textularia agglutinans*	2.26
25	*Triloculina oblonga*	0.23
26	*Astomonema sp.*	1.79
27	*Daptonema sp.*	0.63
28	*Desmodora sp.*	7.73
29	*Desmoscolexsp*	3.56
30	*Halalaimus sp.*	2.37
31	*Haplaomus sp.*	4.4
32	*Neochromadora sp.*	2.26
33	*Oxystomina sp.*	4.87
34	*Pandolaimus sp.*	3.71

35	*Polygastrophora sp.*	5.39
36	*Quadricomasp*	0.75
37	*Rhynchonema sp.*	4.87
38	*Synonchus sp.*	6.03
39	*Viscosia sp.*	7.71
40	*Para stenocypris canaliculata*	0.81
41	*Strandesia elongata*	1.27

Table 5. Diversity indices of macro benthos

Seasons	d	J'	H' (log2)	1-λ'
Pre-Monsoon	7.6894	0.9835	5.7365	0.9811
Monsoon	7.4454	0.9875	5.7089	0.9807
Post-Monsoon	7.4667	0.9805	5.7192	0.9808

Table 6. Diversity indices of meio benthos

Seasons	d	J'	H' (log2)	1-λ'
Pre-Monsoon	5.2299	0.9378	4.7708	0.9564
Monsoon	5.6795	0.9	4.6887	0.9505
Post-Monsoon	4.9921	0.9433	4.7585	0.9568

Table 7. Correlation of macro benthos with hydrological factors

	pH	Conductivity	Phosphate	Nitrate	Sulphate	Chloride	Salinity	Oxygen	Water temperature	Atmospheric temperature	Polychaetes	Gastropods	Bivalves
pH	1												
Conductivity	0.081	1											
Phosphate	-0.126	-0.106	1										
Nitrate	-0.4	0.157	-0.249	1									
Sulphate	-0.26	-0.431	0.228	-0.312	1								
Chloride	0.491	0.326	0.229	-0.081	0.009	1							
Salinity	0.486	0.325	0.213	-0.134	0.052	.988**	1						
Oxygen	-0.013	-0.281	-0.116	0.41	0.175	0.165	0.161	1					
Water temp.	-0.131	-0.417	0.165	0.187	0.296	0.237	0.192	-0.011	1				
Atmos. temp.	-0.191	-0.053	0.33	0.015	0.007	0.407	0.388	-0.056	.669*	1			
Polychaetes	0.135	-0.161	-0.525	-0.376	-0.046	-0.446	-0.447	-0.228	-0.417	-0.471	1		
Gastropods	0.517	0.132	-0.311	-0.179	-.645*	0.298	0.324	-0.226	-0.049	0.235	0.146	1	
Bivalves	0.205	-0.107	-0.127	0.095	-0.176	0.066	0.038	-0.231	0.13	-0.288	0.262	0.247	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 8. Correlation of macro benthos with sedimentological factors

	pH	Organic Carbon	Organic Matter	Nitrate	Phosphate	Sulphate	Sand	Silt	Clay	Polychaetes	Gastropods	Bivalves
pH	1											
Organic Carbon	-0.49	1										
Organic Matter	-0.486	1.000**	1									
Nitrate	0.008	-0.302	-0.301	1								
Phosphate	-.580*	0.433	0.43	-0.049	1							
Sulphate	.609*	-0.417	-0.409	0.504	-0.329	1						
Sand	-0.029	-0.372	-0.378	-0.536	-0.091	-.586*	1					
Silt	0.127	0.306	0.312	0.202	0.041	.593*	-.675*	1				
Clay	-0.009	0.341	0.345	0.574	0.095	0.498	-.967**	0.463	1			
Polychaetes	-0.492	0.222	0.214	-0.412	0.524	-.836**	0.55	-.644*	-0.437	1		
Gastropods	-0.136	0.236	0.229	-0.016	-0.053	-0.381	-0.001	-0.086	0.032	0.146	1	
Bivalves	-0.059	0.125	0.12	0.069	-0.132	-0.25	0.115	-0.022	-0.131	0.262	0.247	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 9. Correlation of meio benthos with hydrological factors

	Water temp.	Atmos. Temp.	Oxygen	Chloride	Salinity	pH	Conductivity	Nitrate	Phosphate	Sulphate	Foraminiferans	Nematodes
Water temp.	1											
Atmos. Temp.	0.765	1										
Oxygen	-0.189	0.421	1									
Chloride	-0.076	-0.058	0.43	1								
Salinity	-0.075	-0.057	0.43	1.000**	1							
pH	-0.442	-0.411	0.324	.895*	.895*	1						
Conductivity	-0.785	-0.436	0.476	0.392	0.392	0.571	1					
Nitrate	0.495	.871*	0.654	0.17	0.171	-0.176	0.028	1				
Phosphate	0.115	-0.157	-0.191	0.309	0.308	0.379	-0.457	-0.474	1			
Sulphate	0.385	-0.267	-.969**	-0.332	-0.332	-0.33	-0.613	-0.533	0.264	1		
Foraminiferans	0.231	0.13	-0.525	-.875*	-.875*	-.813*	-0.319	-0.03	-0.459	0.431	1	
Nematodes	-0.745	-0.79	0.028	0.517	0.517	0.717	0.793	-0.413	-0.097	-0.126	-0.468	1

*. Correlation is significant at the 0.05 level (2-tailed).

**. Correlation is significant at the 0.01 level (2-tailed).

Table 10. Correlation of meio benthos with sedimentological factors

	pH	Organic Carbon	Organic Matter	Nitrate	Phosphate	Sulphate	Sand	Silt	Clay	Foraminiferans	Nematodes
pH	1										
Organic Carbon	-0.692	1									
Organic Matter	-0.69	1.000**	1								
Nitrate	0.083	-0.268	-0.266	1							
Phosphate	-0.742	0.215	0.212	0.067	1						
Sulphate	0.678	-0.506	-0.503	0.639	-0.449	1					
Sand	0.01	-0.334	-0.337	-0.697	0.138	-0.568	1				
Silt	0.019	0.301	0.303	0.231	0.001	0.544	-0.677	1			
Clay	-0.018	0.296	0.299	0.758	-0.166	0.492	-.966**	0.462	1		
Foraminiferans	-0.368	-0.381	-0.384	0.075	0.759	-0.246	0.501	-0.294	-0.5	1	
Nematodes	-0.314	0.661	0.659	-0.59	-0.143	-0.733	0.184	-0.381	-0.087	-0.468	1

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

Fig. 1 Study Area